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Environmental Security Assessment and Management of Obsolete Pesticides in Southeast Europe

Edited by
Lubomir I. Simeonov
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Environmental Security Assessment and Management of Obsolete Pesticides in Southeast Europe

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Series C: Environmental Security

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Preface

The book is developed on the basis of the contributions at the NATO Advanced Study Institute on Environmental Security Assessment and Management of Obsolete Pesticides in Southeast Europe, which took place in Golden Sands Resort near Varna, Bulgaria, between 11 and 17 of September 2012. The main goal of the NATO Advanced Study Institute was to evaluate the current extent of improper disposal of obsolete pesticides in the region of Southeast Europe, to estimate the associated impact on environmental health and to develop recommendations to mitigate or eliminate threats posed to the environment, biodiversity and human life.

The industrial production and use of pesticides has a relatively short but controversial history. Pesticides are chemicals specifically manufactured to be toxic to target organisms and pests and are deliberately introduced in the environment in large amounts. Yet without pesticides, the modern development of agriculture would be impossible and public health could, in some cases, be compromised. The extensive use and/or improper use of pesticides can entail risks for human health, for non-target organisms and for the environment on a whole. Risk assessment and management of pesticide exposure should be a key activity in any country, particularly in countries with economies in transition. In such cases, special attention must be given to the issue of obsolete pesticides. Examples of obsolete pesticides are those that are unwanted because the manufacturer's expiration date has passed, the material is “left over” after the pest problem has been resolved, the material was purchased in excess quantities, the material is banned because of environmental or public health reasons or the material has undergone degradation and alteration to create new hazardous chemical by-products as a result of improper storage. Because of the enormous chemical complexity, at present the only accepted management option for obsolete pesticides is their immediate environmentally safe removal and destruction.

The objectives of the NATO Advanced Study Institute included the following issues in more detail: to consider the transport and fate of pesticides and associated contaminated materials in different environmental media and to identify the principal sources, emission routes and patterns of environmental pollution with pesticides; to identify the most suitable methods for environmental sampling analysis and sample preparation; to evaluate current methods and techniques for chemical analysis of environmental and biological samples and discuss the metrological and quality aspects of trace analysis and issues such as method validation, data processing and uncertainty evaluation for pesticide pollutant determination; to characterize the environmental and human health impacts of pesticide pollution, the health effects associated with acute and chronic exposure, and associations between pesticide pollution and human diseases; to present advanced research technologies and specialized computer models relating to exposure assessment, chemical fate and transport, and risk assessment; to discuss of issues such as model sensitivity, availability, uncertainty and predictive value; to present the advantages and drawbacks of conventional vs. emerging technologies for elimination and destruction of obsolete pesticides and by-products; to survey the international conventions, directives and standards concerning pesticide use.

The NATO Advanced Study Institute contributed for the exchange of knowledge between experienced scientists and young researchers from NATO and partnering countries; for the identification of data gaps and opportunities for international collaboration on research priorities; for the priority and response action setting for contaminated environmental media; for the risk-based management efforts to reduce the impact of obsolete pesticides on the environment; for the prioritization and recommendation for urgent, short-term and long-term policies intended to prevent, or limit, the impacts of obsolete pesticides on the environment and public health; for the stimulation of national policies and strategies for chemical safety and pollution prevention based on risk analyses; and for the harmonization of regulatory policies, personnel training and education and increasing general public awareness of the problems of obsolete pesticide use.

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Chapter 1
Obsolete Pesticides – A Threat to Environment, Biodiversity and Human Health

Ezra J. Mrema, Federico M. Rubino, and Claudio Colosio

Abstract Since Rachel Carson's passionate warning in the seminal 1962 book '*Silent Spring*', which is now celebrating 50 years from its publication, concern on the steadily increasing accumulation in the environment of chemically robust, biologically persistent and possibly toxic organochlorine pesticides led to their substitution with less threatening products and finally to stop or limit their production and to severely restrict their use. In particular, the Stockholm Convention on Persistent Organic Pollutants (POPS) signed in 2001 banned or greatly restricted 12 chlorinated organic compounds or classes due to their toxicity and ability to accumulate in the environment and to magnify through the global trophic network. Among them are 11 pesticides namely aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachloro-benzene, kepone, lindane, mirex, toxaphene. These pesticides played a historical role in mitigating the health impact of parasite-borne human pathogens such as malaria parasites and in protecting food crops to allow better feeding of an increasingly raising population especially in sub-tropical and tropical areas. Concern for human and environmental health is mainly due to long-term effects of some substances, in particular through endocrine disruption, interference with reproduction, carcinogenicity, although the actual size of effects of real-life exposure is still an active and debated research topic. Risk assessment and risk-benefit analysis of some key pesticides such as DDT still need a thorough understanding of the toxicity mechanisms and of its relevance to humans in the different life-stages. As a consequence of the Stockholm ban, large stockpiles of unusable pesticides accumulate in some countries and thus present considerable threat to the environment and to human

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health, also due to unavoidable degradation of the active formulated substances into poorly tractable materials. To avoid environmental damage through contamination of water and agricultural land resources by leaching or improper disposal of repositories, inventories of existing stockpiles are needed to plan and carry adequately safe disposal interventions. Methods for disposal need to tackle the peculiar chemical characteristics of these highly chlorinated aliphatic and aromatic compounds, *i.e.*, very low water solubility and an unusual stability towards acid, basic and oxidizing conditions. Research and technology development in this field is currently exploiting the most advanced 'green chemistry' approaches, aimed at an as complete mineralization of organo-chlorine substrates with as negligible production of toxic waste. We will draw a brief historical perspective of the genesis of this pivotal environmental problem, review the use of the '*dirty dozen*' pesticides and their contribution to the Green Revolution improvement of agricultural food availability, the ecotoxicological and human health concern and the current efforts to front the problems raised by their use.

Keywords 'Dirty dozen' • Organochlorine pesticides • Persistent Organic Pollutants • Stockholm Convention • Aldrin • Chlordane • Dieldrin • Endrin • Heptachlor • Hexachlorobenzene • Mirex • Toxaphene • DDT • α-Hexachlorocyclohexane • β-Hexachlorocyclohexane • γ-hexachlorocyclohexane • Lindane • Chlordecone • Endosulfan • Pentachlorobenzene • Kepone

1.1 Introduction

Agricultural progresses played a pivotal role in sustaining the exponential increase of world population since the late nineteenth century and protection of crops –along with mechanization of processes, chemical fixation of nitrogen and availability of improved cultivars- has since been one of the key factors into the process. In particular, the introduction of synthetic organic chemicals in the second half of the twentieth century not only boosted the capability to counter crop- and food-spoiling organisms but also allowed to eradicate or control parasite-borne health- and life-threatening diseases such as malaria, both improving the quality of life of large populations in temperate and semi-tropical areas and allowing better utilization of agricultural areas.

The ability of early twentieth century synthetic chemists to exploit the large availability of industrial chlorine as a cheap source of chemical diversity resulted in the preparation of a wide array of compounds and materials with large utility in most fields of industrial technology. Several classes of highly-chlorinated organic molecules were found to possess strong insecticidal activity, since Muller's (re) discovery of dichloro-diphenyl-trichloroethane (DDT) in 1939, then a chemical curiosity prepared more than 60 years before as a graduate chemist's exercise in organic synthesis. Many more daughters of the intellectual creativity of industrial chemists followed within two decades and the new, powerful, cheap and relatively safe organo-chlorine pesticides were prepared and applied on agricultural fields in the millions of tons in the following decades. The effect on agricultural landscape was striking since the beginning, both in the beneficial reduction of parasites in cultures and in the improvement of living conditions of the agricultural population, but also, in a soon much less appreciated way, as an immediately apparent decrease of landscape biodiversity. A prominent Italian poet, writer and film-maker, Pier Paolo Pasolini, even metaphorized the observation that the flash of summer fireflies had quickly disappeared from summer nights to sorrowfully point the dangerous side-effect of industrialization on people's most intimate lifestyle. By 1962 [6], since the publication of Rachel Carson's passionate book '*Silent Spring*', concern for the ecological consequences of uncontrolled application of pesticides and for their unstoppable dispersion in the general environment quickly rose, especially when highly populated regions of developed countries started to suffer the effects of widespread contamination as a threat to their own food sources. Awareness was also facilitated by the timely, although unrelated, availability of sensitive and specific methods for chemical analysis at trace level, such as gas chromatography and electron-capture detection of halogenated compounds, which allowed to empirically observe the phenomena of bioconcentration and biomagnification of these poorly degradable chemicals through their measurement in environmental compartments and in living organisms, all up the trophic levels.

As a consequence of a more than decennial effort, industrialized countries started to limit application of organochlorine pesticides, to ban their production and to enforce limits to their presence as ubiquitous contaminants of water and food. Finally an international environmental treaty, the Stockholm Convention on Persistent Organic Pollutants was signed in 2001 (effective from May 2004; amended in 2009 and 2011 with the addition of some more products), to eliminate or restrict the production and use of some priority persistent organic pollutants (POPs). Since then, while most developed countries and some of those which have trade relationship with them have in practice complied to the bid of the Convention, production, use and stockpiling were not discontinued and due to the low cost and still acceptable efficacy of some active substances, their use still continues, although on a much lesser scale.

The reduction of trace contamination from POPs at the global scale needs only to wait for their natural degradation in the environment, long as it will be the process. Two are the worthwhile scientific and technological efforts: (***a***) to better understand the natural processes of degradation of poly-chlorinated organic compounds which occur in the environment and to exploit them to accelerate the bioremediation process and (***b***) to improve our knowledge on their toxicity, especially towards the more sensitive groups of the human population, to perform a sound risk assessment aimed at forecasting their related sanitary and medical needs.

The existence of stockpiles in formerly producing or utilizing countries is a continuing threat to water and agricultural land resources, due to the possibility of contamination by illegal or inappropriate dumping of unmarketable formulated products or the unusable products which were generated over time on stocking. This in turn generates the necessity to address efforts to their safe disposal with the use of state-of-the-art knowledge and cost-effective technology.

1.2 The Stockholm Convention on Persistent Organic Pollutants

Early concern on the long-term consequences of the large, uncontrolled release of organochlorine pesticides into the environment led to restriction or ban to their use in an increasing number of developed Countries, along with the availability of less environmentally persistent insecticides, such as those of the organophosphate and carbamate classes. In particular, 10 years after the publication of '*Silent Spring*', the USA banned DDT.

In May 1995, UNEP Governing Council initiated a global action to reduce and eliminate the release of POPs. As per UNEP definition POPs are "*chemical substances that persist in the environment, bio-accumulate through the food web, and pose a risk of causing adverse effects to human health and the environment*". Following this, the Intergovernmental Forum on Chemical Safety (IFCS) and the International Programme on Chemical Safety (IPCS) produced the initial list of 12 POPs for assessment based on their properties and the urgency for action. These compounds were named, in journalistic jargon, as "*the dirty dozen*" and were listed in Annex A, B and C, respectively of compounds bound for elimination, restriction and limitation of unintentional production. Due to the efficacy of some poly-chlorinated pesticides, such as DDT and lindane, in fighting parasite-borne diseases such as malaria and scabies, respectively, exemptions allowing to continue production from registered parties and use for specific purposes are embedded into the Convention (Annex B), the enforcement of which is however a task of the individual countries.

Of the concerned chemicals, nine are poly-chlorinated pesticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachloro-benzene, mirex, toxaphene), the two remaining being polychlorinated biphenyls (a class of technological substances not used as pesticides, listed twice) and polychlorinated dibenzo-dioxins and –furans (two classes of substances without known uses but unintentionally produced as contaminants in several technological processes) [26].

After a long elaboration and negotiations, the Convention was completed on 23 May 2001 in Stockholm and entered into force on 17 May 2004 with ratification by an initial 128 parties and 151 signatories (http://chm.pops.int/). Co-signatory States agree to outlaw nine of the dirty dozen chemicals, limit the use of DDT to malaria control, and curtail inadvertent production of dioxins and furans.

Parties to the convention agreed to periodically reviewing the list to add more compounds or classes, if they meet certain criteria for persistence and trans-boundary threat. The second set of nine new chemicals to be added to the Convention was agreed at a conference in Geneva on 8 May 2009. Of these, four are pesticides, three isomers of hexachlorocyclohexane (among which is Lindane) and chlordecone (kepone). Others are brominated flame retardants and perfluorinated organic acids, both without any connection with pesticides. In May 2011 technical endosulfan and its related isomers were added in the list. As of April, 2011, there are 173 parties to the Convention and by October 2011 three more Parties were added.

1.2.1 Chemical and Toxicological Aspects of the 'Dirty Dozen' *Organochlorine Pesticides*

Table 1.1 lists the persistent organochlorinated pesticides, along with the exemptions agreed upon for further controlled production and for restricted uses. The chemical structures, names and CAS numbers of the eleven (twelve) organochlorine pesticides (OCPs) listed in the Annexes of the Stockholm Convention are reported in Table 1.2.

OCPs can be divided according to their chemical structure into cyclodienes, dichlorodiphenyl-ethanes and hexachlorocyclohexane isomers [18]. Aldrin, Dieldrin, Endrin, Heptachlor and Chlordane belong to cyclodiene pesticides. They are synthesized by a chemical reaction technically defined as 'cycloaddition of dienes' (or Diels-Alder reaction, from the names of the 1950 awardees of the Chemistry Nobel Prize). Cyclodienes are prepared by reacting of the diene, hexachloro-cyclopentadiene, with other unsaturated organic compounds, dienophiles, following the general scheme shown in Fig. 1.1, which illustrates the preparation of Aldrin. The key starting product, hexachloro-cyclopentadiene, in turn is prepared by chlorination of cyclopentadiene (derived in its turn from the naphtha fraction of oil).

Mirex and Kepone are also synthesized from hexachloro-cyclopentadiene but through a different chemical process ('*cycloaddition*'), so they are not classified as 'cyclodienes'. Lindane and Toxaphene (also known as camphechlor) are polychlorinated aliphatic hydrocarbons derived from the photochemical chlorination of benzene and camphor, respectively. DDT and hexachloro-benzene are organic substances containing chlorinated benzene rings in their structure.

As can be appreciated from their structures, their common chemical characteristics are a very poor solubility in water and a much higher solubility in organic solvents and in the lipid compartments of the living matter; most compounds also react poorly with environmental agents such as oxygen, water and light and are fairly to very resistant to biotransformation by plants, micro- and upper organisms. These properties lead to an accumulation of the compounds in the lower organisms and to the progressive increase of their presence in higher trophic levels, in marine, air and land predators, as typically exemplified by large marine mammals, deep-sea fish, circumpolar birds, penguins and polar bears. Due to poor biotransformation, saprophytic organisms which degrade animal carcasses release the pool of unaltered OCPs back into the food chain. Some compounds are subject to long range transboundary air pollution (LRTAP) due to their volatility or semivolatility properties [35]. Moreover they are able to stick (adsorb) to particulate matter in the environment. Thus they can be carried far from their application regions by means of planetary-scale air movements and ocean currents; also long-range migration of oceanic fish schools and migratory birds continuously contribute to the dispersion of the OCPs pool across the globe (long-range transportation).

From the toxicological point of view, their chemical inertness and bulky shape point at ligand-receptor interaction as the main way to display biological activity,

Table 1.1 An extended list of persistent organochlorinated pesticides regulated by the Stockholm Convention on Persistent Organic Pollutants

Name	Exemptions	Annex
Aldrin[a]	Production none Use as a local ectoparasiticide and insecticide	A
Chlordzane[a]	Production by registered parties Use as a local ectoparasiticide, insecticide, termiticide (including in buildings, dams and roads) and as an additive in plywood adhesives	A
Dieldrin[a]	Production none Use in agricultural operations	A
Endrin[a]	None	A
Heptachlor[a]	Production none Use as a termiticide (including in the structure of houses and underground), for organic treatment and in underground cable boxes	A
Hexachlorobenzene[a]	Production by registered parties Use as a chemical intermediate and a solvent for pesticides	A&C
Mirex[a]	Production by registered parties Use as a termiticide	A
Toxaphene[a]	None	A
DDT[a]	Disease vector control in accordance with Part II of Annex B Production and use as an intermediate in the production of dicofol and other compounds	B
α-Hexachlorocyclohexane[b]	None	A
β-Hexachlorocyclohexane[b]	None	A
γ-hexachlorocyclohexane (Lindane)[b]	Production none Use Human health pharmaceutical for control of head lice and scabies as second line treatment	A
Chlordecone[b]	None	A
Technical endosulfan and its related isomers[c]	As a broad-spectrum insecticide, endosulfan is used currently to control a wide range of pests on a variety of crops including coffee, cotton, rice, sorghum and soy	A
Pentachlorobenzene[b]	Used in PCB products, in dyestuff carriers, as fungicide, a flame retardant and as a chemical intermediate in the production of quintozene. Produced unintentionally during combustion, thermal and industrial process. Also present as impurities in solvents or pesticides	A&C

Annex A – Intentionally produced chemicals that need to be eliminated; **Annex B –** Intentionally produced chemicals with restrictions; **Annex C** – Unintentionally produced chemicals

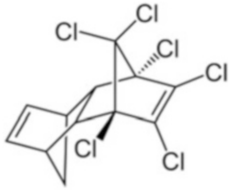
[a] Present in the initial list produced in 2001

[b] Added by the Fourth Conference of Parties, May 2009

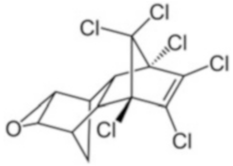
[c] Added by Fifth Conference of Parties, May2011

the main exception being the radical reactivity of pentachloro- and hexachlorobenzene [19], due to its rather unique chemical characteristics of lipophilicity and possibly to conversion into the redox-active tetrachloro-1,4-benzoquinone [32]. Insecticidal activity is mostly due to interaction with ion channels in the target

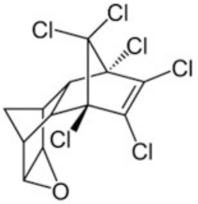
Table 1.2 Chemical structures, names and CAS numbers of the organochlorine pesticides listed in the Annexes of the Stockholm Convention.



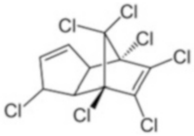
Aldrin; CAS: 309-00-2



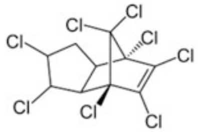
Dieldrin; CAS: 60-57-1



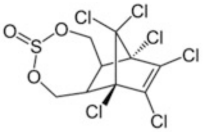
Endrin; CAS: 72-20-8



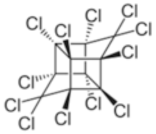
Heptachlor; CAS:76-44-8



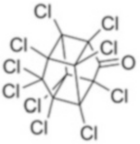
Chlordane; CAS: 57-74-9



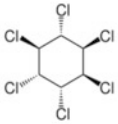
Endosulfan; CAS No:115-29-7



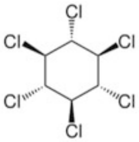
Mirex; CAS: 2385-85-5



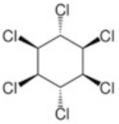
Chlordecone (Kepone); CAS: 143-50-0



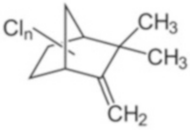
α: Cyclohexane, 1,2,3,4,5,6-hexachloro-, (1α,2β,3α,4β,5α,6β) CAS: 319-84-6



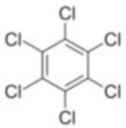
β-1,2,3,4,5,6-hexachlorocyclohexane; CAS: 319-85-7



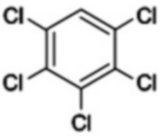
γ: **Lindane;** CAS: 319-84-6, 319-85-7, 58-89-9



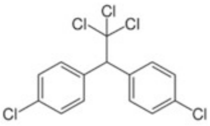
Toxaphene; CAS: 8001-35-2



Hexachlorobenzene; CAS: 118-74-1



Pentachlorobenzene; CAS: 608-93-5



DDT; CAS: 50-29-3

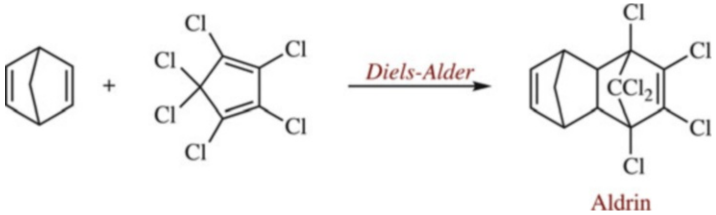


Fig. 1.1 General scheme for preparation of Aldrin

organisms' nervous system, a phenomenon which leads to disruption of bioelectric signalling at most organs as the pathway to organisms' death [17]. The same mechanism applies to higher animals and the human, but at considerably higher doses than the insecticidal ones. This property leads to very low toxicity towards humans, who can be harmed only in case of massive, usually accidental or deliberate exposure, and is the key reason for which OCPs were quickly accepted as key means of parasite elimination.

Concern for animals' and human health stems from interference of OCPs with biological processes leading to impairment of individuals' health or reproduction. One of the best known mechanisms is interference of DDT with calcium ion metabolism in birds, which leads to insufficient deposition in bird eggs' shells and to impaired development and early death of chicks hosted in eggs with a thinned softened shell [33]. The bald eagle (*Haliaeetus leucocephalus*), the heraldic animal of the U.S.A and the victim of widespread use of DDT, was brought to the verge of extinction by 1971 and it was only in 2007 that it was withdrawn from the list of endangered species [31].

Due to the limitation or ban to production and use, current concern for human health mainly stems from exposure of the general population to trace amounts of PCPs present as ubiquitous contaminants of the environment, in water and in food. Levels of individual exposure and of body burden depend on subjects' age, on their residence and on levels in the consumed food and water. Most toxicological information on the effects on humans at environmental levels of exposure derive from epidemiological studies. Examined groups were plant production workers, workers who applied the products for agricultural and civil and industrial, non-agricultural purposes and the general exposed populations. Although scientific and regulatory literature in the field is abundant, debate on the real extent, if any, of effects is still vocal and specific risk-benefit analyses are therefore conditioned by diverging opinions.

In the following, a necessarily brief and selective presentation of the chemical and toxicological information on each of the eleven (twelve) polychlorinated pesticides regulated by the Stockholm Convention is reported.

1.2.1.1 Aldrin

Aldrin is the Diels-Alder adduct of hexachlorocyclopentadiene with norbornadiene. It has been used in industry, agriculture and in public health control. Aldrin was widely used to treat seed and soil until it was banned in most countries in the 1970s. It was applied to soils to kill termites, grasshoppers, corn rootworm and other insect pests. An estimated 270 thousands of tons of aldrin and related cyclodiene pesticides were produced between 1946 and 1976. Aldrin is not toxic to insects but it is metabolically oxidized in the insect to form the epoxide **dieldrin** which is the active compound. The oxidation can also occur in soil and on plant surfaces. Aldrin residues are rarely found in foods and animals as it is rapidly converted to dieldrin. Aldrin is toxic to humans; the lethal dose of aldrin for an adult man has been estimated to be about 5 g, equivalent to 83 mg/kg b.wt. Signs and symptoms of aldrin intoxication may include headache, dizziness, nausea, general malaise, and vomiting, followed by muscle twitchings, myoclonic jerks, and convulsions. Although this may be somewhat relevant in accidental or voluntary acute intoxication, it has little, if any, relevance with regard to environmental exposure.

1.2.1.2 Chlordane

Chlordane is a more highly chlorinated analogue of Heptachlor, also prepared from hexachlorocyclopentadiene and cyclopentadiene, followed by chlorination. Insertion of three chlorine atoms in the molecule of the Diels-Alder adduct yields a technical mixture of two isomers, α and β, the β isomer being more bioactive. The technical mixture contains chlordane, heptachlor, nonachlor and related compounds. Chlordane was first synthesized in 1944 [13] and entered the United States market from 1948 to 1988 both as a dust and an emulsified solution.

1.2.1.3 Chlordecone (Kepone)

Chlordecone is a degradation product of Mirex in which one *gem*-dichloride ($>CCl_2$) group has been converted to a carbonyl ($>C{=}O$) function and has been used for the same purposes. In the USA it was produced by Allied Signal Company in Hopewell, Virginia, and dumping of the substance into the James River in the 1960s and 1970s caused toxic effects on wildlife to the point that in 1975 the Governor of the State raised a ban to fishing down the James River for 100 miles, from Richmond to the Chesapeake Bay for 13 years, until efforts to clean up the river started to yield successful results. Production in the USA was stopped in 1976. In the French island of Martinique it was used without restrictions in banana plantations arguing that no alternative pesticide was available and despite a 1990 ban of the substance by France. Since 2003, the local authorities restricted cultivation of crops because the soil has been seriously contaminated by kepone. Similarly, the nearby island of Guadeloupe was also contaminated, but to a lesser extent and has one of the highest prostate cancer rates in the world. Kepone was found to be carcinogenic in rats and mice. It induced malignant tumors in the liver of rats and mice where female rats were more susceptible to kepone exposure than male rats [24, 25]. Cannon et al. [5] observed a number of workers in Kepone production plant with clinical illness characterized by nervousness, tremor, weight loss, opsoclonus, pleuritic and joint pain, and oligospermia. The illness incidence rates for production workers (64 %) were significantly higher than for nonproduction personnel (16 %). The mean blood Kepone level was significantly higher among workers with illness than those without disease (mean: 2.53 and 0.60 ppm, respectively; $p < 0.001$). Moreover, blood Kepone levels in current workers (mean, 3.12 ppm) were higher than those in former employees (1.22 ppm). However the authors did not observed apparent association between frequency of symptoms and proximity to the plant in the survey of the community population.

1.2.1.4 DDT

Dichloro**d**iphenyl**t**richloroethane (DDT) was first synthesized from the reaction of chlorobenzene with chloral hydrate by a young Austrian chemist, Othmar Zeidler, a doctoral student with Adolf von Baeyer, in 1874. The substance, in fact is a mixture of *ortho*- and *para*- substituted isomers, the main one (*approx.* 77 %) being that with *para-, para-* substitution. Its insecticidal properties were discovered in 1939 in Paul Hermann Müller's laboratory at Ciba in Basel, Switzerland. Müller was awarded the Nobel Prize in Physiology and Medicine in 1948 "for his discovery of the high efficiency of DDT as a contact poison against several arthropods." DDT was used with great success in the second half of World War II to control malaria and typhus among civilians and troops. At the time of its discovery, the advantages of DDT that made it the best known and most useful insecticide were its stability, greater persistence, low cost, low mammalian toxicity and broad spectrum of insecticidal activity. Following more than 15 years of liberal use in agricultural and household (in the 1960s, 400,000 tons where applied annually worldwide, about 70 % for agricultural use), which resulted in the almost complete eradication of malaria in several previously endemic areas, it was Rachel Carson's passionate warning in the seminal 1962 book '*Silent Spring*' to raise concern on the ecological consequences.

1.2.1.5 Dieldrin

Dieldrin is the active epoxide of aldrin and is industrially prepared by chemical epoxidation of Aldrin [4]. It is used principally to control termites and textile pests, as well as insect-borne diseases and insects living in agricultural soils. Dieldrin is toxic to humans. Based on anecdotal evidence, the lethal dose of dieldrin has been estimated to be 10 mg/kg b.wt, so it is more toxic than its metabolic precursor, Aldrin. Signs and symptoms of acute dieldrin intoxication are essentially the same as for aldrin (*see* above). Hunter et al. [12] studied the pharmacodynamics of dieldrin in a human volunteer study. The study subjects with no recent occupational exposure to dieldrin, received 0, 10, 50, or 211 μg dieldrin per day for 2 years. All volunteers continued in excellent health, and clinical, physiological and laboratory findings remained essentially unchanged through the 24 month exposure period and an 8-month follow up. However, workers of a chemical plant which produced several pesticides (Aldrin, Dieldrin, Endrin as well as the known animal carcinogen, dibromochloropropane) showed an increase of liver and biliary tract cancers. In 1987 IARC concluded that there is inadequate evidence for the carcinogenicity in humans, and limited evidence in experimental animals and that therefore Dieldrin is not classifiable as to its carcinogenicity in humans (Group 3).

1.2.1.6 Endosulfan

Endosulfan is a close structural analogue of aldrin, chlordane, and heptachlor and is the only sulphur-containing chlorinated pesticide in the Stockholm Convention list. It is synthesized via a Diels-Alder addition of hexachloro-cyclopentadiene and cis-butene-1,4-diol in xylene, followed by cyclization of the adduct with thionyl chloride. The annual production is estimated to range between 18,000 and 20,000 tonnes worldwide. In USA, 400 tonnes are estimated to be used annually for domestic purpose. The main uses include control of pests in vegetables, fruits, cereal grains and cotton, ornamental shrubs, trees, vines and ornamental plants. It was also used to control ectoparasite on beef and lactating cattle. In Africa it is commonly used in cotton production at a dose rate between 1,000 and 2,000 g/ha while in India it is used to control pests on cashew plantations at the dose rate of 1200 g/ha. The estimated annual production in India is 9,500 tonnes, of which 4,500–5,000 tonnes are consumed domestically. Endosulfan is one of the most toxic pesticides still on the market and is responsible for many fatal pesticide poisoning incidents around the world. Since it is a GABA-gated chloride channel antagonist and a Ca^{2+}, Mg^{2+} ATPase inhibitor, endosulfan is a strongly neurotoxic compound. Symptoms of acute poisoning include hyperactivity, tremors, convulsions, lack of coordination, staggering, difficulty breathing, nausea and vomiting, diarrhea, and in severe cases, unconsciousness. Doses as low as 35 mg/kg have been documented to cause death in humans, while many cases of sub-lethal poisoning have resulted in permanent brain damage. Farm workers with chronic endosulfan exposure are also at risk of rashes and skin irritation. Endosulfan is also considered a human endocrine disruptor, with the ability to delay development of the male characters in children exposed through the early age, as demonstrated by an epidemiological and monitoring study on Indian children of Kerala villages [28]. Results along the same trend were also observed in a Northern European study [7].

1.2.1.7 Endrin

Endrin is a stereoisomer of dieldrin, prepared by stepwise reaction of hexachlorocyclopentadiene with acetylene, followed by cyclopentadiene, followed by epoxidation [21]. About 80 % of endrin was consumed for controlling insect pests of cotton. It was also used on rice, sugar cane, grain crops, sugar beets, tobacco and cole crops. It was occasionally used in orchards as a control of rodents and as a treatment for cotton and beans seeds. It was also used as a rodenticide. Endrin has been shown to be carcinogenic at several body sites in the rat, and possibly in the mouse and in the dog [24, 25]. Several cases of acute and fatal intoxication were reported from consumption of Endrin-contaminated food in different Countries [15, 27], along with poisoning of animals and pets. One puzzling cluster of cases of seizure occurred in 1988 in the USA was reported as '*The tale of the toxic taquitos*'. Traces of Endrin were found in tortilla shells (2.4–4.6 ppm, corresponding to less than 0.1 mg per piece) purchased at an individual store by several families in Orange County (California, USA) and, based on estimates of intake from affected individuals, in this case toxic effects were observed at doses (50 micrograms/kg b. wt) four times below those previously known [34]. In comparison with dieldrin, endrin is less persistent in the environment.

1.2.1.8 Heptachlor

Heptachlor is prepared from hexachlorocyclopentadiene and cyclopentadiene, followed by mono-chlorination. It has both insecticidal and fumigant activity [8]. As per the Stockholm Convention, the sale of heptachlor products has been limited to the specific application of fire ant control in underground transformers. Heptachlor is rapidly oxidized to 2,3-heptachlor epoxide both photochemically in the environment, and biologically in soils and plant tissue by microsomal oxidation. Heptachlor epoxide is a more potent insecticide than heptachlor itself. Heptachlor epoxide remains in the soil for decades without significant further degradation and also dissolves more easily in water. Heptachlor and its epoxide can be absorbed to soil particles and evaporate. Compared to the more chlorinated analogue chlordane, heptachlor is about 3–5 times more active as an insecticide and more inert chemically, since it is resistant to caustic alkali. The half life of Heptachlor in the environmental compartments is ~1.3–4.2 days in air, ~0.03–0.11 years in water and ~0.11–0.34 years in soil, although one study claimed for soil a much higher half life of 2 years and that its residues were measured in soil 14 years after its initial application. Soil microorganisms transform heptachlor according to complex pattern into a range of different epoxidation, hydrolysis and reduction products. Like other POPs, heptachlor is lipophilic and poorly soluble in water (0.056 mg/L at 25 °C), thus it tends to accumulate in the body fat of humans and animals. When the compound was incubated with a mixed culture of organisms, chlordene (hexachlorocyclopentadine, its precursor) formed, which was further metabolized to chlordene epoxide. Other metabolites include 1-hydroxychlordene, 1-hydroxy-2,3-epoxychlordene, and heptachlor epoxide. Soil microorganisms hydrolyze heptachlor to give ketochlordene. Rats metabolize heptachlor to the epoxide 1-exo-1-hydroxyheptachlor epoxide and 1,2-dihydrooxydihydrochlordene. When heptachlor epoxide was incubated with microsomal preparations form liver of pigs and from houseflies, the products found were diol and 1-hydroxy-2,3-epoxychlordene [23]. Metabolic scheme in rats shows two pathways with the same metabolite. The first involves following scheme: heptachlor → heptachlor epoxide → dehydrogenated derivative of 1-exo-hydroxy-2,3-exo-epoxychlordene → 1,2-dihydrooxydihydrochlordene. The second involves: Heptachlor → 1-exo-hydroxychlordene → 1-exo-hydroxy, 2,3-exo-epoxychlordene → 1,2-dihydrooxy-dihydrochlordene [3]. Heptachlor was designated as a Class 2B or "possible human carcinogen" by The International Agency for Research on Cancer (IARC).

1.2.1.9 Hexachloro-Benzene

Hexachlorobenze is a fully chlorinated derivative of benzene which was used until its 1966 ban as a fungicide for seed treatment, especially on wheat to control the fungal disease bunt. Its half life in human is estimated to be in the range of 6 years [30]. The most sensitive target organs are the liver, the ovary and the central nervous system [1]. HCB is well known to induce porphyria through a free-radical generation mechanism [19], due to its rather unique chemical characteristics of lipophilicity and possibly to conversion into the redox-active tetrachloro-1,4-benzoquinone. Eating wheat treated with hexachlorobenzene has been associated with human dermal toxicity which can result in blistering of the skin. In 1955 and 1959 consumption of bread produced with HCB-treated seeds caused epidemic of chemical porphyria in Anatolia, Turkey where 500 people were fatally poisoned and >4,000 people fell ill. The victims were affected with a liver condition called *porphyria cutanea tarda*, which disturbs the metabolism of hemoglobin and results in skin lesions. Most breastfeeding children <2 years of age, whose mothers had eaten tainted bread, died from a condition called "pembe yara" or "pink sore," suspected to be due to high doses of HCB in the breast milk [22].

1.2.1.10 Hexachloro-Cyclohexane

This product is also incorrectly referred to as benzene hexachloride, but must not be confused with hexachloro-benzene, also a pesticide listed in Annex A of the Stockholm Convention. Hexachloro-cyclohexane exists in at least nine stereoisomers, of which three are of insecticidal importance and are: the α-, β- and γ- isomers, the latter, which is the most active one, being also known as lindane. The crude mixture of products obtained by photochemical chlorination of benzene consists of 10–18 % of the γ-isomer, 55–70 % of the racemic α-isomer, 5–14 %

of the β-isomer, 6–8 % of the δ-isomer, 3–4 % of the ε-isomer, and a trace of the η-isomer, along with traces of hepta- and octa-chlorocyclohexane which contribute to the unpleasant odour of technical lindane.

1.2.1.11 Mirex

Mirex is the product of 'cycloaddition' dimerization of the hexachloro-cyclopentadiene and thus is not classified as a cyclodiene pesticide. It was popularized to control red imported fire ants (*Solenopsis saevissima richteri* and *Solenopsis invicta*) but by virtue of its chemical robustness and lipophilicity it was recognized as a bioaccumulative pollutant in aquatic and terrestrial food chains to harmful levels. As an example, after six applications of mirex bait (0.3 % mirex, corresponding to 4.2 g mirex/ha) at 1.4 kg/ha in the field, turtle fat contained 24.8 mg mirex/kg; kingfishers 1.9 mg/kg; coyote fat 6 mg/kg; opossum fat 9.5 mg/kg; raccoon fat 73.9 mg/kg. In a model ecosystem with a terrestrial-aquatic interface, sorgum seedlings were treated with mirex at 1.1 kg/ha. Caterpillars fed on these seedlings and their faeces contaminated the water which contained algae, snails, Daphnia, mosquito larvae, and fish. After 33 days, the ecological magnification value was 219 for fish and 1,165 for snails. Mirex is highly resistant to microbiological degradation. It only slowly dechlorinates to a monohydro derivative by anaerobic microbial action in sewage sludge and by enteric bacteria. Degradation by soil microorganisms has not been described. Ironically, the use of Mirex encouraged the spread of the red imported fire ants since it also kills native ants that are highly competitive with the fire ants. The US EPA prohibited its use in 1976 after *approx.* 250,000 kg of Mirex was applied to fields between1962-75. IARC [14] evaluated the carcinogenic hazard resulting from exposure to Mirex and concluded that "there is sufficient evidence for its carcinogenicity to mice and rats. In the absence of adequate data in humans, based on above result it can be said that it has carcinogenic risk to humans".

1.2.1.12 Pentachlorobenzene

Pentachlorobenzene (PnCB) is not used as a pesticide itself but was the chemical precursor and production by-product and the degradation product of the fungicide pentachloro-nitrobenzene (PCNB, quintozene), a compound that is currently prepared by a different chemical route, no more involving the use of pentachlorobenzene. PCNB has itself been restricted in several Countries but still finds use in others. The chemical characteristics of PnCB allow long-range transport in air and through soil, while very poor water solubility allows bio-accumulation from water into food organisms and bio-concentration in the fat body compartments of biota. On the contrary, bio-magnification through the aquatic trophic levels is not a particularly important phenomenon. PnCB is estimated to have a half-life of 194–345 days (experimentally contaminated soil samples) and residues can persist for least 2–3 years in soil from agricultural sites [2].

Pentachlorobenzene is poorly metabolized in most species, probably because its high degree of chlorine substitution inhibits formation of the arene-oxide reactive intermediates. On the contrary, reductive dechlorination is a likely pathway for degradation and mineralization both by environmental agents (photochemical processes) and by enzymatic pathways. Its poor bio-reactivity has also consequences in explaining its carcinogenic activity [11, 20, 29], since PnCB is a "Phenobarbital-type" inducer of P450 enzymes and most likely an inducer of oxidative stress through mechanisms centred on its bio-transformation into tetrachloro-*p*-benzoquinone similar to those shown by hexachloro-benzene (*see* above).

1.2.1.13 Toxaphene

Toxaphene is a complex mixture obtained by chlorination of the natural terpene camphor, in which at least 670 chemicals, including chlorobornanes, chlorocamphenes, and other bicyclic chloroorganic compounds more, have been identified. At least some of them are volatile enough to be transported for long distances through the atmosphere. Toxaphene was used as an insecticide. Between 1970 and 1995, cumulative global usage of toxaphene was estimated to be 670 thousand tons. In USA during the early to mid 1970s, toxaphene became the most heavily used pesticide. Production peaked in 1975 at 30,000 tons. It was chiefly used in the cotton and soybean growing areas in the southeastern region. In California it was used to treat acariasis in cattle and this caused some deaths in cattle treated by spraying. In 1982, it was banned for most uses, and in 1990 it was banned for all uses in the United States. However, in 2010, toxaphene was still available from eleven suppliers worldwide, including seven in the U.S.A. It is classified as an IARC Group 2B carcinogen.

1.3 Obsolete Pesticides as a Problem of Global Concern

Obsolete pesticides are stocked pesticides that can no longer be used for their intended purpose or any other purpose and therefore require disposal [9]. Obsolete pesticide stockpiles are an international environmental policy issue thus its management needs to be given a global attention to save our resources. In the developing countries and in many Central and Eastern European countries, there are huge stockpiles of pesticides, estimated to be several hundreds of thousands of tones [16]. Estimates show that >500,000 tons of obsolete pesticides are accumulated globally, especially in developing countries [10]. A considerable amount of the accumulated obsolete pesticides are in the group of persistent organic pollutants (POPs). The cause of accumulation of these chemicals has been attributed to lack of appropriate management, misuse of chemicals, uncoordinated chemical donations, substandard storage, poor storekeeping, lack of expertise and financial resources. Other factors include banning or restriction of products for health or environmental reasons e.g. through banning as for the case of those which were banned or restricted by Stockholm Convention; withdrawal of registration; or policy decision by the Ministry of Agriculture or other authorized ministries. As a result of improper or prolonged storage some products can no longer be used according to its label specifications and instructions for use, nor can it easily be reformulated to become usable again and thus lead to deterioration of these products. A product is considered deteriorated when:

- it has undergone chemical and/or physical changes that result in phytotoxic effects on the target crop;
- causes an unacceptable hazard to human health or the environment;
- the product has undergone an unacceptable loss of biological efficacy because of degradation of its active ingredient and/or other chemical or physical changes;
- its physical properties have changed to such an extent that it can no longer be applied with standard or stipulated application equipment.

To determine whether a pesticide is obsolete and need for disposal a decision tree has been constructed to assist this decision making process (see Fig. 1.1 from [9]).

The accumulation and bad management of obsolete pesticides constitute a threat to human health and the environment, locally, regionally and globally. When located near water sources, obsolete pesticides can pose a high risk of contaminating drinking water sources as well as irrigation water. In turn contaminated irrigation water might introduce pesticide residues into crops and fish, making them unfit for trade as well as for local consumption. Leaking of stockpile can contaminate a significant land area making it unfit for human habitation or for any agricultural activities [36].

1.4 Safe Disposal of Stockpiles and Bioremediation of Contaminated Areas

Industrial production of polychlorinated organic compounds such as pesticides is far from being an innocuous activity, in that it generates large quantities of toxic waste per unit of marketable active ingredient, even if the formulated products contain technical mixtures of active substances. As an example, the manufacture of lindane, which is still authorized by the Stockholm Convention as a scabicide entails the chlorination of benzene in the presence of UV light (‘photo-chlorination’) to form a mixture of isomers, among which α- (70 %), β- (12 %), γ- (10 %, the desired active substance) and δ- (7 %) HCH, respectively. Lindane is purified from rest of the HCH isomers and the rest becomes an environmental liability. Therefore manufacture of 1 tonne of lindane produces at least 9 tonnes of waste just from synthesis yield, not even considering solvents used for extraction from the raw product and crystallization of the desired isomer to a suitable purity of 99.9 %.

Once off-products of the fine chemicals industry accumulate in production and formulation plants, they are at best stored in containers steel or plastic drums and stockpiled in ground holes, usually within or close to production yards. Solids may be directly dumped into the ground. Long-term disposal used to be of lesser concern until very recent times, especially in Countries where regulations and controls were lacking and safe technologies poorly available.

The problem of disposal of hazardous chemical wastes is common not only to chemical plants, but also to other sectors of concern for public health and safety such as disposal of spent ammunition, of banned weapons such as chemical warfare agents, of expired pharmaceuticals. Most of the involved chemicals can harm the environment and adversely influence human health by contaminating water streams and tables, agricultural land and dwelling sites.

Remediation is a costly and often unsolvable process and may leave to several future generations ahead an economic and human burden which is difficult to cope with. However, currently available technology is at least in part able to address such problems or to stabilize current situations in the view that in near future better technologies will be available for permanent solutions.

Dumping into long-term disposal sites has long been the preferred way to get rid of solid industrial waste. At least in the past, little if any care was given to prevent leaching of the contents from corroded or pierced vessels by isolating the walls of the dig or even to take note of the position of filled up digs. This caused the unexpected and uncontrolled release or leaching of hazardous chemical waste into lower-lying water tables and downstream carryover of toxic products. Nowadays intermediate- to long-term dumping sites can be established by means of comprehensive technologies which include geological, geochemical and hydrogeological assessment of the selected sites with reference to ground permeability and to presence of reachable water sources. Clay layers are mostly impermeable to water and counter diffusion of leachates. The walls of digs are usually lined with multiple layers of waterproof synthetic materials such as polyvinyl-chloride. Since sand and gravel readily absorb water, layers can be employed to drive the flow of leached materials to inspection drills, which are commonly employed to monitor the permeation of leachates and to draw the permeated liquids towards safe treatment and disposal.

High-temperature incineration is still the technique of choice for the permanent solution to disposal of toxic chemical wastes. At sufficiently high temperature any organic substance reacts with air or oxygen to yield the thermodynamically favoured products carbon dioxide and water, while sulphur, phosphorus and the halogen are converted into the corresponding acidic products. The very problem of incineration is that a high proportion of hetero-atoms, especially the halogens impair the natural processes of combustion by quenching the free radical propagation processes which lead to the extensive decomposition of complex molecules into the final combustion products. As a consequence, new, kinetically stable molecules generate from the radical species in the combustion environment, such as polycyclic aromatic hydrocarbons and carbon soot, chlorinated dibenzo-dioxins and –furans, which are of a major concern for human health and for that reason are also considered by the Stockholm Convention. To minimize emission into the environment especially of chlorinated dibenzo-dioxins and –furans from incineration of chlorinated organic waste, which include not only pesticides (which are mostly banned) but mainly organic polymers used for packaging or as structural material for industrial goods specific techniques have been developed. In principle, factors which lead to *de-novo* formation of these products are quite large in number, yet to be subdivided into two groups with various mutual interactions: (***a***) those related to operational factors (poor combustion conditions, locally insufficient oxygen and mixing of oxygen-rich and oxygen-lean combustion products, or inadequate temperature, residence time, and turbulence, the so-called 3 T's) and (***b***) those related to chemical and catalytic factors (elemental composition of waste and local composition of combustion products, catalysis from chemical species, especially metals such as copper, present in ashes). Among the strategies adopted to lower the net emission of dioxin-like compounds from incinerators, the use of alkaline conditions, such as by using chlorinated waste as an additive to fuel in the manufacture of Portland cement in kilns (co-combustion). However, the destruction of stockpiles of obsolete chlorinated pesticides is mostly considered a remediation intervention, rather than a routine process in waste management (as in the case of chlorinated plastics) also due to the very high proportion of chlorine in the mixtures, which is well over 60 % on a by-weight basis. As a consequence, dedicated facilities aimed at complete destruction with minimal production of toxic end-products are employed.

Supercritical Water Oxidation (SCWO) is an emerging and promising technique for the mineralization of organic substances which are not easily amenable to incineration or biodegradation. This technique exploits the unique properties of water at supercritical conditions, *i.e.*, at high temperature (above 370.15 °C) and pressure (above 221.2 bar), when the vapor and liquid are indistinguishable and several properties of water suddenly change. Supercritical water exhibits high gas-like diffusion rates along with high liquid-like collision rates, the behavior of water as a solvent is reversed in comparison to that of subcritical liquid water and, as a result, the solubility of organic non-polar compounds such as chlorinated hydrocarbons greatly increases while polar substances such as inorganic salts precipitate out of solution. As a consequence, reaction of dissolved organic compounds with oxidants, and even with dissolved oxygen occurs under essentially single-phase conditions, and complete mineralization of organic substance to carbon dioxide, water and inorganic salts can be achieved with great efficiency and in shortened time. Heteroatoms, such as chlorine, that are bound to the organic will produce hydrochloric acid; organic-bound sulfur and phosphorous are oxidized to sulfuric acid and phosphoric acid respectively and metals are oxidized to their highest oxidation state (*e.g.*, iron to iron rust, chromium to chromium oxide). SCWO for destroying hazardous wastes involves supercritical water as the reaction medium, and an oxidant such as air, oxygen or nitrogen oxide at a relatively high temperature and pressure (235 bar and 650 °C), yet much lower than that required in gas-phase incineration. Among the drawbacks which still hamper the large scale application of SCWO is corrosion of reactors by the very reactive conditions, which dictates the use of resistant materials. In practice, no operative facilities beyond investigational stage are known to be active in this field.

Bioremediation is a process in which indigenous or inoculated micro-organisms such as fungi, bacteria and other microbes degrade (metabolize) organic contaminants. Nutrients, oxygen or other amendments may be used to enhance bioremediation and contaminant desorption from subsurface materials. This procedure have been successfully used to remediate soils, sludges and ground water contaminated with petroleum hydrocarbons, solvents, pesticides, wood preservatives, and other organic chemicals. Bioremediation is especially effective for remediating low level residual contamination in conjunction with source removal. It does not require heating; rather it requires relatively inexpensive inputs, such as nutrients. Thus it is a more cost effective as compared to incineration and physical and chemical methods.

Enhanced bioremediation does not generate residuals requiring additional treatment or disposal. When conducted in situ, it does not require excavation of contaminated media. Treatability or feasibility tests are performed to determine whether enhanced bioremediation is feasible in a given situation, and to define the remediation time frame and parameters. The length of time required for treatment can range from 6 months to 5 years and is dependent on many site-specific factors.

Cleanup goals may not be attained if the soil matrix prohibits contaminant microorganism contact. The circulation of water-based solutions through the soil may increase contaminant mobility and necessitate treatment of underlying ground water. Preferential colonisation by microbes may occur causing clogging of nutrient and water injection wells. Preferential flow paths may severely decrease contact between injected fluids and contaminants throughout the contaminated zones. The system should not be used for clay, highly layered, or heterogeneous subsurface environments because of oxygen (or other electron acceptor) transfer limitations. High concentrations of heavy metals, highly chlorinated organics, long chain hydrocarbons, or inorganic salts are likely to be toxic to some microorganisms. Those compounds that resist biodegradation are often not capable of efficient treatment using bioremediation and an alternative must be found. Bioremediation slows at low temperatures. Many of the above factors can be controlled with proper attention to good engineering practice.

1.5 Conclusions

Polychlorinated pesticides played an important role in the Green Revolution to increase agricultural production and in eradicating or controlling parasite-borne diseases such as malaria in temperate and sub-tropical areas of the world. However, their impact on environmental biodiversity was not negligible due to their environmental persistence, poor biodegradation and biomagnifications into and along the planetary trophic network. Also their effect on the long-term health of the different sub-groups of the human population needs a thorough assessment based on sound toxicological knowledge, on accurate dosimetry in the different scenarios and on responsible evaluation of risk/benefit relationship.

The burden of final disposal of stockpiles and of remediation of contaminated sites is a benchmark of international cooperation both on openness in the circulation of information and in sharing state-of-the-art technology.

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Chapter 2
Designing Good Sampling Plans for Characterizing Pesticide Pollution

Thomas A. Lewandowski

Abstract The first stage in addressing chemical pollution typically involves sampling and analysis to understand the nature of the pollution, its extent, and potential effects. A detailed sampling and analysis plan is a crucial component of this first stage; it not only ensures that data of appropriate quality will be obtained but also forces one to ask and address important questions about the range of potential data needs and the expected remedial objectives. The plan must specify the procedure (s) by which samples are collected, the sample locations, and the appropriate sample number. It must also describe how samples are to be handled, transported and analyzed. Proper consideration of these elements of the plan will yield data that provide a solid foundation on which to based all subsequent activities.

Keywords Sampling • Analysis • Investigation

2.1 Introduction

A well conceived plan for assessing the presence and/or extent of chemical pollution is a critical component of efforts aimed at environmental protection and remediation. The data obtained *via* the sampling and analysis plan (or SAP) provides the basis for all subsequent activities, including determining whether a problem exists, how significant the problem is, and how it is best remedied. Problems arising from a poorly designed SAP can therefore spread like ripples throughout all subsequent stages of environmental remediation and decision making.

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A well thought out SAP tells you what, where, when and how to collect samples, and defines procedures for analyzing the samples once they have been collected. It also helps to ensure that the data collected are of appropriate quality. The SAP therefore serves as an instruction manual for field and laboratory staff. But the SAP serves another very important purpose – development of the SAP forces one to ask and attempt to answer many important questions about the site in question. Such questions may include:

1. What types of chemicals are thought to be involved?
2. What is currently known about the site in terms of contamination of environmental media and past waste practices? It is a rare site indeed where some current knowledge is not driving the desire to investigate.
3. Is there concern about past or future pesticide pollution?
4. How do you expect human health or ecological systems may be affected by this problem?
5. Is the work intended to comply with or achieve regulatory requirements?
6. How will the data collected during sampling activities be used: to support risk assessment, to define the limits of contamination, to help develop a remedial action plan, or some combination of these?

Attempting to answer these questions may seem more difficult and time consuming than simply "going out and getting some data", however, the answers obtained will not only help develop a robust SAP, but may also ensure that the data obtained (usually at considerable time and expense) are of the greatest use.

2.2 Defining the Objectives

A number of the questions posed above relate to the objectives of the proposed sampling activities. It is critical when defining the objectives that all potential data users (to the extent that they can be known in advance) be consulted. Failure to obtain agreement from all interested parties on the objectives and requirements for the SAP may result in repeating the work later at additional expense and delay of action. For example, if the collected data will be used in developing a site-specific risk assessment or remedial design, the downstream data users should be consulted as to their particular needs. From the standpoint of risk assessment, sampling data should be obtained that are directly informative of potential human or ecological exposures; if the concern is exposure *via* soil ingestion or dermal contact, then more surface sample coverage will be preferable than sampling of subsurface soils. If exposure off-site is the concern, than sampling subsurface soils or groundwater may be more relevant. Samples should also be located in areas where potentially exposed individuals are likely to be present. From the remedial design standpoint, it may be useful while sampling soils for chemical contaminants to also collect data on their geophysical properties or to gather data that delineate the groundwater table or preferential migration pathways. Thus by conferring with potential downstream data users, the most can be made of sampling efforts.

2.3 Making Use of Existing Information

Another critical element in SAP design is to take advantage of already available information about the site in question. Questions that can be asked include the following:

1. What have the past uses of the site been? Have past owners contributed to the problem? Is there a history of waste dumping at the site or nearby properties?
2. Are there potentially multiple sources of contamination (both in terms of time and place)?
3. Based on what is known about prior activities, what types of compounds might be expected? Based on the timeframes involved, are breakdown products of chemicals of concern also likely to be present in appreciable amounts?
4. Has the land been altered in ways (*e.g.*, regraded, filled) which could influence pollutant movement or transformation?
5. What sampling and analysis data have already been collected? Can these be used to rule out the presence of certain chemicals? Were prior SAPs deficient in some regard that can be remedied?

With respect to sampling and analysis specifically for obsolete pesticides, substantial information is available concerning their environmental properties. This information can be used to help understand pollutant movement in the environment and the types of environmental media that should be sampled (refer to Table 2.1). For example, if contamination with a relatively water soluble and readily degraded pesticide like 2,4-D is suspected then historical pollution may have left only residual traces in surface soils but groundwater would likely be affected. Due to its relatively low bioaccumulative potential (log BCF = 0.3), 2,4-D would be unlikely to be present at high levels in biota (*e.g.*, fish, soil invertebrates). Conversely, while

Table 2.1 Environmental properties of some obsolete pesticides

Pesticide	Half-life in soil (days)	Koc (L/kg)	Water solubility (mg/L)	H (atm-m^3/mole)	Log BCF (daphnia) (unitless)
2,4-D	10–30	19.6–109.1	500	1.02 E-8	0.3
DDT	2,000	677,934	0.025	8.10 E-06	4.2–4.4
Chlordane	4,300	10,811	0.25	7.52 E-06	3.13–4.0
Chlorpyrifos	600	95,816	0.74	6.00 E-06	3.49–4.84
Dieldrin	1,000	25,546	0.195	1.51 E-05	4.1
Heptachlor	250	30,200	0.18	1.09 E-03	4.08
Lindane	400	1,352	6.8	1.4 E-05	1.2–3.2
Methoxychlor	350	51,310	0.056	4.86 E-05	4.4
Toxaphene	120	80,000	0.045	1.58 E-05	“low”

Sources:
http://www.epa.gov/superfund/health/conmedia/soil/pdfs/part_5.pdf
http://water.epa.gov/drink/contaminants/basicinformation/historical/upload/Archived-Technical-Fact-Sheets
http://npic.orst.edu/ingred/ppdmove.htm

persistent and highly lipophillic pesticides such as chlordane and DDT would be expected to be bound preferentially in soil and present in biota, they would be expected to have relatively limited impacts on groundwater. In terms of air pollution, the Henry's Law Constant (H) provides an indication of the partitioning between air and water (including soil pore water). Consideration of the Henry's Law Constant suggests that a relatively volatile pesticide such as heptachlor may be more likely to be present in soil gas samples than a pesticide such as DDT which will tend to remain dissolved in the aqueous phase. Information of this nature will also be useful in interpreting the results of soil sampling. For example, the finding of minimal concentrations of toxaphene in groundwater samples says little about overall pollution levels at a site because most of the chemical would be expected to remain in the soil matrix.

2.4 Sample Collection

Methods for the collection of environmental samples from different media are well established [4, 7, 8]. For soil and other solid media (*e.g.*, sludge, waste piles), sample depth is an important consideration. Surface samples can be collected using a simple stainless steel shovel, trowel or scoop. For intermediate depths (up to approximately 7 m) a hand auger or thin-walled tube sampler can be used. A hand auger consists of a steel pole and "T" handle to which a bladed auger head or sample collection tube (used to obtain unambiguous soil core samples) has been attached. The auger is twisted to the desired depth at which point either the auger head itself is pulled up to obtain the soil sample or the collection tube is attached and pushed into the bottom of the hole to retrieve the sample. Care must be exercised not to dislodge the sample when removing the auger, an outcome that may be unavoidable in very wet and/or loose materials. The maximum depth that can be sampled will be soil-type dependent, both in terms of the difficulty in penetrating the ground and the ability to maintain the bore hole. For samples deeper than 7–10 m, a drill rig will likely be required. While requiring a more stable surface, larger footprint and better site access than hand sampling, the drill rig can allow for the collection of samples at much greater depth and can also facilitate the installation of groundwater sampling wells. It may also allow for continuous sampling of the soil profile from the ground surface downwards, which may be useful for understanding how soil strata affects contaminant levels. Drill rig sampling involves hammering a split-spoon sampler to the desired depth and then pulling the sample (24–60 cm long) to the ground surface for collection. More details about soil sampling methods can be found in USEPA [7].

The choice of soil sampling method must be guided by the data needs of downstream users and the available resources. Shallow soil samples (*e.g.,* obtained with a hand auger) can be taken much more quickly and inexpensively than samples requiring a drill rig so shallow samples may be more appropriate in cases involving suspected surface contamination. On the other hand, shallow samples would be virtually useless in situations involving subsurface contamination (*e.g.,* from buried drums) and more involved sampling methods will have to be employed.

Shallow groundwater samples can be collected by simply digging down to the water table and extracting water as it accumulates, but such a sample may also contain a substantial amount of suspended coarse particulate which may not be indicative of actual water concentrations. A more optimal method of collecting groundwater samples involves installing groundwater monitoring wells which have screens at the depth of interest that will exclude soil particulates and yield samples that consist only of groundwater (field filtering to remove fine particulate may also be appropriate if, for example, the concern is contamination of drinking water supplies). For surface water samples, simple scoop or "grab" collection is appropriate unless stratification is suspected (this is unlikely in shallow water bodies). Efforts to avoid contamination of the sample with sediment are particularly important when collecting surface waters in shallow water bodies.

If the pesticides in question have substantial vapor pressures, air sampling may be appropriate; for example, to assess risks to remedial personnel. Sampling can either be conducted using area monitoring stations or personal sampling pumps. Both sampling methods rely on pumps to draw air through a sample medium (aqueous solutions or solid sorbents) which is then analyzed for the pollutant of interest.

Sampling of biota presents particular challenges and should not be undertaken without consulting a wildlife ecologist or toxicologist. One needs to consider not only what organisms to sample (invertebrates, birds or mammals inhabiting different media) but also which tissues to sample (muscle, fat, reproductive tissues). Sampling of invertebrates (worms, insect larvae, small crustaceans) is easiest but data for higher trophic level species may give a clearer indication of potential adverse ecosystem wide effects. Sampling of species with only limited periods of residence in the area of concern (*e.g.*, migratory periods, mating periods) may present particular challenges. Details on methods for sampling biota can be found in WHO [9].

Timing of sampling is another important consideration. Static systems may only need a single sampling period whereas dynamic systems (those in constant change) may require continuous sampling. In reality, no environmental system is entirely static and unchanging, but when pollutants have relatively long half-lives (true for most obsolete pesticides) and conditions at the site are expected to remain relatively stable, the assumption of static conditions is not an unreasonable approximation. An important exception would be when use of the site in question varies with time (*e.g.*, seasonal use of the area by hunters or vacationers or use by migratory species). The effect of season on ambient conditions should also be considered. Periods of high temperature may mean more volatilization of the chemicals of interest, enhanced biodegradation, and more active biota. In addition, periods of high precipitation may mean greater dilution of contaminants in water samples and also greater difficulty in obtaining samples.

Finally, one must decide between obtaining discrete (grab) or composite samples. Discrete samples are those taken at a single location, whereas composite samples involve combining individual discrete samples, mixing them thoroughly, and taking a representative sample of the combined material. Data for discrete samples have the advantage of being traceable to a particular location but also have the disadvantage of providing coverage for only a very limited area. Thus, many discrete samples may be required to characterize the extent of contamination in a large geographical area or determine whether the area is uncontaminated. Composite samples have the advantage of providing a result indicative of a wide area; thus minimizing analysis costs. However, interpretation of composite sample data can be difficult. For instance, does the result indicate the whole area is "clean" or is it overlooking particular hot spots (*i.e.*, the phenomenon of dilution)? A composite sample that returns an unacceptable result (*i.e.*, a concentration above clean-up criteria or acceptable health levels) also presents a problem since it is impossible to determine where the unacceptable concentrations actually occur. One possible solution is to composite a portion of each discrete sample but retain the remainder for follow up if the result for the composite sample indicates a concern.

2.5 Decontamination

An environmental sample is intended to indicate the concentrations of chemicals from the exact media location where it was collected. It is therefore extremely important that sampling equipment be cleaned and decontaminated between sampling events to prevent cross-contamination. Decontamination also prevents the introduction of contaminants to clean areas and prevents the mixing of potentially incompatible substances. After physically removing bulk materials from sampling equipment, surfaces should be washed with a solution of non-phosphate detergent and then thoroughly rinsed with distilled/deionized water. Use of pressure sprayer equipment (*i.e.*, hand pumped or motor driven) during these steps, as well as the use of plastic bristle brushes, may facilitate equipment cleaning. Where specific contaminants are suspected, other rinses may be appropriate (*e.g.*, rinses with dilute nitric acid followed by distilled/deionized water for metal contaminants). Decontamination waste solutions should be collected and disposed of appropriately (this may require sampling and testing of the solution). Sampling equipment should also be frequently inspected to be sure it has not been damaged or degraded to the extent that it can contaminate the sample. Further details concerning decontamination of sampling equipment can be found in USEPA [6].

2.6 Sampling Strategies

The question of how many samples to collect is one of the most difficult to address. In an ideal world, every particle of soil could be sampled and analyzed which would yield an absolute certainty of the nature and extent of pollution. Given practical limitations on time and resources, something less than absolute certainty must be accepted. The goal in determining sample number must therefore be to balance the level of certainty required with the limitations imposed on available resources.

Various strategies have been developed to determine sample number based on the assumption that pollutant concentrations are normally (or log normally) distributed across the site of interest. With this assumption, statistical theory can be used to identify the number of samples required to characterize the average pollutant concentration (or to be sure we are below some value) with a given degree of statistical confidence. These formulae often rely on existing information or default estimates about the variance in pollutant concentrations in the media of interest. Thus it is helpful in such cases to have preliminary data from earlier studies or data obtained from similar sites to characterize the variance. Where such data are lacking, some regulatory agencies have proposed default values based on contaminant concentrations assessed at multiple sites in the past.

As an example, if we want to discover the average concentration of a particular pesticide at a site and if we assume pollutant concentrations (or log transformed concentrations) are normally distributed then the minimum number of samples to be collected (n_{min}) can be calculated as:

$$n_{min} = \left(Z_{1-\alpha/2} \times \sigma/d\right)^2$$

Where:

n_{min} = the minimum number of samples to collect
d = the maximum error in measuring the true mean that will be tolerated (*e.g.,* in mg/kg)
σ = the estimate of the standard deviation based on prior data (*e.g.,* in mg/kg)
$Z_{1-\alpha/2}$ = the value of the standard normal deviate as it relates to the upper tail of a standard normal distribution

(from Gilbert [1])

Thus, suppose we need to estimate the average concentration of DDT in near surface soil to compare to a regulatory limit and we can accept a 5 % chance (*i.e.*, $\alpha = 0.05$) of being 5 mg/kg off from actually measuring the true mean ($d = 5$). Suppose that prior sampling in the area (or at similar sites) tells us that the standard deviation (σ) of DDT measurements in soil should be about 200 mg/kg. Then:

$$n_{min} = (1.96 \times 200/5) = 78\,\text{samples}$$

Now suppose that this exceeds the available resources for conducting site sampling. One may then want to consider less stringent criteria for identifying the true site mean. For example, changing the value of $\alpha = 0.10$ and d to 10 mg/kg results in the following:

$$n_{min} = (1.65 \times 200/10) = 33\,\text{samples}$$

Note the significant role played by the estimate of the concentration variance across the area to sampled. Another formula, using similar statistical principles, allows one to calculate the number of samples needed to have a specified degree of certainty of being below a health or cleanup criterion [3]:

$$n_{min} = \delta^2 \times \left(\frac{Z_{1-\beta} + Z_{1-\alpha}}{Cs - \mu_1}\right)^2$$

Where

$Z_{1-\beta}$ = the critical value from the normal distribution with probability $1-\alpha$
$z_{1-\alpha}$ = the critical value from the normal distribution with the probability $1-\beta$
Cs = the criterion to be measured against
μ_1 = the acceptable false positive rate (α)
β = the false negative rate (power)
σ = the measured or estimated standard deviation in pollutant concentration at the site

In addition to the number of samples required to determine the level of chemical contamination, one should not forget to include field duplicates, laboratory split samples, or blanks. Field duplicate samples are separate samples collected from the same sampling location; they provide an indication of sample variability (both due to site conditions and analytical variability). Laboratory split samples typically involve dividing the same individual sample in half after first ensuring it is well mixed, and sending the split samples to two different laboratories for analysis. This can provide an indication of variability of laboratory analysis (since, in theory, the two splits contain the same homogenous material). Finally, blank samples (*e.g.*, field blanks, trip blanks) can help identify sample contamination that may occur as a result of improper equipment decontamination or insufficiently washed sample containers. Although these quality assurance/quality control (QA/QC) samples will increase sample number (*e.g.*, a duplicate sample might be collected for every 10 or 15 standard samples) they are essential for developing a credible data set.

Beyond the issue of sample number, one also must decide the order and placement of samples across the area of interest. Several approaches for sample allocation are available, each of which has distinct advantages and disadvantages depending on the level of knowledge and characteristics of the site:

2.6.1 Judgmental Sampling

As implied by the name, this technique is based on using personal judgment and opinion to select sample locations. To put it simply, samples are placed where one thinks they ought to go based on what is known about the site and the particular interests in defining the contamination. Thus in Fig. 2.1 below, samples might be

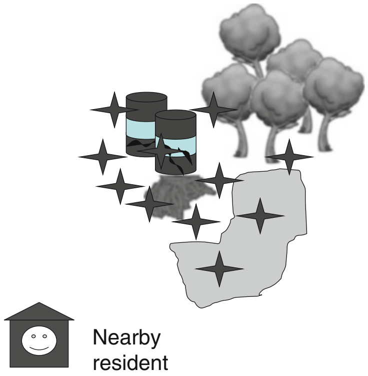

Fig. 2.1 An example of judgmental sampling

placed around the area of waste drums because these are the most apparent hazard and source of possible pollution. The advantages of judgmental sampling are that it takes advantage of site-specific knowledge and experience and is fairly easy and straightforward to implement. It is the most intuitive approach to sampling. On the negative side, this method is subjective and depends on one's knowledge being accurate and complete. What if some other source of pollution, not readily apparent, is also involved? This would likely be missed by judgmental sampling. This method of sampling also does not permit statistical evaluation of the results because the sample is by its very nature, biased.

2.6.2 Systematic Sampling

Under this approach, one allocates samples in a consistent and pre-determined pattern. Thus in Fig. 2.2 below, samples are located every 10 m from a preselected starting point in all directions (the sampling interval could be expanded as one moves further out, the important factor is for it to be consistent). The advantages of systematic sampling are that it is largely unbiased and objective (entirely objective aside from the selection of starting point) and the strategy is easy to explain. The negatives are that it either requires a substantial number of samples (*e.g.*, to sample every square in the grid) or supplies only limited coverage of the area in concern. Hot spots of potential contamination can be easily missed. Systematic sampling makes only limited use of site-specific information.

2.6.3 Random Sampling

Random sampling is generally preferred as a sampling technique because it produces an unbiased data set that can be analyzed using standard statistical

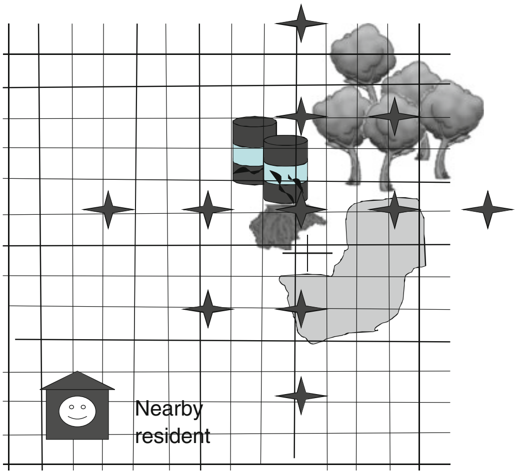

Fig. 2.2 An example of systematic sampling

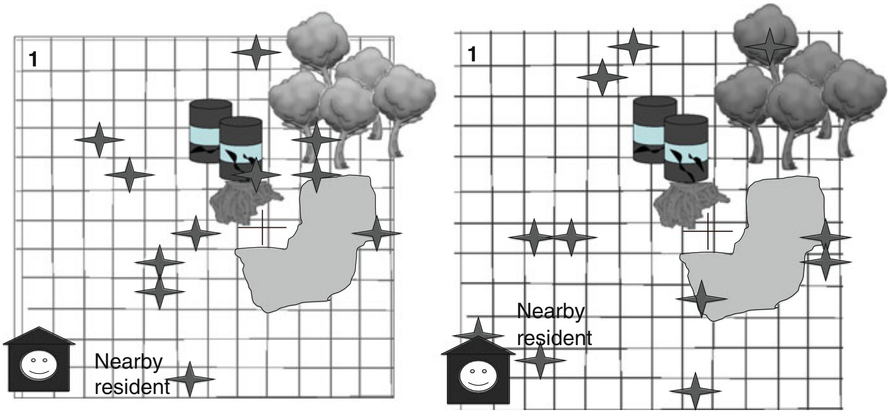

Fig. 2.3 Two possible outcomes of random sample allocation

techniques. In random sampling, one starts at a randomly chosen point in the sampling grid and then uses a random number generator to identify the locations to be sampled (random number generators are easily found on the internet). In addition to the advantage of being able to reliably perform statistical operations on the collected data, random sampling is also recognized by regulators as being objective and unbiased. Use of random sampling cannot, however, make up for having extremely small sample numbers. One is just as likely to miss a hot spot with small numbers of random samples as with small numbers of systematic samples. Another potential negative of random sampling is that, depending on the combination of random numbers generated, the sampling pattern can look rather counterintuitive and clumped (Fig. 2.3). While statistically valid, such a pattern can be difficult to explain to individuals unfamiliar with statistical theory.

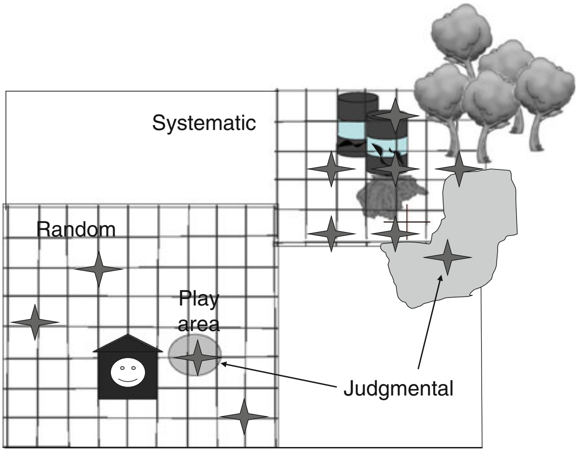

Fig. 2.4 An example of systematic sampling

2.6.4 *Stratified Sampling*

Stratified sampling combines several of the best features of the prior three approaches. As shown in Fig. 2.4, using site specific knowledge, one can divide the site in question into different strata and then use different sampling strategies, as appropriate, with each. Thus for identifying the extent of contamination, systematic sampling might be appropriate whereas to determine if the much larger remaining area of the site is affected, a random approach might be preferred. Specific samples could also be located *via* judgmental sampling to address specific concerns, in this case children's exposure during play and possible sediment contamination. Stratified sampling has the advantage of reflecting site-specific information (in terms of how the site is stratified) while still providing unbiased samples where appropriate. It does require site specific knowledge and depends on that knowledge being correct; otherwise the strata selected may be inappropriate.

A final strategy for sampling involves search sampling. In this approach, a small number of initial samples are collected which then inform subsequent rounds of sampling and analysis. This type of method is most useful for "chasing" areas of contamination to identify their source and is less relevant for characterizing site risks. It has the advantage of being fairly frugal with sample number but the disadvantage of being fairly time consuming as each round of analysis must wait on sample collection, analysis and data evaluation from prior rounds.

2.7 Sampling Grids

Several of the sampling location approaches identified above require that a sampling grid be imposed on the area of concern. The dimensions of such a grid are of course a critical consideration, since a grid that is too large may miss localized hot

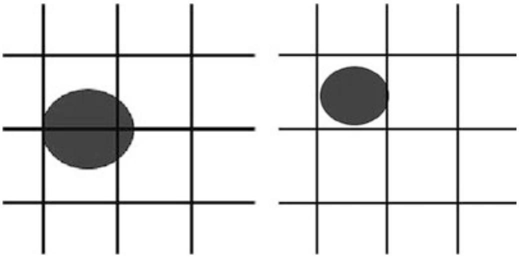

Fig. 2.5 The likelihood of missing a pollution hot spot depends on the sample grid interval

spots of contamination and lead to an erroneous conclusion (Fig. 2.5). There are a number of formulae for determining the appropriate grid interval to avoid missing a "hot spot" of a given size. One such formula takes the following form:

$$G = R/0.59$$

Where

G = distance between two sampling points (the grid size, m)
R = radius of the smallest hot spot of potential interest (m)
0.59 = factor derived from 95 % detection probability assuming circular hot spots

(from NZMFE [2])

2.8 Sample Handling

Determining where, when and how the samples are collected is only part of the information required in the SAP. It is also necessary to specify how the samples should be handled after collection and how they should be transported to the analytical laboratory. Ideally, sample containers (including any necessary preservatives) should be obtained in advance from the analytical laboratory and used "as is" for collecting samples in the field. Field work is complicated and chaotic enough without the need to require field staff to add preservatives or choose sample containers during the sample collection process. Samples may also need to be split according to the needs of handling and storage for particular analytes. For example, some semi-volatile organic compounds require storage in amber rather than clear glass containers and volatile organic carbons (VOCs) may require special headspace containers to facilitate analysis by gas chromatography. The SAP should therefore specify the appropriate container(s) for each sample and chemical of interest.

Each sample should also be assigned a clear and unambiguous sample number and this should be recorded on both the sample container and the chain-of-custody (COC) form. COCs are critical when sampling and analysis is being conducted for regulatory or legal matters but should also be employed for more routine cases. The ability to track the handling and transit of a sample may prove useful in explaining unexpected results or may help identify errors in the sample handling process. The SAP should also specify how samples are to be transported (typically, on ice, using sealed ice packs) and identify the address of the analytical laboratory.

2.9 Sample Analysis

Finally, the SAP should specify how the samples are to be analyzed. It makes little sense to carefully specify the procedures for locating and collecting samples if unclear directions to the analytical laboratory result in unsatisfactory data. The directions should include not only the analytical method but also whether any sort of pre-analysis digestion or treatment (*e.g.,* compositing, extraction) is required. Analysis methods (GC-MS, ICP, HPLC) should ideally reference specific protocols (*e.g.,* SW-846, USEPA [5]) unless some specialized analysis is being requested. When specifying the analytical procedures to be followed, one should consider whether breakdown products or contaminants should also be included (*e.g.,* 2,4,5-T, 2,4-D and dioxins). It is vital that the SAP specify the necessary detection limits for all analyses to be conducted. Data reported by the laboratory as below the detection limit will be useless if the detection limit is higher than the relevant regulatory or risk criterion. Finally, analytical data quality objectives (percent recoveries, acceptable results for blanks, *etc.*) should be specified.

2.10 Conclusions

In summary, a well designed sampling and analysis plan is a critical component of any remedial strategy. Such a plan provides the fundamental data on which additional assessments and decisions can be made. Successful design and implementation of such a plan requires the participation and input from all involved stakeholders, to the extent that such parties can be identified earlier in the remedial process. By maximizing the use of pre-existing information, determining the sample location strategy that best fits with the project needs, and thorough attention to the details of sample collection, handling and analysis, a sound sampling and analysis plan can be developed and carried out.

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Chapter 3
Analytical Approaches for Sampling and Sample Preparation for Pesticides Analysis in Environmental, Food and Biological Samples

Elisabeta Chirila and Camelia Drăghici

Abstract Residues of pesticides, especially organochlorine pesticides (OCPs) in sediment and aquatic biota have been an environmental concern since the 1960s. Widely used in agriculture in the past, most of OCPs are resistant to photochemical, biological and chemical degradation for a long period of time. For their determination in different environmental media, sampling and sample preparation represents a time consuming stage, but a key factor in the entire pesticides trace analysis procedure. Scientific efforts directed towards the sample pretreatment issue are focused on developing methods for enriching and isolating components present in complex sample matrices. Due to the differences of pesticides properties (volatility, polarity), to the complexity of the sample matrix and to the required degree of preconcentration, there is no unique strategy for the sample preparation. This chapter presents different techniques available for samples preparation for pesticide analysis in environment, in food or in biological samples.

Keywords Pesticides • Sampling • Extraction • Preconcentration • Clean-up

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3.1 Introduction

Pesticides and their metabolites have received particular attention in the last few years in environmental trace organic analysis because they are regularly detected in surface and ground waters especially throughout Europe and North America as a consequence of their widespread use for agricultural and non-agricultural purposes [16].

The determination of pesticides in food and environmental samples at low concentrations is always a challenge. Ideally, the analyte to be determined would be already in solution and at a concentration level high enough to be detected and quantified by the selected final determination technique (i.e., HPLC or GC) [36]. However, in environment, in food or in biological samples they are present at trace levels and despite the advanced techniques available for separation and quantification, no sample can be directly analyzed, therefore an extraction and concentration stage is required.

Even when the analyte is already in a liquid media (i.e. water, juice, serum), the presence at different concentration levels of the matrix-interfering compounds, imposes the need to overcome several difficulties related to the required selectivity and sensitivity of the analytical technique. Consequently, the selection of an appropriate sample preparation procedure involving extraction, concentration and cleanup steps becomes mandatory to obtain a final extract, enriched in the target analyte, as free as possible of the interfering compounds.

This contribution presents different sample treatment techniques currently available and most commonly used in specialized laboratories for the analysis of pesticides in environmental, food and biological samples. Depending on the nature of the sample (solid or liquid) and the specific application (type of pesticide, concentration level, multiresidue analysis), the final procedure might involve the use of only one, or a combination of several different described techniques.

3.2 General Procedures for Samples Preparation

Environmental analysis, like any other analytical process, must follow three major steps: (i) sampling and sample preparation for measurements; (ii) measuring; (iii) data processing [7]. Sampling for organic compounds analysis, like pesticides, is based on the principle to extract them from the sample matrix in a soluble and stable form [11].

One key problem in pesticide analysis comes from the diversity of their chemical functional groups with varying polarity and physicochemical properties. Sampling and sample preparation represents a time consuming step, very important in the whole procedure for trace analysis of pesticides. The methods for extraction and concentration of pesticides are mainly liquid-liquid extraction and solid-phase extraction. Table 3.1 presents comparative information about the general pesticides extraction techniques from solid and liquid samples, that will be further presented.

Table 3.1 Comparison of general pesticides extraction techniques from liquid and solid environmental samples

				Solvent
Extraction method	Application	Cost	Time of extraction	Volume (mL)
Purge & trap	V (L, S)	High	30 min	–
Headspace	V (L, S)	Low	30 min	–
LLE	V, SV, NV (L)	Low	1 h	500
SPE	V, SV, NV (L)	Medium	30 min	50–100
SPME	V, SV, NV (L)	Low	30 min	–
Soxhlet/Soxtec	SV, NV (S)	Low	12–48 h	300–500
USE	SV, NV (S)	Medium	15–30 min	5–30
ASE (PSE)	SV, NV (S)	High	20–30 min	30
MAE	SV, NV (S)	Medium	15 min	40
SFE	SV, NV (L, S)	High	30 min	5–20

ASE accelerated solvent extraction, *LLE* liquid-liquid extraction, *MAE* microwave assisted extraction, *PSE* pressurized solvent extraction, *SFE* supercritical fluid extraction, *SPE* solid phase extraction, *SPME* solid phase microextraction, *USE* ultrasonic solvent extraction, *L* liquid, *NV* nonvolatile, *S* solid, *SV* semivolatile, *V* volatile

3.2.1 Purge and Trap Extraction

Purge and trap extraction is used for organic non-polar volatile compounds to be further used for GC analysis. An inert gas is bubbled in the water sample, transferring the organic volatiles into the vapor phase. These are trapped in an active carbon and/or condensed. The trap containing the adsorbent is passed into a heated desorption chamber that allows desorption of the retained compounds. This is not always a fast process (as needed for GC) but cryogenic focusing may be used. It is very important to use highly pure purge gas. Purging water media may raise difficult problems because usually low water quantities are allowed in the column.

3.2.2 Headspace Extraction

Headspace extraction is used for the pollutants trapped in a matrix that cannot be introduced, as such, in a chromatographic system. There are two techniques, depending on the way to introduce the sample in the measuring equipment:

- **Static headspace technique** is probably the simplest solvent-free sample preparation technique, has been used for decades to analyze volatile organic compounds; the sample (liquid or solid) is placed in a vial; the vial is sealed, then heated and the volatile compounds are driven into the headspace; equilibrium between the headspace and the sample matrix is reached (Fig. 3.1a); a portion of the vapor from the headspace is injected then into a GC;
- **Dynamic headspace technique** uses a carrier gas (helium) for eluting the volatile parts to a collector where they are adsorbed and concentrated; a thermal desorption follows in the collector, allowing the gas components to enter the GC (Fig. 3.1b); the sample can be recovered by stripping.

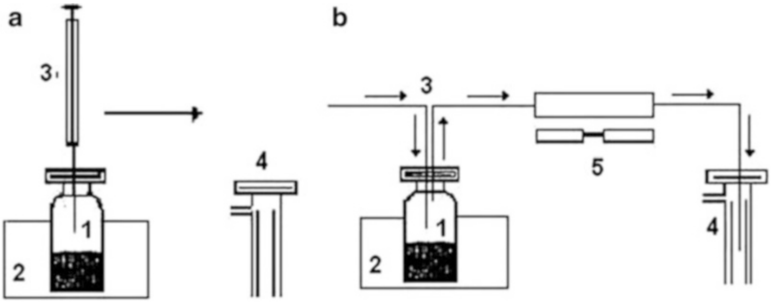

Fig. 3.1 Headspace extraction techniques: (**a**) static; (**b**) dynamic. *1* – headspace vial; *2* – termostate; *3* – sample prelevator; *4* – GC injector; *5* – collector

3.2.3 Liquid-Liquid Extraction

Liquid-liquid Extraction (LLE) has been widely used for the extraction of pesticides from aqueous liquid samples and, although to a lesser extent, for the purification of organic extracts. LLE is based on the partitioning of target analyte between two immiscible liquids. The efficiency of the process depends on the affinity of the analyte for the solvents, on the ratio of volumes of each phase, and on the number of successive extractions. Hexane and cyclohexane are typical solvents for extracting non-polar compounds, such as organochlorine and some organophosphorus pesticides, whereas dichloromethane and chloroform are the common solvents used for the extraction of medium-polarity pesticides.

The use and evaporation of large volumes of solvent, often toxic and flammable, are the main drawbacks. Therefore, the trends in reducing the use of organic solvents in analytical laboratories and the low performances in extracting polar compounds explain the increasing replacement of LLE by liquid phase micro-extraction and/or solid-phase extraction.

Liquid Phase Micro Extraction (LPME) is a miniaturized implementation of conventional LLE in which only microliters of solvents are used instead of several hundred milliliters in LLE. The technique is quick, inexpensive and can be automated [19].

There are two sampling modes that can be used with LPME: two-phase and three-phase. In two-phase LPME, the analytes are extracted from the aqueous sample solution (donor phase) into the organic solvent (acceptor phase) which either consist of a micro-drop (ca. 1–3 μL), suspended from the needle of a micro-syringe (**single drop micro-extraction – SDME**, Fig. 3.2a, b) or it is present in the pores and/or inside the lumen of a hydrophobic membrane, respectively a **hollow fiber (HF LPME)**, Fig. 3.3a, b.

Basically, in liquid phase micro extraction using hollow fiber membranes (HF-LPME) technique, the piece of porous polypropylene hollow fiber is impregnated with a water-immiscible solvent and the analytes are extracted by passive diffusion from the sample into the hydrophobic organic solvent supported by the fiber (two

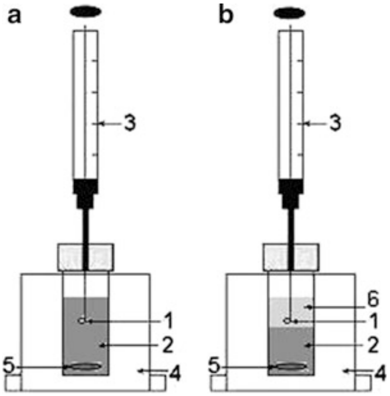

Fig. 3.2 The schematic representation of SDME: (**a**) two phase; (**b**) three phase: *1* – solvent drop; *2* – aqueous phase; *3* – chromatographic micro-syringe; *4* – water bath; *5* – stir bar; *6* – organic solvent layer

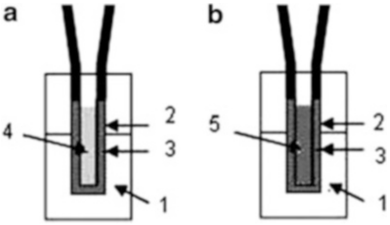

Fig. 3.3 HF – LPME (**a**) two – phase; (**b**) three – phase: *1* – sample; *2* – hollow fiber; *3* – supported liquid membrane; *4* – acceptor phase (organic solvent); *5* – acceptor phase (aqueous solution)

phase HF-LPME). On the other hand, the analytes can be extracted through the organic solvent immobilized in the pores of the fiber and further into a new aqueous phase in the lumen of the fiber (three phase HF-LPME) [30].

Liquid Membrane Extraction Techniques, supported liquid membrane, (SLME), and micro porous membrane liquid–liquid (MMLLE) extractions are based on the use a hydrophobic membrane, containing an organic solvent, which separates two immiscible phases. These extraction techniques are a combination of three simultaneous processes: (1) extraction of analyte into organic phase; (2) membrane transport (3) re-extraction in an acceptor phase. SLME and MMLLE have been successfully applied for enrichment of phenoxy acid, sulfonylurea, and triazine herbicides from environmental water samples.

3.2.4 Solid–Liquid Extraction

Solid–liquid extractions processes are based on the extraction of the analytes from a liquid sample in a solid material (solid phase extraction), or are based on the extraction of the analytes from solid samples with liquid solvents.

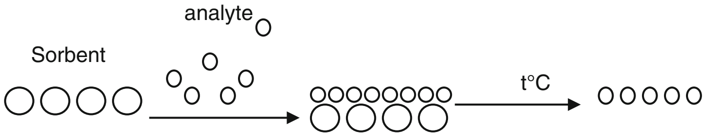

Fig. 3.4 Solid phase micro extraction process

3.2.4.1 Extraction from Liquid Samples

In **Solid Phase Extraction (SPE)**, a liquid phase (liquid sample or liquid sample extracts) is loaded onto a solid sorbent (polar, ion exchange, non-polar, affinity), which is packed in disposable cartridges or enmeshed in inert matrix of an extraction disk. Those compounds with higher affinity for the sorbent will be retained on it, whereas others will pass through it unaltered. Subsequently, if target analytes are retained, they can be eluted using a suitable solvent with a certain degree of selectivity [6]. Understanding the mechanism of interaction between the sorbent and the analyte is a key factor on the development of a SPE method, since it will ease choosing the right sorbent from the wide variety of them available in the market: polar (silica, alumina, florisil), non-polar (n-alkyl-bonded silica, styrene-divinylbenzene based polymers, graphitized carbon), ion-exchange, affinity (immunosorbents, molecularly imprinted polymers).

SPE has demonstrated to be a very useful procedure for the extraction of a great variety of pesticides in food and environmental analysis. However, although in a lower extent than LLE, this technique still requires the use of toxic organic solvents and its applicability is restricted to liquid samples.

Solid Phase Micro Extraction (SPME), introduced in 1989 [3] eliminates these drawbacks. The SPME device contains a melted silica fiber, coated with an adsorptive material which adsorbs the pesticides from liquid samples or those contained in headspace. After adsorption, the silica fiber is extracted from the flask and then is coupled to a GC injector, where the analytes are thermally desorbed, cryogenic focused at the entrance of the column, then separated. The SPME process is presented in Fig. 3.4.

The SPME can use **split-splitless** or **on column injectors.** A proper selection of the SPME sorbent is a key factor in the success of the analysis. In general, the polarity of the fiber should be as similar as possible to that of the analyte of interest. In this sense, there are nowadays a great variety of fibers commercially available that cover a wide range of polarities: carbowax/DVB for polar compounds, or polydimethylsiloxane (PDMS) for hydrophobic compounds.

Stir Bar Sorptive Extraction (SBSE) is similar with SPME, based on the partitioning of target analytes between the sample (mostly aqueous-based liquid samples) and a stationary phase-coated stir bar. The experimental procedure followed in SBSE is quite simple. The liquid sample and the PDMS-coated magnetic stir bar are placed in a container. Then, the sample is stirred for a certain period of time (30–240 min) until no additional recovery for target analytes is observed, even when the extraction time is further increased. Finally, the stir bar is removed and placed in a specially designed unit in which thermal desorption and transfer of target analytes to the head of the GC column takes place.

3.2.4.2 Extraction from Solid Samples

Solid–liquid extraction is probably the most widely used procedure in the analysis of pesticides in solid samples and includes various extraction techniques based on the contact of a certain amount of sample with an appropriate solvent. The steps that take place in a solid–liquid extraction procedure are: (i) solvent penetration inside the pores of the samples' particles; (ii) desorption of the analytes bound to matrix active sites; (iii) diffusion of the analytes through the matrix; (iv) dissolution of the analytes in the extracting solvent; (v) diffusion of the analytes through the solvent and (vi) recovery of external solvent containing analytes. The final extraction efficiency is influenced by the proper selection of the solvent to be used and also by other parameters such as pressure and temperature. Working at high pressure facilitates the solvent to penetrate sample pores (step 1) and, in general, increasing temperature increases solubility of the analytes on the solvent. Moreover, high temperatures increase diffusion coefficients (steps 3 and 5) and the capacity of the solvent to disrupt matrix–analyte interactions (step 2). Depending on the strength of the interaction between the analyte and the sample matrix, the extraction will be performed in soft, mild, or aggressive conditions. Table 3.2 shows a summary and a comparison of advantages and drawbacks of the different solid–liquid extraction techniques most commonly employed in the analysis of pesticides in food and environmental samples.

Supercritical Fluid Extraction (SFE) uses as solvents supercritical fluids which can be considered as hybrid between liquids and gases, and possess ideal properties for the extraction of pesticides from solid samples. Supercritical fluids have in common with gases the ability to diffuse through the sample, which facilitates the extraction of analytes located in not easily accessible pores. In addition, the solvation power of supercritical fluids is similar to that of liquids, allowing the release of target analytes from the sample to the fluid.

Carbon dioxide is the most used in SFE because it can be obtained with high purity, it is chemically inert, and its critical point (31.1 °C and 71.8 atm) is easily accessible. Its main drawback is its nonpolar character, limiting its applicability to the extraction of hydrophobic compounds. In order to overcome, at least to a certain extent, this drawback, the addition of a small amount of an organic solvent modifier (i.e., methanol) has been proposed and permits varying the polarity of the fluid, thus increasing the range of extractable compounds. However, the role of the modifier during the extraction is not well understood. Once target analytes are in the supercritical fluid phase, they have to be isolated for further analysis, which is accomplished by decompression of the fluid through a restrictor by getting analytes trapped on a liquid trap or a solid surface. With a liquid trap, the restrictor is immersed in a suitable liquid and thus, the analyte is gradually dissolved in the

Table 3.2 Solid–liquid extraction techniques

Technique	Description	Advantages	Drawbacks
Shaking	Samples and solvent are placed in a glass vessel; shaking can be done manually or mechanically	Simple Fast (15–30 min) Low cost	Filtration of the extract is necessary dependent of matrix type moderate solvent consumption (25–100 mL)
Soxhlet	Sample is placed in a porous cartridge and solvent returns continuously by distillation–condensation cycles	Standard method No further filtration of the extract necessary independent of kind of matrix Low cost	Time-consuming (12–48 h) high solvent volumes (300–500 mL) solvent evaporation needed
USE	Samples and solvent are placed in a glass vessel and introduced in an ultrasonic bath	Fast (15–30 min) Low solvent consumption (5–30 mL). Bath temperature can be adjusted Low cost	Filtration of the extract is necessary dependent of kind of matrix
MAE	Sample and solvent are placed in a reaction vessel; microwave energy is used to heat the mixture	Fast (~15 min) Low solvent consumption (15–40 mL) Easily programmable	Filtration of the extract is necessary addition of a polar solvent is required moderate cost
PSE	Sample is placed in a cartridge and pressurized with a high temperature solvent	Fast (20–30 min) Low solvent consumption (30 mL) Easy control of extraction parameters (temperature, pressure) High temperatures achieve	Initial high cost dependent on the kind of matrix

USE ultrasound – assisted extraction, *MAE* microwave-assisted extraction, *PSE* pressurized solvent extraction

solvent, while CO_2 is discharged into the atmosphere. In the solid surface technique, analytes are trapped on a solid surface (i.e., glass vial, glass beads, solid-phase sorbents) cryogenically cooled directly by the expansion of the supercritical fluid or with the aid of liquid N_2. Alternatively, SFE can be directly coupled with gas chromatography or with supercritical fluid chromatography, and its successful online coupling depends on the used interface, which determines the quantitative transfer of target analytes to the analytical column [40].

3.2.5 Solid-Solid Extraction

Matrix Solid Phase Dispersion (MSPD), introduced by Barker [2], is based on the complete disruption of the sample (liquid, viscous, semisolid, or solid), while the sample components are dispersed into a solid sorbent. Most methods use C8- and

C18-bonded silica as solid support. Other sorbents such as Florisil and silica have also been used although to a lesser extent. Experimentally, the sample is placed in a glass mortar and blended with the sorbent until a complete disruption and dispersion of the sample on the sorbent is obtained. Then, the mixture is directly packed into an empty cartridge as those used in SPE. Finally, analytes are eluted after a washing step for removing interfering compounds. The main difference between MSPD and SPE is that the sample is dispersed through the column instead of only onto the first layers of sorbent, which typically allows the obtainment of rather clean final extracts avoiding the necessity of performing a further cleanup.

MSPD has been successfully applied for the extraction of several pesticides from fruit juices, honey, oranges, cereals, and soil, and the obtained results, compared with those obtained by other classical extraction methods, has been found superior in most cases. The main advantages of MSPD are the short extraction times needed, the small amount of sample, sorbent, and solvents required, and the possibility of performing extraction and cleanup in one single step.

3.3 Air Sampling and Samples Preparation for Pesticides Determination

The atmosphere is known to be a good pathway for the worldwide dissemination of pesticides. Pesticides can enter into the atmosphere by "spray drift" during application, post application volatilization from soils and leaves, and by wind erosion when pesticides are retained to soil particles and entrained into the atmosphere on windblown particles. Pesticides are present in the atmosphere in the gas phase (from volatilization processes) and in the particle phase (including aerosols) [22].

Due to the very low concentrations of pesticides in the ambient air, appropriate sampling and pre-concentration techniques are necessary to achieve the sensitivity of the analytical instruments. The most common sampling techniques for pesticides in ambient air can be grouped into two categories: active and passive (diffusive) samplers.

Active sampling is carried out by pumping the air through sorbents, glass fiber or quartz filters. The pesticides in the particulate phase are retained in the filter, whereas those present in the gas phase are trapped by the adsorbent (Fig. 3.5). Before analysis, the filters and sorption materials are submitted to extraction and clean-up steps.

Passive air sampler (PAS) is based on a device that collects chemicals from the atmosphere without the aid of a pump, and consists in an accumulating medium that has a high-retention capacity for the target analytes. Such samplers allow for integrative (time-averaged concentrations, TWA) sampling in locations where active samplers would not be practical over long periods, due to lack of electricity supply in remote locations. Nevertheless, passive samplers are able to collect only the free gaseous phase pollutants and the duration of sampling ranges from few

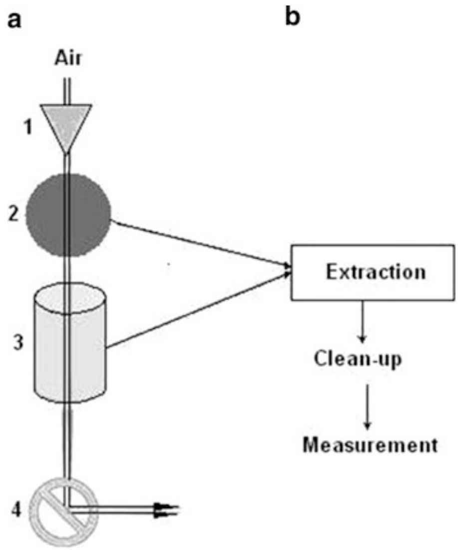

Fig. 3.5 Schematic diagram of a typical active air sampling and the analysis steps of filters and adsorbents: (**a**) – sampling; (**b**) – sample preparation; *1* – sample inlet; *2* – filter; *3* – sorption material; *4* – aspiration pump (Adapted from Yusa et al. [39])

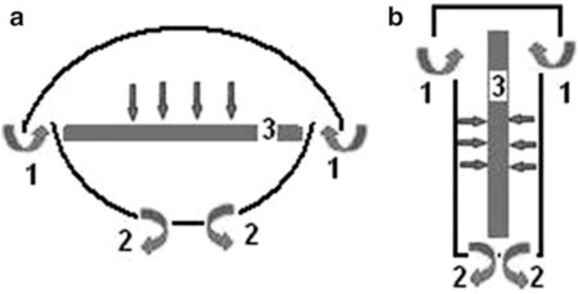

Fig. 3.6 Schematic diagram of passive air samplers: (**a**) planar; (**b**) axial; *1* – air inlet; *2* – air outlet; *3* – adsorption surface

weeks to several months, significantly larger than the usual time required for active samplers. After sampling, the adsorbed analytes are desorbed off the adsorbent by solvent or thermal desorption.

A range of PAS are available for different chemical species sampling from air. Most commercially available passive/diffusive samplers have a planar or axial shape (Fig. 3.6).

The used **adsorbent materials** are mainly polyurethane foam (PUF) disks, semi-permeable membrane devices (SPMDs), polymer-coated glass (POG), and styrene – divinylbenzene resin (XAD) [39]. In the dynamic air sampling techniques there are used glass-fiber filters (GFF) or quartz-fiber filters (QFF) followed by the adsorption on materials like XAD or PUF. The types of materials used for passive air sampling devices (PAS) and sampling duration are presented in Table 3.3.

SPMDs comprise a low density polyethylene (LDPE) bag, of 70–90 μm wall thickness, filled with triolein (1,2,3-tris-*cis*-9-octadecenoyl glycerol). Standard devices are 106 cm long, 2.54 cm wide, and contain 1 mL of triolein. The operation

Table 3.3 Types of passive air samplers

Number	Type	Sampling duration
1.	XAD-2	5–12 months
2.	PUF disks	4 weeks–4 months
3.	PDMS	14 days
4.	POG	7 days
5.	SPMD	7 days

Adapted after Kosikovska and Biziuk [18]

Table 3.4 Examples of air samples preparation for pesticides analysis

Location, samplers, materials	Extraction, solvent	Clean-up	Measurement	Reference
Luxemburg, passive, XAD-2	PSE, acetonitrile	SPME-PDMS	GC/MS-MS	[34]
Algeria, passive, PUF	Soxhlet, hexane/diethylether	Not specified	GC/MS-MS	[24]
Algeria, active, GFF	Direct, methylene chloride	Not specified		
Spain, active, QFF	MAE, ethyl acetate, dichloromethane	GPC, hexane	GC/EI/MS-MS	[9]
France, greenhouse, active, SPME/PDMS	Not necessary	Not necessary	GC/MS-MS	[37]
France, greenhouse, active, glass cartridge with PUF	Soxhlet, hexane/diethylether	Kuderna Danish concentration, alumina, hexane	GC/MS	

GFF glass fiber filter, *GPC* gel permeation chromatography, *MAE* microwave assisted extraction, *QFF* quartz fiber filter, *PDMS* polytimethylsiloxane, *PSE* pressurized solvent extraction, *PUF* polyuretane foam, *SPME* solid phase microextraction, *XAD* styrene-divinylbenzene resin

of a SPMD as passive sampler is based on the diffusion of compounds through the polymeric membrane bag and their accumulation in the lipophilic solvent. POG samplers are created by applying a thin polymeric stationary phase (ethylene vinyl acetate-EVA) to a solid glass surface. XAD have been previously used to collect a variety of pesticides including diazinon, chlorpyrifos, disulfoton, fonofos, mevinphos, phorate, terbufos, cyanazine, alachlor, metolachlor, simazine, atrazine, deethyl atrazine, deisopropyl atrazine, molinate, hexachlorobenzene, trifluralin, methyl parathion, dichlorvos, and isofenphos.

Solid Phase Micro Extraction (SPME) has significant advantages over the traditional methods: is a solvent-free technique, convenient coupling with field analytical instruments, cost effectiveness and simplicity of operation. Depending on the purpose of the study, sampling time with SPME can range from a few seconds to days, for assessment of short-term and long-term exposures, respectively. For peak concentration, the SPME fiber is exposed to the sample. If a TWA concentration is needed, the fiber is kept retracted in the needle [37]. Table 3.4 presents examples of air samples preparation for pesticides analysis in studies published during the last years.

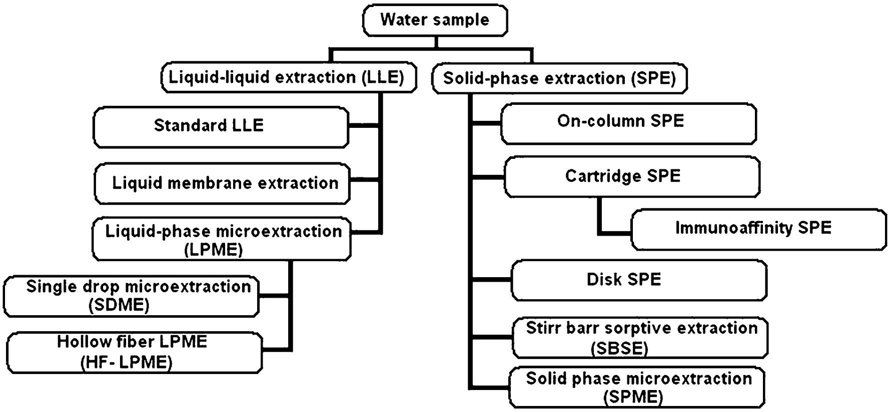

Fig. 3.7 Sample preparation methods for pesticides analysis in water (Adapted after Gan and Bondarenko [12])

3.4 Water Samples Preparation for Pesticides Determination

The need for detecting pesticides at trace levels means that a water sample must be reduced many times in such a volume that a small aliquot of the final sample may provide adequate sensitivity for detection. The concentration magnification is achieved through phase transfer by using liquid-liquid extraction or solid-phase extraction. Many other methods may be considered as variations of the traditional LLE and SPE methods (Fig. 3.7).

For example, micro-LLE or single-drop extraction can be considered as a miniaturization of the standard LLE procedure. Variations of cartridge SPE include SPE disks and SPME. The available methods can also be classified based on the mechanisms used for pesticide detection. However, as detection methods are usually common among different sample matrices and are not limited only to water, this chapter will mostly focus on sample preparation methods for water analysis, with exceptions made only for immunoassays and capillary electrophoresis (CE) because of their significant deviations from conventional chromatographic methods [12]. Table 3.5 presents examples of water samples preparation for pesticides analysis.

3.5 Soil, Biota and Food Samples Preparation for Pesticides Determination

Traditional pesticide residues analysis requires a large amount of organic solvent for sample extraction and a series of steps for preconcentration and clean-up, which are complicated, tedious and expensive [10]. The most commonly used methods for

Table 3.5 Examples of water samples preparation for pesticides analysis

Water type, location	Sampling and preparation	Measurement	Reference
Lake water, USA	Passive – SPE, extraction in methanol, evaporated to 1 mL, filtered, adjusted to 1 mL with ethyl acetate	GC-MSD	[5]
River water, Spain	100 mL water spiked with standard mixture; 5 mL extracted with automated on-line SPE	LC-MS/MS	[17]
River water and tap water, Brazil	SDME – toluene	GC-MS	[29]
River water, Botswana	HS-SPME	GC-ECD	[23]
River water, Iran	SDME – hexyl acetate and derivatization	GC-MS	[32]
Groundwater, Spain	SPME; acetonitrile/water	HPLC-PIF-FD	[27]
Surface water, Europe	Passive with Chemcatcher devices, extraction in acetonitrile/methanol, evaporation to dryness, redissolved in acetonitrile	GC-MSD	[33]

FD fluorescence detection, *HS* headspace, *MSD* mass selective detector, *PIF* photochemically induced fluorimetry, *SDME* single drop microextraction, *SPE* solid-phase extraction, *SPME* solid phase microextraction

solid sample preparation include pressurized solvent extraction (PSE), solid phase extraction (SPE), supercritical fluid extraction (SFE), solid phase microextraction (SPME), headspace – solid phase microextraction (HS-SPME), liquid phase microextraction (LPME), microwave assisted extraction (MAE), matrix solid phase dispersion (MSPD) etc. In addition, Quick Easy Cheap Effective Rugged Safe (QuEChERS) is another very popular analytical method in pesticide residue analysis, while a direct solid sample introduction is also available, and will further be presented.

3.5.1 QuEChERS Method

The recently introduced [1] QuEChERS method avoids the use of nonpolar solvents inducing LLP by addition of $MgSO_4$ and NaCl to acetonitrile extracts, which leads to removal of majority of water and highly polar matrix components, yet achieving high recoveries of wide range of both GC- and LC-amenable pesticides. Additional SPE clean-up is performed to remove mainly sugars and fatty acids.

QuEChERS is a sample preparation approach entailing solvent extraction of high-moisture samples followed by clean up using d-SPE. Basically, the sample is firstly extracted with a water-miscible solvent (for example, acetonitrile–ACN) in the presence of high amounts of salts (for example, sodium chloride and magnesium sulfate) and buffering agents (for example, citrate) to induce liquid separation and stabilize acidic and basic labile pesticides, respectively. Upon shaking and

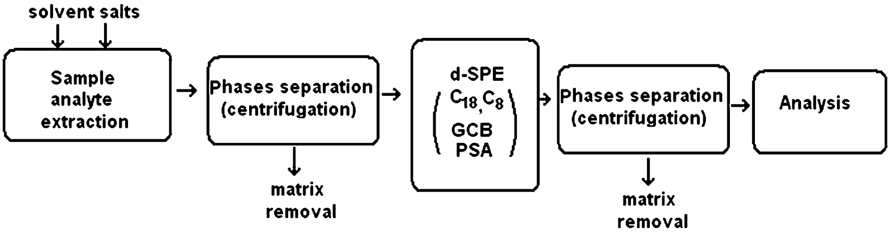

Fig. 3.8 Schematic diagram of QuEChERS sampling method

centrifugation, an aliquot of the organic phase is subjected to further clean up using dispersive – solid phase extraction (d-SPE), by adding small amounts of bulk SPE packing sorbents to the extract. After sample clean up, the mixture is centrifuged and the resulting supernatant can be directly analyzed, or can be subjected to another concentration, when solvent exchange step if necessary. The sorbents used for d-SPE depend on the matrix compounds to be removed, as following:

- non-polar sorbents (C_{18} or C_8) are retaining lipids from the ACN extract, on which the majority of subsequent studies have demonstrated that it does not negatively affect pesticide recoveries, but helps to obtain cleaner extracts;
- graphitized carbon black (GCB) remove sterols and pigments and provide a greater degree of clean-up, giving less colored extracts;
- primary secondary amine (PSA) has been found as the most effective sorbent for removal of various matrices, significantly reducing matrix-enhancement effect.

The schematic diagram of QuEChERS method is presented in Fig. 3.8.

This type of solvent extraction method have shortened the whole analytical time and enhanced the extraction efficiency. However, these methods are not simple and quick enough with regard to the problem that more and more samples needed to be analyzed [15, 26].

3.5.2 Direct Solid Sample Introduction

In 2005, Zhang and coworkers have developed a new method for pesticide residue analysis in vegetables. Sample without any pretreatment was directly introduced into the split/splitless injector for GC–MS determination. This method was proven to be quick, convenient and accurate. It also worked well for rapid detection of pesticide residues in food and large scale screening of samples in field detection. In addition, the GC split/splitless injector was simply modified to quickly remove oxygen and low boiling point matrices of the sample by adding a venting gas line on the original pneumatic system. No sample pretreatment was needed and the sampling procedure required less than 5 min. The injector's modification can be conducted on portable GC. Hence this method is potential for field analysis of pesticide residues in crops and large scale screening of samples [25].

Table 3.6 Examples of solid samples preparation for pesticide analysis

Sample	Sample preparation	Measurement	Reference
Tea	QuEChERS + LLE	GC/MS-MS	[4]
Lettuce	SPME	HPLC/DAD	[21]
Seaweeds	MSPD	GC/MS	[14]
Fruits	QuEChERS	GC/MS	[8]
Plant materials for medicine	QuEChERS	GC/ECD	[38]
Vegetables	Ultrasonic extraction dichloromethane	GC/MS	[20]
Crops	Direct solid sample introduction	GC/ECD	[25]
Cow milk	HS-SPME	GC/MS	[31, 35]
Bovine meat and liver	MSPD	HPLC/DAD	[13]
Tea	HS-SPME	GC × GC/TOF/MS	[33]
Cucumber, watermelon	SPME	HPLC/PIF/FD	[28]

FD fluorescence detection, *PIF* photochemically induced fluorimetry, *TOF* time of flight

A known quantity of crushed solid sample having a diameter >1.5 mm is added in the middle of a glass liner and supported by a little bit glass wool. Finally the glass liner is installed back to the injector for GC analysis. The solid sample is disposable after each test. Next sample could be transferred into the liner for another experiment immediately.

Table 3.6 presents examples of solid samples preparation for pesticides analysis in papers published in last years.

3.6 Conclusions

This chapter presents an overview of the available sample pretreatment techniques to be used for pesticides analysis from all types of gaseous, liquid and solid samples. Actual trends in analytical techniques are the simplification and miniaturization of sample preparation as well as the minimization of the solvents volumes used. There are several novel micro-extraction techniques which improve the sample preparation steps. These require the design and formulation of new materials capable to provide selective extraction of the organic pollutants, like pesticides are.

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Chapter 4
The Problem of Seeking Environmentally Sound Development: The Case of Obsolete Pesticides Assessment Models

Ganna Kharlamova

Abstract The purpose of this paper is twofold: firstly, to indicate how the environmental safety issues grow rapidly in the convergence with economic aspects in modern world, and, secondly, to present analyses of implementation of economic-mathematical methods for assessment analysis to OPs management issues. For management (destruction and recycling) of OPs a comprehensive approach is required that includes also modeling, safety assessment and identification of the pesticides, which further will be the determining factor when choosing a method of destruction for the particular region. We offer an economic-mathematical approach to use ecological-economic models, taking into account particularly the density and type of contamination of OPs in synergy with pure economic factors.

Keywords Obsolete pesticides • Environmental security • Sustainable development • Model

4.1 Introduction

> Appeal to the industrialists and politicians of the world: if you please. Stay the Planet alone, similar to that how it was when you got it.
>
> (F. Beigbeder)

The widespread use of pesticides in agricultural activity has led to the fact that all countries in some extent have problems with pesticide wastes. In highly industrialized countries (Europe, North America), the problem of pesticides wastes are mainly related to waste water, recycling, and (or) the elimination of packaging

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(containers, etc.) after the use of pesticides and the remediation of contaminated soils. For developing countries, the main problem is the elimination of unused (forbidden) and unusable pesticide stocks [2, 15, 22].

Thus, environmental pollution by pesticides – one of the major agro-ecological problem – therefore there is degradation of soil, violated environmental and productive functions of soils, reduced productivity and deteriorating quality of plant and animal products. Particular danger goes from prohibited for the use obsolete pesticides (OPs) [20]. The gap, which has formed between the volume of the waste OPs accumulation after agricultural activities and the measures to prevent its formation, expansion of recycling, disposal and removal OPs, deepens the environmental crisis giving it a progressive nature and is an inhibitory factor for the economy of the region. Therefore urgent environmental problems of recycling and disposal of OPs becomes important for solving and appears to be global challenges. So, it is necessary to choose the right direction to solve this problem and to take into account all impact-factors, including environmental safety, project costs, and environmental, social, legal risks. As any region that has OPs on its territory faces the economic aspects of this problem – costs on re-packaging, storage stocks and remediation/utilization. Most such costs are clearly seen for majority population of the region, than even ecological damages which volume as well is calculated mostly in money indicators (costs). So the assessment tools that could cover all practical aspects of OPs management for a region is in demand from ecological point of view and under terms of sound economic development strategy of the region.

4.2 Ecological-Economic Synergism: Ecology, Economy and Environment

Transition to the “environmental” type of economic development requires a review of priorities, fundamental restructuring of the global economy and the transition to a qualitatively new stage of international cooperation [14]. If in the future the support of conditions for environmentally sound development does not become the primary concern of government, then the ongoing destruction of natural systems that provide the economy brings to naught all their efforts to improve the human life condition. One of the components of solutions to global environmental problems is the need to develop a set of measures for environmental safety and its consistent implementation at various levels – from local to global [12, 13].

Currently the prime position belongs neither the economy nor the ecology itself but their mix, synergetic combination of effects and impacts, so called ***ecological economics***. Ecological economics is referred to as both a transdisciplinary and interdisciplinary field of academic research that aims to address the interdependence and coevolution of human economies and natural ecosystems over time and space [9]. The global engine works in the interdependence of economic laws and ecological challenges, what results in the more broad term – Environment (Fig. 4.1).

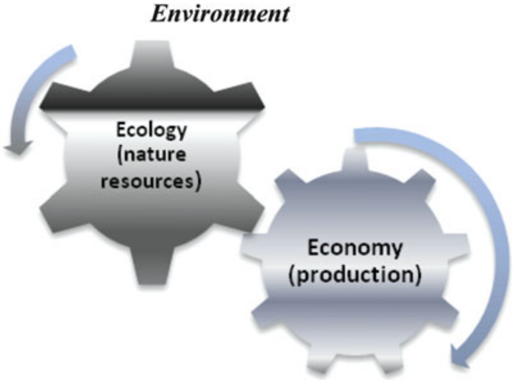

Fig. 4.1 The synergism of ecology and economy results to environment

Healthy ecosystems are the foundation for sound economic development, sustaining and enhancing human life with services ranging from food and fuel to clean air and water. Ecologically sustainable development must maintain ecosystem resilience – the continued ability of ecosystems to provide future generations with easily access to various services (conveniences) in spite of natural and human-driven disturbances. Many current ecosystem management strategies are unsustainable, focusing on a single service/convenience – such as the production of food, fuel, or fiber – in the neglecting of others. Such strategies can reduce biodiversity and ecosystem resilience by eliminating native species, introducing new and harmful species, converting and simplifying habitat, and polluting the surrounding environment.

The agriculture has the tensest connection to the Nature in comparison with other economic fields. From the one side, it uses natural resources (lands, water, etc.), from the other hand – it gives food so the resources for reproduction of human population. But currently, there is a tendency that more yields to agricultures reflects in more ecological damages.

4.3 Obsolete Pesticides and Their Specific Features in Aspect of Its Management and Estimation

Pesticides are the classic example of the twofold nature of the Environment as we termed it: here we can clearly see the dichotomy of the ecological-economic process. A man resorts to the help of pesticides in order to save the crop grown. However, pesticides affect the ecology in damaging direction. On the cost side of pesticide use there can be a cost to the environment and human health, as well as the cost of the development and research of new pesticides (Fig. 4.2).

But the usage of pesticides brings not as much damage to the ecology, than the obsolete pesticides and persistent OPs (POPs). That’s why the accumulation of OPs

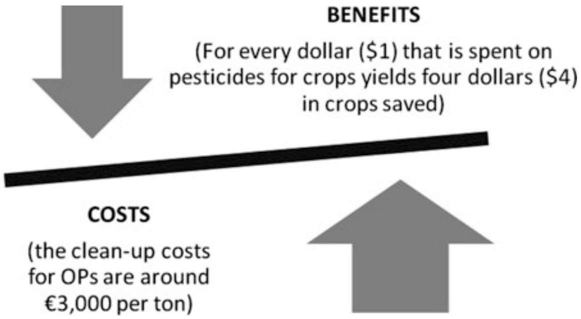

Fig. 4.2 Dichotomy effect of OPs: cost weights (Data source: Pimentel [17], Pimentel et al. [18])

became a twenty-first century challenge. According to the United Nations' Food and Agriculture (FAO), in developing countries now have accumulated about 100,000 tons of obsolete pesticides (a large group of banned pesticides (so-called "dirty dozen") and a group of POPs). Some pesticides have accumulated for 50 years. The main factors that have led to the accumulation of unusable stocks of pesticides in developing countries are the following:

- Inadequate storage and inventory management;
- Improper handling during transportation;
- Creation over-storage of pesticides;
- Reservation of resources in the event of the need to destroy large quantities of insects;
- Prohibiting the use of certain pesticides;
- The purchase of inappropriate formulations of pesticides;
- Poor quality of purchased pesticides and lack of quality control;
- Excessive donor funding and poor coordination among the sponsoring agencies;
- Change of national policy.

But despite the OPs damages there is other source of dramatic side affect – storages of OPs. Thus each stage of the technology of pesticides leads to waste.

Such complex problematic of OPs remediation and utilization reflects in not simple techniques of its management and assessment. The investigation of environmental contamination by OPs consists of three main parts [1]:

- Determination of the emission sources,
- Sampling/measurement,
- Modelling.

The possible strategic plan for the management choice to OPs utilization could be as it is shown on Fig. 4.3.

The modeling of OPs management aims mainly, as any modeling process, to create the model with maximum adequacy to the original process (object). So such model should be homorphic reflection of the object to the reality, which is modeled. According to the main principals of modeling, the OP's management as process is considered as a "black-box", so the classic procedure should be applied:

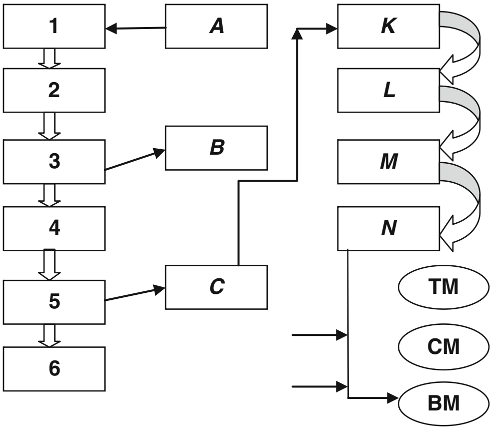

Fig. 4.3 The possible strategic plan for the management choice to OPs utilization, where: *1* – modelling of soil (water, etc.) contamination and assessment of environmental safety, *2* – identification of the OPs, *3* – usage/processing, *4* – processing/destruction, *5* – options filtration, taking decision process, *6* – failed options, *A* – identification of OPs (POPs) storages, *B* – preservation, *C* – possible option for taking decision management, *K* – human safety and ecology security (risk management), *L* – factors of risk management (political, social, economic, legal), *M* – costs (project costs), *N* – elimination method, which is preferred, *TM* – thermal method, *CM* – chemical method, *BM* – biological method

- identification of controlled variables;
- choosing choice criterion function for variables' problem;
- choosing restrictions via variables' problem.

The specific approach to the OPs assessment and management goes from the complexity, multi-criteria aspect of the problem as well as the necessity to use not only objective calculated variables but also the subjective ones, like the expert estimation, binary data, dummy variables, stochastic variables.

So taking in the account the specific features of OPs for the management and assessment aims, the ecological-economic estimation methods for the case of OPs belongs to:

- applied methods (mission criteria),
- stochastic methods (criteria of cause-and-effect net reflection),
- dynamic methods (criteria of time reflection),
- non-linear (criteria of mathematic forms of interactions),
- aggregated (macro-ecological-economic models) (criteria of resolution).

So the techniques for OPs analyses should be applied in the correspondence with said above. Models are thus useful for testing the hypotheses on the complex system under consideration. By means of a sensitivity analysis, models show the most influential factors that should be thoroughly investigated. Finally, such models guide our understanding of complex systems that are not fully accessible in the real scale [16].

4.4 Models and Economic-Ecological Methods of Estimation: Application for the Case of Obsolete Pesticides

The most popular methods in the application to OPs analysis are box models and risk assessment. In addition to the box models, atmospheric transport models with much higher spatial and temporal resolution are also being used for investigating the environmental fate of POPs [21]. Multimedia models describing the global transport and fate of POPs basically serve two purposes [1, 19]:

- understanding of the environmental processes leading to global contamination through POPs and development of the indicators that would allow a clear definition of the constituents of a POP (this can be achieved with idealized release scenarios such as a single point source);
- assessment of the actual risk to the environment and human health, requiring the determination of environmental concentrations, based on reliable emission estimates with a view to toxicity thresholds.

Risk management. Analysis and evaluation of risks in technological safety is the foundation of safety management system of technical and technological systems of different types and levels [8]. The following major tasks are under consideration:

- study goals and objectives of the risk analysis,
- analysis of technological features of production facility and identification of all sources of possible hazards,
- identifying events that could initiate an accident occurring,
- the formation of plausible scenarios of accidents,
- scenario analysis,
- evaluation of the probability of failure for each event that triggers an accident;
- determination of damage factors,
- modeling and predicting the extent of consequences of accidents for human population, environmental scenarios of accidents;
- evaluation of probabilities of external factors that do not depend on the operating conditions of potentially hazardous objects;
- evaluation and risk analysis regarding its admissibility;
- construction of the potential risk field around each of the selected hazards,
- identifying the adequacy of preventive measures to ensure the stability of objects to internal and external impacts.

This list of major tasks look rather general but this approach allows using quantitative safety numbers similar in mathematical terms to the indicators of reliability theory, methods which are sufficiently developed and widely used in practice for the OPs management.

Special approach of the case of OPs reflects that risk assessment should be applied not on the level of OPs identification and following utilization but not less on the level of storage and re-packing, so called *risk assessment of obsolete pesticide storage sites*. Basically all the former pesticides storage sites present a potential contamination risk, but the volume and extent vary a lot. On the basis of the data on the pesticide storage sites the methodology for the risk assessment of the obsolete ones helps develop a priority list of the sites which are potentially most hazardous to the environment and need to be properly managed. According to the methodology each pesticides storage site is assessed by [1]:

- technical condition;
- pollution potential;
- factor of groundwater vulnerability;
- land use and environmental value;
- potentiality of pollutant to access the water reservoirs;
- data on the soil and/or groundwater contamination.

The aim of the Environmental risk assessment tool in its application for the case of OPs is to gather sufficient information to provide decision-makers, experts, project managers and planners with the data necessary for:

- ranking the stores and characterizing the situation in each according to simple environmental and public health risk criteria;
- pinpointing the magnitude of the risk and prioritizing practical actions and measures for reducing it;
- devising an implementation plan based on priority actions for the disposal of OPs;
- pre-selecting intermediate and regional collection centres for OPs, for which a separate assessment may be required under national risk assessment procedures;
- final selection of centres is based on additional logistics, economic, public, social or political factors.

Besides, existing computer models of fate of OPs are helpful tools because they make it possible to combine theoretical description of different processes into a consistent mathematical framework that is open to expansion and adoption if new processes are to be included. Models are also the tools supporting the decisions taken while implementing Stockholm Convention requirements. The tools based on a systematic and integrated approach to the POPs management difficulties in the region are deficient [1].

4.5 Model

There exist many different models for the assessment of fate of OPs in the environment; however, the model which could help systematically assess the OPs management situation in a country and develop integrated measures for the OPs risk reduction is deficient.

The aim of the paper is to offer reasonable, from a theoretical and methodological point of view, method of the OPs assessment based on the ranking assessments of its components (toxicity, life-time, storage conditions). Logic of method's developing defines the following stages:

(I) Draft components of the OPs assessment.
(II) Identify the conceptual range and clarify categories.
(III) Match a system of indicators and methods for OPs assessment.
(IV) Conduct a comprehensive and systematic monitoring of the OPs with further developing of strategies to strengthen state's ecological policy in its synergism with economic sustainable development.

As to 1st stage: analysis of the structure and function of the various classifications shows that the number of indicators (components) included in the classification is calculated from a few to dozens of tests, which reflects the state of science in this period of time. This was especially evident in the last decade due to severe growth trend of indicators of process of interaction between pesticides (OPs particularly) and the human body.

In the structure of the classifications are essential:

- Informative test;
- Approach to ranking the indicators;
- The assessment of hazard class.

Criteria for selection of statistical indicators for further calculation the assessment rating of OPs of regions (states) are following:

1. indicator must be relevant, adequate to the realities;
2. indicator should have a clear quantitative expression, be compact, dynamic, and relatively predictable;
3. indicator should be available for rating-making person. This requirement is, firstly, because of the need to save time and financial costs for monitoring, and secondly, guarantee reliability and adequacy of the results;
4. ability to get "fresh" data for monitoring areas (monthly, quarterly, semi-annually, annually).

The stock of factors can differ according to the aims of the analysis and further decision making but the core factors-indicators could be as shown in Table 4.1.

So the challenge is to model and to optimize (minimize) the function of possible costs from ecothreats and ecoviolence (case of OPs). If to put the latter in math symbols, we can propose some form of function:

$$\mathrm{F(E)} = (\text{ecological impact, economic costs}) \rightarrow \min .$$

There should be four main steps within the complex model on three levels:

1. state level:
   - assessment of the OPs management situation in a country (including assessment of OPs distribution in the environment, wastes, products and POPs pollution sources) [1];
   - identification and prioritization of OPs management problems in a country [1];

Table 4.1 Possible environmental, economic and social factors for the model

Environmental factors							Economic factors		Social factors		
Diseases	Impact on living organisms	Soil pollution	Air pollution	Water pollution	Waste reduction	Use of natural resources	Investment demand	Risk costs	Innovations	Storage indicators	Image improvement (informative component)

2. region level:
    - development of integrated measures for the solution of prioritized problems and OPs risk reduction [1];
    - the first step of the model should be an assessment of OPs current situation in a region. The principal aim of the model application being the reduction of the OPs risk in a region, the result of its application is a developed actions plan;
3. storage level:
    - evaluation of the effectiveness of chosen measures in regard with the identified indicators (environmental/economic) [1].

So **the Algorithm for estimation of safety (reliability) of OPs contamination** could be proposed:

1. Analytically calculate the probability of different types of system states:

    H_0 – serviceability of all sub-systems;
    H_1 – refusal of exactly one subsystem,
    H_k – refusal of exactly k subsystems.

2. The probability of H_k event calculated using the binomial distribution:

$$P_k = C_n^k q^k p^{n-k},$$

   where p – the probability of subsystem operability ($q = 1 - p$).
3. Using the method of statistical tests we can estimate conditional probabilities Φ_κ – that indicate the operation state of the system. Calculate the total probability of operability of the system:

$$h = h_0 + \sum_{k-1}^{n} \Phi_k P_k.$$

4. If the system is constructed so that the refusal j any element does not lead to system failure in general then the full probability of system operability is given by the formula:

$$h - \sum_{k-0}^{j} P_k + \sum_{k=j+\mathrm{L}}^{n} \Phi_k P_k$$

   The bigger j, the higher efficiency of analytical and statistical evaluation method's implementation.
5. In quantitative terms the risk is determined by the conditional probability of causing harm to humans (ecosystem) from OPs (W(I)) and by the probability of occurrence of adverse events (P(I)) and is calculated by the formula:

$$R = \sum_{i=1}^{m} [W_i(I_i])P_i(I_i)$$

where m – the amount of hazards (I) of the same level.

6. In the current risk assessment methodology risk assessment for human health and life under OPs exposed better to use logarithmic-normal distribution of the form:

$$R(Prob_i) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{Prob_i} [\exp\left(-\frac{t^2}{2}]\right) dt$$

7. The upper limit of integration is the so-called probit function ($\mathrm{Prob_i}$), which reflects the relationship between the probability of lesions and absorbed (influential) dose (concentration). For its calculation we use the logarithmic dependence:

$$Prob_i = \alpha_i + \beta_i \lg C_i,$$

where α and β – parameters that depend on the toxicological properties of the substances (OPs) and types of effects, C – the concentration of harmful substances (OPs).

8. So we can calculate for the any state (region) the probit-panel-econometric model:

$$\mathrm{I} = \mathrm{f}(\mathrm{Prob} * \mathrm{Dens}, \mathrm{Gov}, \mathrm{PP}),$$

where I – costs of the utilization, Dens – density of OPs relocation in the state, Gov – costs on governmental project implementation, PP – the price (net cost) of the OPs utilization itself.

For complex modelling process that is proposed here, all statistical indicators of OPs (regions, countries) should be systematized due to three components of assessment: human safety and ecology security (risk management) – toxicity, factors of risk management (political, social, economic, legal), economic costs (on detection, elimination and taking decision procedure itself), which in its turn include such relevant factors (mostly binary variables [3, 4]):

- Storage conditions: management procedures,
- Storage conditions: safety,
- Environmental conditions: hazards affecting the store,
- Environmental conditions: human settlements,
- Environmental conditions: water sources and soil,
- Environmental conditions: agriculture, livestock activities, wildlife and biodiversity,
- Store conditions,
- Storage conditions: content of the store,
- Store conditions: security.

Table. 4.2 Procedure of calculating an integral assessment of OPs

# of a stage	Description
Stage 1	Selection of statistics: $\{x_{ni}\}$
Stage 2	Standardization of indicators: $x^*_{ni} = \frac{x_{ni}}{x_n}$
Stage 3	Correlation analysis (ρ) as a criterion for selection of indicators: $\rho \geq \pm 0.5$, with exclusion of multicollinearity
Stage 4	Establishment of the database $\{x^*_{ni}/\rho_{x^*ni} \geq \pm 0{,}5\}$ – adjusted and standardized data set
Stage 5	Clustering of objects by each indicator using k-means cluster analysis. Considering singularity of the region, each factor evaluating the option is given the value from 1 to 5. The more the option is friendly to the environment of the region, the more it is acceptable economically and socially, the more points it will be given
Stage 6	Application of the author's ranking technique for objects on the level of factors
Stage 7	Apply authorial ranking system for giving an integral assessment to each object at the level of three integral components of storage, region, state (human safety and ecology security (risk management) – toxicity, factors of risk management (political, social, economic, legal), economic costs (on detection, elimination and taking decision procedure itself)), putting a certain place in the ranking to each object
Stage 8	Calculation of the effectiveness of OPs management realization for the object (E) as the ratio of its costs to its potential hazard
Stage 9	Presentation of the results, application of a taking decisions approach to develop ecological strategy for further positioning and elimination of rated objects

As well, in order to make an optimal (benefit-cost) decision on how best to eliminate stockpiles of OPs there is a requirement of data of the qualitative and quantitative composition of the waste.

We offer such procedure for calculating an integral estimation of OPs based on formalizing of operations of comparison, carried out in nine stages (Table 4.2) [10, 11].

Our approach can be considered as adequate to the stated objectives of the study. Not engaging of subjective "expert weights" are the clear advantage in favor of the use of author's ranking system for integral assessment of OPs, because of undoubted objectivity of the results.

From other hand, it's always possible to include the expert estimation to the objective result of monitoring: to define a subjective assessment (weighting) factors $(\mathrm{T} - \lambda_1)$ and $(\mathrm{C} - \lambda_1)$ for a criterion of the test system, which has the form:

$$\mathrm{E} = \lambda_1 \mathrm{T} + \lambda_2 \mathrm{C},$$

where T – time (duration of the test), and C – the cost of testing; λ_1, λ_2 – weights determining influence of T and C on the effectiveness of the tests. We can expect that λ_1 and λ_2 will vary according to the setting in which are used the issue and the degree of its influence on the identity of the expert. Thus, the authorial method, despite its simplicity, makes it possible to receive in-line rating product.

4.6 Conclusions

The basis of an extensive factual material proves that all, without exception, global economic problems of today have a definite ecological context. Environmental security cannot be ensured only by means of environmental actions in isolation from social, economic, political and demographic problems. They are all so interconnected and interdependent, that the solution for each of them can be found only in their joint consideration. In future challenging and essential should be issues of not recycling of OPs but preventive methods, such as actions against excessive use of pesticides, promotion of sustainable agriculture and environmentally safe integrated plant protection (IPM).

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Chapter 5
Exposure Assessment to Hazardous Pesticides – Strategies to Reduce Human and Environmental Risks

Maria de Lurdes Dinis and António Fiúza

Abstract In a generic way, obsolete pesticides can be defined as those that can no longer be used for their intended purpose or any other purpose and thus need to be disposed. Almost every developing country and economy in transition has stocks of obsolete pesticides. Due to many factors, obsolete pesticides have been stored under conditions that do not meet safe and responsible requirements, posing a significant risk of leaking. The assessment of the storage and containment conditions for obsolete pesticides implies a site-by-site evaluation in order to detect these eventual leakages. Leaked pesticides will be dispersed in the environment by wind, evaporation, spillage into water, surface runoff or leaching through the soil. The exposure effects to humans and animals will be similar to non-obsolete pesticides, although the chemical composition is different from the original product. Strategies to reduce human and environmental exposure require more that an isolated exposure assessment and it is not possible to standardize "how to eliminate pesticides obsolete stockpiles", as a site by site approach is needed. Regardless of the local legislation, environmental condition and diversity in the techniques that may be applicable for particular sites at different countries, there are four basic steps that should work as a start to the obsolete pesticides safe containment and removal process. An inventory is required as first, followed by a risk characterization, site stabilization and finally the disposal. This work will focus in particular on the methodology for the inventory and for the risk characterization such as the site stabilization and disposal steps for all obsolete pesticides stockpiles rely on these two key activities.

Keywords Obsolete pesticides • Storage and leakages • Exposure • Human and environmental risks

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5.1 Introduction

Obsolete pesticides can be defined as those that can no longer be used for their intended purpose or any other purpose and thus need to be disposed. The definition includes pesticides that have past their expiry date, banned pesticides, unidentified pesticides products, damaged and degraded products, buried pesticides and containers.

Over the years, a significant amount of obsolete pesticides have been stockpiled all over the world. Due to many factors, obsolete pesticides have been stored under unsafe conditions and substandard requirements posing a significant risk of leaking, leading to human and environmental exposure. Pesticide stockpiles in these conditions require urgent action.

In general, the problem does not concern the use of pesticides but the pesticides that have not been used and became obsolete and the associated risks resulting from their inadequate management and storage. The problem dates back to the 1950s and 1960s when the use of pesticides was increased in order to raise agricultural production. Pesticides were distributed free of charge to farmers, leading not only to overuse but also to unsound management of residuals and packaging materials [12]. And if the storage sites were once located away from residential areas they are now surrounded by urban communities where people living and working nearby are exposed to these obsolete pesticides and suffer consequent health problems.

The exposure effects to humans and animals will be similar to the one originated by non-obsolete pesticides, although the chemical composition is different from the original product.

The assessment of the storage and containment conditions for obsolete pesticides implies a site-by-site evaluation. Regardless of the local legislation, environmental condition and diversity in the techniques that may be applicable for particular sites at different countries, there are four basic steps that should work as a start to the obsolete pesticides safe containment and removal process.

An inventory is required as first followed by a risk characterization, site stabilisation and finally disposal. The inventory will allow determine which products should be categorized as obsolete pesticides and which are usable. The risk derived by obsolete pesticide stockpiles is a combination of toxicity or hazard of the product and an exposure assessment. The site stabilisation has as purpose to decrease the environmental contamination, reducing both risks and accidents. The disposal represents the solution for those products that can no longer be used for their intended purpose and cannot be reformulated to become viable again.

This work focuses in particular on the inventory and on the prioritization of the stores and regions based on environmental risks characterization as the site stabilization. Disposal steps for all obsolete pesticides rely on these two key activities: inventory and environmental assessment. A step by step methodology for a risk assessment, specific for obsolete pesticides stockpiles, is outlined here. Data on the store (location, structure, management and organization) are combined with information on obsolete pesticides (quantity, toxicity and packaging conditions) and ranked according to the risk calculated both to the store and to a region in a comparative criterion [8].

5.2 The Legacy Problem

Stocks of obsolete pesticides occur in most of the developing countries and economies in transition. It is estimated that about 500,000 t are stockpiled worldwide [3], half of which are located in countries of the former Soviet Union. In the African continent, the total obsolete stocks is about 50,000 t, while Latin America has at least 30,000 t [5–7, 13].

The exact quantity of obsolete pesticides is unknown as many of these products are very old and documentation is often lacking. Many times the problem is only known when an inspection is conducted and the real situation is brought up; in October of 2011, a mission to Mongolia from several international pesticides organizations discovered obsolete pesticides stocks stored under substandard conditions in different locations in the country. The scale of the problem, however, is difficult to determine without a further research [11].

Obsolete pesticides are placed mostly at 10,000 locations of the former Soviet Union, the Southern Balkans and new EU member states [12]. According to FAO [9], in Central Europe, the highest amounts of obsolete pesticides are placed in the Russian Federation (100,000 t); Macedonia (38,000 t); Ukraine (25,000 t); Uzbekistan (12,000 t); Belarus (11,000 t); Kazasthan (10,000 t). Outside Central Europe, about 27,400 t are placed in Africa; 6,500 t are placed in Asia; 241,000 t in Eastern Europe and 11,300 t in Latin America and the Caribbean area.

5.2.1 Obsolete Pesticides Occurrence and Exposure

There are many factors that may have led to the accumulation of obsolete pesticides in developing countries : (i) product bans; (ii) inadequate storage and poor stock management; (iii) unsuitable products or packaging donation or purchase in excess of requirements; (iv) lack of coordination between donor agencies and commercial interests of private sector and hidden factors [8, 13].

Obsolete pesticides result from the degradation of non-obsolete pesticides and the resulting by-products are usually more toxic than the original product. Obsolete pesticides are chemically complex as about 1,000 of active ingredients compose many thousands of pesticides formulations.

Persistent Organic Pollutants (POPs) represent more than 20 % of the obsolete pesticides stocks worldwide. In particular, chlorinated hydrocarbons (organochlorides) persist in the environment are highly toxic and bioaccumulate in humans, wildlife and fish. Besides to POPs, obsolete stocks also include organophosphates (less persistent but more toxic than POPs), carbamates and synthetic pyrethroid insecticides, fungicides and herbicides, and even botanical and microbial groups [10].

Over time obsolete pesticides often leak from corroded or otherwise damaged containers into the surrounding environment which is the main pathway for contamination. The resulting environmental hazards will be caused by the dispersion in soil, leaching into the groundwater through the contaminated soil, surface water contamination by surface runoff and wind dispersion of pesticides dusts or pesticide contaminated soil particles and widespread through natural disasters like hurricanes and floods [1].

While the stockpiles remain where they are and continue to leak, any damage caused will increase. The unsafe storage also leads to vandalism: these products are repackaged, provided with a new label and sold at regular markets; the theft of products often occurs as well as illegal digging and burning at large burial sites and these sites are easily accessed by children to play in obsolete pesticides sites [3].

People living and working nearby may be exposed to these pesticides suffering acute or chronic exposure. Long-term exposure have been associated with a range of adverse health effects from problems of the nervous, immune, reproductive and endocrine systems causing birth defects, injury of a specific organ body and cancer. The short-term acute effects are usually associated with nauseas, headaches, sore eyes, skin rashes and dizziness.

5.2.2 Progress on Obsolete Pesticides Sites Clean-Up

5.2.2.1 European Union Member States

The common practice in European member countries is to “return to sender” the unused or outdated products whereby the ownership of any obsolete stocks is clearly defined. The EU law obliges the producers to manage the obsolete pesticides, including their collection and destruction. This extends to new member states under the enlargement process [12].

The principal international agreements that regulate obsolete pesticides are the Stockholm Convention on Persistent Organic Pesticides 2001 (POPs), the Basel Convention on the Control of Trans-boundary Movements of Hazardous Wastes and their Disposal (1989), the UNECE Convention on Long-Range Trans-Boundary Air Pollution (1979 and the 1998 Protocol on POPs) and the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals (1998).

In particular, the Rotterdam Convention is a global treaty upon pesticides and industrial chemicals that are banned or severely restricted in participating countries. Besides the exchange of information on those chemicals between the countries it gives them the right to refuse imports of certain chemicals which they cannot manage safely. The EU legislation covers all countries whether they are part of the convention or not and includes a broader range of chemicals [4].

The Stockholm Convention only bans the use of a selected number of POPs and barely addresses obsolete pesticides. In the EU, obsolete pesticides are regulated by Regulation (EC) Nº 850/2004 of 29 April 2004 which complements earlier Community legislation on POPs. To a certain extent, this regulation goes further than the international agreements emphasizing the aim to eliminate the production and use of the internationally recognized POPs. The Regulation contains provisions regarding production, placing on the market and use of chemicals, management of stockpiles and wastes, and measures to reduce unintentional releases of POPs [4].

5.2.2.2 Non-European Union Member Countries

For non-EU member states the problem has been and continues to be an issue. In these countries, national legislation is less developed, ownership of land is not always defined, producers have disappeared or cannot be held accountable and infrastructure for effective remedial treatment is non-existent [12].

However, a few actions have been taken: in the framework of the Arctic Council Action Plan to Eliminate Pollution of the Arctic (ACAP), around 2,000 t of obsolete pesticides have been repackaged in North-Western Russia; in 2002 Albania received financial support from the EU PHARE programme to eliminate all obsolete pesticides; the World Bank has initiated the cleanup of around 2,200 t of POPs, PCBs and soil-contaminated with PCBs, in the Republic of Moldova which was concluded by the end of 2007; the Dutch Ministry of Foreign Affairs has financed a project to eliminate acute risks of obsolete pesticides in Moldova, Kyrgyzstan and Georgia from 2005 to 2008; in 2011 a consortium of the International HCH and Pesticides Association (IHPA), Milieukontakt International and the independent legal expert Helle Husum was established to conduct a pilot remediation of obsolete pesticides sites in Vietnam [11]. Still, the main problem arises from the fact that activities remain partial as there is no systematic approach across all countries concerned and not all potential sources are targeted.

5.3 Measures to Reduce or to Mitigate Exposure to Obsolete Pesticides

Strategies to reduce human and environmental exposure require more that an isolated exposure assessment; it is not possible to standardize “how to eliminate pesticides obsolete stockpiles” as a site by site approach is needed.

Regardless of the local legislation, environmental condition and diversity in the techniques that may be applicable for particular sites at different countries, there are four basic steps that should work as a start to the obsolete pesticides safe containment and removal process.

As first an inventory is required, followed by a risk characterization, site stabilisation and finally disposal. The inventory will allow determine which products should be categorized as obsolete pesticides and which are usable. The risk derived by obsolete pesticide stockpiles is a combination of toxicity or hazard of the product and an exposure assessment. The site stabilisation has as purpose to decrease the environmental contamination, reducing both risks and accidents. The disposal represents the solution for those products that can no longer be used for their intended purpose and cannot be reformulated to become viable again. The following sections will focus on issues related to the inventory and the prioritization of stores based on environmental risk.

5.3.1 Inventory

The inventory is an accurate record of the pesticides in stocks, enabling to determine which products should be categorized as obsolete pesticides and which are usable. A comprehensive inventory of obsolete pesticides stocks is important in order to understand the scope and nature of the problem. Cleanup and safe disposal of obsolete pesticides have high costs therefore the interventions must be prioritized on the basis of a detailed inventory of pesticide stockpiles and contaminated sites. An inventory should be reliable as it is the solid basis for planning, budgeting and executing removal activities determining the identity of the contaminant, its proximity to people and to the environment. It should be based on actual field data gathered at every site where stocks exist. The collected information should then be inserted in the Pesticide Stock Management System (PSMS), a web-based application developed by FAO used to record, monitor and manage stock of pesticides, including obsoletes.

5.3.2 Risk Characterization Methodology

Risk assessment of pesticide stockpiles impact on human health is not an easy and accurate process due to the differences in the exposure period and frequency, type of pesticides (toxicity) mixtures or cocktails, geographic and meteorological characteristics of the sites where stockpiles are located. Such differences affect mainly the population that lives near the stockpiles storage facilities with obsolete pesticides leakage [2].

The difficulties in assessing risks of obsolete pesticides on human health and the complexity make the usual approach for risk assessment a very hard task to apply to obsolete pesticides stockpiles. In particular, the magnitude of the exposure to obsolete pesticides depends on the chemical properties of pesticides, their toxicity and storage conditions in addition to the exposure characteristics. As these factors vary from site to site and from year to year, the results from any field study, on the fate and behaviour of the pesticide are specific for any particular location and season. Moreover, when dealing with obsolete pesticides in such a huge scale of the stockpiles it is absolutely necessary to consider the risks resulting not only from the pesticides' chemical toxicity but also from the storage conditions [2].

In this context, the Food and Agricultural Organization (FAO) has developed a tool specific for obsolete pesticides stockpiles prioritizing sites and regions which should receive prior attention. The tool provides a set of practical methodologies to develop a risk based management of obsolete pesticides based on a very objective chemical and environment criteria that can be used to develop an effective environmental management plan for obsolete pesticides. It is a system for prioritizing affected storage locations based on the comparative risk posed by each of the locations where obsolete pesticides are currently stored [8].

This tool was based on real situations experienced in locations where obsolete pesticides are currently found and validated by FAO with extensive field tests in several countries. The methodology considers three major components for the risk characterization. The first one collects and analyses additional information in order to appraise the environmental and public health risks associated with each stock of pesticides (risk assessment). The second one identifies the most dangerous stores by ranking them according to environmental and public health priorities based on risk assessment (prioritization of the stores). The last one provides a methodology for identifying, classifying and selecting the regions where stocks of obsolete pesticides are accumulated, which should receive prior attention (regional prioritization) [8].

5.3.2.1 Risk Assessment

The main goals of the risk assessment are to collect information and calculate two risk factors to characterize each site.

The first step is to gather information on the store: its location (region, district, map coordinates and altitude); the structures of the store (roof, walls, floor and ventilation); management and organization of the store (security, safety and management procedures).

Following, it is necessary to collect information on pesticides located at or inside the store including quantity, the toxicity class according to the World Health Organization (WHO) and the packaging conditions of each pesticide. A list of all pesticides contained in a store should be elaborated.

The collected information will be used to calculate a risk factor (F_P) which represents the risk related to the conditions associated with pesticides (conditions prevailing in the store). This risk factor has a linear progression (the higher the F_P the higher the associated risk) and it is used to rank the stores according to the level of risk related with the pesticides contained in each store, toxicity and packaging conditions. The basic principle relies on the more pesticides contained in a store the more toxic those pesticides are and/or the worst the condition of the packaging in terms of leaking, the higher the associated risk [8] (Fig. 5.1).

The last step is to collect information on the store and on the environment around the store, including proximity to human settlements, water sources, agricultural and livestock activities, wildlife and biodiversity. This information is used to calculate a risk factor F_E based on the assessment carried out for each store. This second risk factor F_E is associated with the pesticides contained in each store considering that the worse structure and the greater store's relationship with or proximity to critical

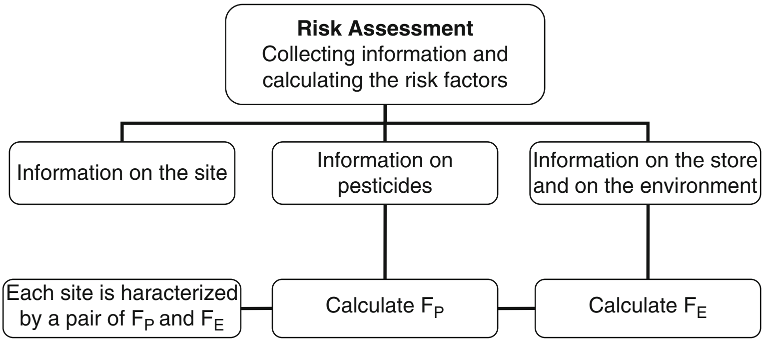

Fig. 5.1 Risk assessment methodology (Adapted from FAO [8])

areas, the higher the risk or potential risk to public health and to the environment, in case of an accident at the store.

In general, for each pesticide identified in a store it is necessary to [8]:

- Assess their quantity (Q);
- Classify them according to their toxic hazard: Ia, extremely hazardous; Ib, highly hazardous; II, moderately hazardous; III, slightly hazardous; U, unlikely to present acute hazard pesticides;
- Score the pesticide toxicity according to the following criteria (S_T): 1 for class U; 2 for class III; 4 for class II; 8 for class Ib and 16 for class Ia;
- Assess the conditions of the containers and give them a score (S_C) according to the following criteria: 1 if none of the containers are damaged; 8 if less than 50 % damaged; and 16 if more than 50 % damaged;
- Calculate the pesticide "*i*" score, S_{Pi} (Eq. 5.1);
- Calculate F_P as the sum of all the S_{Pi} scores (Eq. 5.2) obtained for the individual pesticides in the store.

$$S_{Pi} = (3 \cdot S_T + S_C) \cdot Q \tag{5.1}$$

$$F_p = \sum S_{Pi} \tag{5.2}$$

In Eq. 5.1, the toxicity score (S_T) is multiplied by a factor of three as toxicity class is considered three times as important as the condition of the containers [8].

In the Table 5.1 the calculation of the risk coefficient is presented for all possible situations according to the toxicity and containers conditions scores.

The risk factor F_E is calculated according to nine criteria weighted by a factor to include the relative contribution in the risk assessment calculation [8]:

- Management procedures (max. 4);
- Safety conditions (max. 5);

Table 5.1 Storage containers conditions and toxicity score values

Toxicity score, S_T			Storage containers conditions score, S_C		
			No damage	Minor or moderate damage	Serious damage
WHO	S_T	$3.S_T$	$S_C = 1$	$S_C = 8$	$S_C = 16$
Ia	16	48	$S_P = 49$	$S_P = 56$	$S_P = 64$
Ib	8	24	$S_P = 25$	$S_P = 32$	$S_P = 40$
II	4	12	$S_P = 13$	$S_P = 20$	$S_P = 28$
III	2	6	$S_P = 7$	$S_P = 14$	$S_P = 22$
U	1	3	$S_P = 4$	$S_P = 11$	$S_P = 19$

- Hazards affecting the store (max. 15);
- Human settlements (max. 20);
- Water sources and soil (max. 20);
- Agriculture, livestock activities, wildlife and biodiversity (7);
- Store conditions (max. 20);
- Content conditions (max. 6);
- Security conditions (max. 3).

The most severe weighting factors are applied to store conditions, human settlements, water sources, and environmental hazards affecting the stores. The risk factor F_E is obtained by the sum of these weighting scores and this factor can reach a maximum of 100 which is the worst situation [8].

5.3.2.2 Prioritization of the Stores

The main purpose of this section is to clearly identify the stores that pose a comparatively high level of risk to the general public and to the environment. The methodology allows recognizing the most critical stores based on the calculated risk.

After each store being characterized with the two risk factors F_P and F_E it is possible to identify the most dangerous stores. All available data must be analyzed and used to identify the group of stores that should be considered critical and pose the greatest immediate threat to the public health and to the environment.

The prioritization process is based on a comparative analysis of all the stores studied; however, we must be aware that the analysis does not provide an absolute scale for the risk factor associated with the pesticides. Instead the analysis allows dividing the stores in the following categories [8]:

- **Critical stores**: high scores for both F_P and F_E. In general these stores contain products classified with the most WHO hazardous class, stored in large quantities, badly packaged or in packages that are leaking (high score for F_P). The containment offered by the stores is critical and the dispersion of chemicals would result in a severe impact for the environment or for the general public (high score for F_E).

- **Problematic stores**: high scores for F_E or F_P. A detailed analysis should be done to determine what make them a relatively high risk for either F_E or F_P because the risk of an immediate accident at one of these stores may make the store a high priority.
- **Lower priority stores:** low scores for F_E and F_P. The impact on public health and/or the environment is low. Usually, in this situation, stores contain smaller quantities of less hazardous pesticides that are generally well packed and/or are located in more favorable environments in what concerns to natural accidents or hazards to happen.

A first analysis may be applied to F_E values in order to identify the stores with the worst environmental conditions irrespective of the pesticides they contain. The same analysis may be applied to a modified risk factor ${F_P}^*$ in order to identify the most problematic stores according to the chemicals they contain. The modified risk factor is calculated by the following expression for each store [8]:

$$F_P^* = \frac{F_P}{\max F_P} \cdot 100 \tag{5.3}$$

The risk factors values should then be ranked individually and plotted in a histogram in decreasing order (Fig. 5.2).

In the histogram representation, stores with ${F_P}^*$ higher than 50 % are classified as problematic due to the pesticides that are present: high toxicity, damaged containers and/or large quantities of pesticides. Stores with F_E higher than 50 % are classified as problematic due to conditions of the store, packaging and/or environment. All stores with ${F_P}^*$ and F_E higher than 50 % should be classified as critical [8]. This representation has the advantage that immediately identifies the stores presenting both a very high F_E score and a very high ${F_P}^*$ score.

The next step is to determine the critical, problematic and lower-priority stores. The classification for each store may be easily achieved by plotting F_E and ${F_P}^*$ in a graph. The new risk factor ${F_P}^*$will be the *y* coordinate and the risk factor F_E will be the *x* coordinate. The location of the stores in the graph will classify the stores according to the quadrant of the graph. The stores located in the top right-hand corner of the graph present the highest risk and consequently the highest priority. The stores located at the bottom left-hand of the graph will be classified as lower-priority. The stores located in the two other quadrants will be classified as lower-priority (Fig. 5.3).

When many stores are present at the same location it is necessary to plot the gathered and collected information for each store.

5.3.2.3 Regional Prioritization

This section concerns to the prioritization of the geographic regions by selecting stores where environmental and health risks are at the highest level.

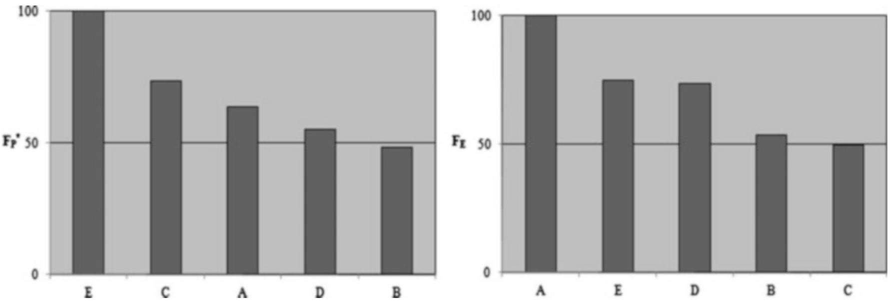

Fig. 5.2 Ranking of the sites according to the F_P^* and F_E risk values

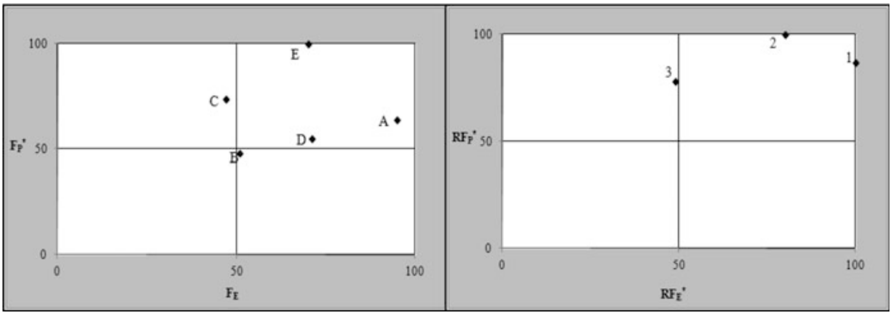

Fig. 5.3 Site and regional characterization according to their priority

Based on the risk factors calculated before, regional risk factors RF_P and RF_E should be calculated for each region in a national level. The regional risk factors, RF_P and RF_E, are calculated by adding the risk factors F_P of all stores and by adding the risk factors F_E of all stores in one region, respectively. These new risk factors will give the cumulative environmental and public health risks associated with all stores existent in a certain region. The regional risk factors should be normalized so that a maximum value of 100 corresponds to the worst scenario [8]:

$$RF_P^* = \frac{RF_P}{\max . RF_P} \cdot 100 \tag{5.4}$$

$$RF_E^* = \frac{RF_E}{\max . RF_E} \cdot 100 \tag{5.5}$$

The two factors RF_P^* and RF_E^* can also be represented in an X-Y graphic to identify the region presenting the worst cumulative and public health risk according to the previous classification: critical, problematic and lower-priority (Fig. 5.3) where the higher the environmental and public health risks, the higher the regional factors RF_P^* and RF_E.

5.4 Risk Characterization Methodology Applied to a Hypothetical Case Study

This section applies the above described methodology on a group of stores, assuming that the first stage of inventory, with the information on the location and types of pesticides that are known to exist, has already been done. A sample of pesticides was used from a real obsolete pesticides stockpile [3] and extended to several hypothetical sites. Five stores have been inventoried (A, B, C, D, E) and the risk factor F_P was calculated using FAO [8] methodology (Table 5.2).

For site A the risk factor F_P is 1,820, for site B is 1,375, for site C is 2,100, for site D is 1,570 and for site E is 2,850.

The risk factor F_E was calculated assuming the following scores for each one of the nine criteria (Table 5.3).

The next step is calculate the modified risk factor (Eq. 5.3), ranking the sites according to the calculated values for both risk factors ($F_P^*$and F_E) and represent them in a histogram (Fig. 5.2).

The ranking of F_E values indicates that sites A, E, D, and B are problematic (F_E is higher than 50 %).

For the regional prioritization of the stores it was considered that stores A, B, and C are located in region 1, stores C and D are located in region 2 and store D is located in region 3. The regional modified risk factors RF_P* and RF_E* are calculated in the Table 5.4.

Both risk factors (R_E and F_P^*) may be plotted in an X:Y graph representing F_E the *x* coordinate and F_P^* the *y* coordinate, for each site (Fig. 5.3). The same representation may be adopted for both regional risk factors (RF_E^* and RF_P^*) where RF_E^* is the *x* coordinate and RF_P^* is the *y* coordinate for each site.

According to this representation sites A, D and E are classified as critical (both F_E and F_P higher than 50 %) and sites C and B are classified as problematic (F_P^* higher than 50 % and F_E higher than 50 %, respectively).

Regions 1 and 2 are classified as critical and these should be the first regions to have an intervention, region 3 is classified as problematic and should be the next one to be remediated.

5.5 Conclusions

In many countries the problem of obsolete pesticides stockpiles remains unsolved. The scale of the problem is still a big issue in a relevant number of European, Eastern European, Caucasus and Central Asian countries. In some locations the situation is described as devastating due to the huge amount of pesticides stockpiled and improperly stored. In 2008 it was estimated that about 260,000 t of obsolete pesticides required urgent intervention with an associated clean-up cost of over 700 million euro [12].

Table 5.2 Pesticides inventory in five stores and risk factor F_P [3]

				Toxicity		Container		Pesticide
	Nº	Pesticide	Q (tons)	WHO	Score S_T	Condition	Score S_C	Score S_P
A	1	Dimecron	15	Ia	18	Minor	8	930
	2	Lannate	12	Ib	8	Serious	16	480
	3	DDT	10	II	4	Minor	8	200
	4	Anteor C3	5	III	2	Serious	16	110
	5	HCB	25	U	1	No	1	100
	F_P							1,820
B	1	Dimecron	3,5	Ia	18	Minor	8	217
	2	Lannate	15	Ib	8	Serious	16	600
	3	DDT	20	II	4	Minor	8	400
	4	Anteor C3	5	III	2	Serious	16	110
	5	HCB	12	U	1	No	1	48
	F_P							1,375
C	1	Decis	50	II	4	Minor	8	1,000
	2	Cuprosan	50	III	2	Serious	16	1,100
	F_P							2,100
D	1	Dimecron	10	Ia	18	Minor	8	620
	2	Lannate	12	Ib	8	Serious	16	480
	3	DDT	15	II	4	Minor	8	300
	4	Anteor C3	5	III	2	Serious	16	110
	5	HCB	15	U	1	No	1	60
	F_P							1,570
E	1	Phosdrin	15	Ia	18	Minor	8	930
	2	Furadan	30	Ib	8	Serious	16	1,200
	3	Novathion	25	II	4	Minor	8	500
	4	Fyfanon	10	III	2	Serious	16	220
	F_P							2,850

Table 5.3 Risk factor F_E for each one of the five stores according to FAO [8] criteria

Criteria	Stores				
(1–9)	A	B	C	D	E
1. Management procedures (max. 4)	4	2	1	3	2
2. Safety conditions (max. 5)	5	3	2	3	3
3. Hazards affecting the store (max. 15)	15	7	5	10	15
4. Human settlements (max. 20)	20	10	8	15	15
5. Water sources and soil (max. 20)	20	10	8	15	9
6. Agriculture, livestock, wildlife, biodiversity (max. 7)	7	5	4	6	6
7. Store conditions (max. 20)	4	2	1	5	3
8. Content conditions (max. 6)	5	5	8	6	3
9. Security conditions (max. 3)	15	7	10	7	15
F_E	95	51	47	70	71

Table 5.4 Regional modified risk factors, RF_P* and RF_E*

Sites	F_E	F_P	F_E^*	F_P^*	Region	RF_E	RF_P	$RF_E^* = x$	$RF_P^* = y$
A	95	1,820	65	57	1	146	3,195	100	87
B	51	1,375	35	43					
C	47	2,100	40	57	2	117	3,670	80	100
D	70	1,570	60	43					
E	71	2,850	100	100	3	71	2,850	49	78

The decision of stores priority in a remediation strategy, when the funding does not reach all the situations in a country, must be based in simple and feasible criteria. However, the most common approach has been based on the assessment of an international expert or consultant, resulting in a relative risk posed by each particular situation but not following a standardize criteria in the prioritization process [8]. This usual risk assessment approach tries to define the magnitude of a particular risk that will be acceptable and while it is focused on known quantities hazards often misses the huge uncertainties regarding both individual sensitive synergies and interactions of multiple exposures. In addition, while there are many studies and regulations on pesticide uses and its effects on human health and on the environment few approaches have been expressly directed to obsolete pesticide stockpiles. In this context, the methodology developed by FAO [8] allows calculating the risk resulting from obsolete pesticides stockpiles in a very realistic way.

This methodology showed to be effective in assessing the relative toxicity risk of stockpiles sites to population and to the environment as an integrated data on "at-risk" population, their proximity to stockpiles and the toxic hazards of the stockpile chemicals. The hot spots analysis shows the priority stores and regions with highly toxic stockpiles and, in this way, the disposal efforts and public resources can be focused on the highest priority areas.

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Chapter 6
Psychosomatic and Psychosocial Aspects of Risk Perception

Vladimir Bencko

Abstract When evaluating perception of environmental risks some psychosocial and psychosomatic factors may be of fundamental importance. This is the case in particular where our knowledge of the true health consequences of exposure to given factor is incomplete or its action is within the range of values where we do not anticipate biological effect. This applies not only in the case of indoor environment related complains but as well e.g. to non-ionizing electromagnetic radiation and electro-ionic microclimate. A serious consequence of the syndrome of mass hysteria is the fact that due to differently motivated disinformation part of the population can really suffer from some psychosomatic symptoms. Those imply objective suffering and deterioration quality of life for those affected.

Keywords Risk perception • Environmental risks assessment • Psychosomatic aspects • Psychosocial aspects of risk • Health an illness • Scientific and social models

6.1 Introduction

6.1.1 Scientific and Social Models of Health and Illness

When contemplating the problem of a healthy environment in relation to a sick one, it is necessary to define the relationship of *health* and *illness* in general. Currently, health is conceived as a condition of physical, psychic, and socioeconomic wellbeing. Contrarily, illness involves an extensive set of different experience or

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behaviour of the affected person. Different experience in the negative sense against the generally accepted standard is implying the deteriorated or endangered subjective condition or social function, feeling of undesirability, of being unwelcome and/or unexpected. The illness induces some activities which aim is an improvement of the condition [6].

Every society responds to such impaired function by charging a number of individuals or institutions, which duties are to evaluate and interpret the actual condition, and provide the necessary measures. Medicine, or more specific public health actors (being an institution and scientific discipline as well) whose representatives are usually the physicians, is expected to react to these social needs. Medicine tries to build up a scientific model of illness, its diagnostics, treatment and prevention, yet this model often is neither identical nor congruent with the social one. There is a difference between *illness* and *disease*, same as difference of views concerning the therapeutic and preventive approach [5].

The priority of the scientific approach is the attempted objectivity and criticism in collecting data and interpreting them. On the contrary, the social model is mostly based on subjective and strongly emotional attitudes. Both, the expert and lay community, are not immune against the harmful influence of myths. Science, however, is closer to truth, but not exceptionally, the science-based as well as lay models tend to misinterpret the situation, as confirmed by history. Science using objective methods is able to reflect its own failures but the subjective views often resist any logical argumentation. Nevertheless, even science operates with some traditional elements. Max Planck had once put it, *the new scientific truth would not win by convincing the opponents, but rather by letting the opponents die, and the new generation then adopts a new, and own truth.*

If rationally removing harmful effects and providing for a healthy living environment we have to consider both the scientific and social aspects, i.e. the views and needs of people living in particular environment.

6.2 Assessment of Ecological and Health Risk Factors/Settings

The assessment of potential ecological and health risks rising from the planned industrial, transport, waste treatment facilities and other construction activities becomes an indispensable part of their audit. Of course, the public health aspect of such activities is no novelty any longer as all above cited projects had legally been controlled and approved by the district or regional public health authorities, within the scope of the preventive supervision.

Whereas the initial phase of risk assessment, its identification or potential human exposure are of pure scientific character, the actual risk assessment increasingly assumes the arbitrary aspects (e.g. safety coefficients), risk communication, its control and management by way of psychological aspects the decision making then becomes a sheer political issue [4]. As illustrating examples we can use problems related to conflicting views concerning e.g. health effect of electromagnetic field and electronic microclimate [9].

The present approach to quantitative risk assessment artificially separating physiologically based pharmacokinetic (PBPK) model and biologically based dose response (BBDR) model needs to be substantially improved. The modelling procedure must go beyond the current organ-tissue based PBPK model as well as the hard-to-modify two-stage BBDR model. It is clear that a model must be flexible and capable of incorporating information about pharmacokinetics and cell signalling response [4].

A limitation of the present approach to risk assessment is low dose extrapolation of cancer incidence data from both animal (experimental) and human (epidemiology) studies that are most frequently based on models that assume linearity atlow doses/exposures. There are situations in which this assumption could be considered unreasonable. However, because of the lack of data and no alternative methodology for risk extrapolation at hand, the model of low – dose linearity continues being used despite existence of qualitative evidence showing otherwise. This is specifically relevant in the case of many non-genotoxic carcinogens modulating mitogenic stimulation or suppression of apoptosis – processes regulated by signalling through its impact on gene expression. Dioxins (TCDD) can serve as example of non-genotoxic carcinogen, endocrine disrupter acting through the Ah receptor. It is a general consensus that to resolve this problem, we need to develop a methodology incorporating biological data on mechanisms operating at cellular or molecular level.

6.3 Psychic Infection and Mass Hysteria

As every expert knows dealing with clients may sometimes bring about a number of both material and psychological problems. Besides, addressing a group of individuals, who, moreover, feel endangered is more complicated still, especially when these groups previously organized in harmony and certain hierarchy start to change in disintegrated ones whose behaviour rather suggest the behaviour of masses or mob. The mass psychology may appear whenever a sufficient number of persons are gathering around one point of common interest.

The psychology of the group never makes a mere sum of the member’s psychology but it has its own individual characteristics. The group as whole, shows better quality than the most inferior members, but the worse judgement and lower IQ compared to the best individuals of the group, and it is prone to getting influenced rather by emotion than reality. Another characteristic is behaviour of the group as a mob (aggressive, panicking, etc.) whose doings are almost always worse than those of an individual [2, 3, 13].

The basic characteristic of mass dynamics is the “psychic infection” due to increased suggestibility responsible for the sensation of symptoms and subsequent chain reactions. A person in the mob then is capable of acts he/she would never have committed as an individual on his own. The cases of mass psychoses are well known from many literary descriptions of “mass hysteria” in real or supposed exposure to the toxic substances, or in health problems symptoms connected with the sick indoor environment (sick building syndrome) usually found in air conditioned houses [7, 10–12, 14].

In such cases the can be considered "objective", i.e. the patient really suffers from them. They remind of symptoms of acute distress but they are less intensive and last for a longer time, e.g. for many days, weeks, or months. The affected is aware of the overall stress and tension, fright, shyness, of sensations of oppressiveness and worries, when addressing other people, and vague stressing uncertainty for the future. All these symptoms are accompanied by chronic fatigue, headache, insomnia and other sub-acute vegetative disorders. As the syndrome is not fully invalidating the patient feels chronically unwell in both his daily duties and his reaction towards other people. Often his capacity of sense making activities becomes reduced as the result of chronic fatigue and impaired concentration.

The symptomatology fully corresponds to the term "somatization" introduced in the ICD-10 international classification. The point is that emotion – here a very strong one – finds its vegetative correlate occurring in the somatic sphere. An important role in further development plays the "interpretative model" of the patient being *xenochtonous* in our case (the cause of all trouble comes from outside) and the patient is aware of it (sick building, nearby radar station, TV tower, waste incineration plant).

The mass reaction can practically manifest by two syndrome levels: in one prevails the state of anxiety, in other prevail motoric symptoms (e.g. the medieval processions of flagellants praying for aversion of pest). The symptoms may appear separately or combined, or occur in turn in the patient. Mass hysteria afflicts men less frequently than women, especially those living in poorer socioeconomic conditions. Mass hysteria is closely connected with the problems of "sick indoor environment" illness. Important here is the firm conviction of outside noxae responsible for any kind of symptoms, further tendency to hypochondria and stress and also hostile attitudes of the patient to anybody to blame for these conditions, in practice materialized by endless weary court trials. In a sense, also collective insistence on UFO and other paranormal encounters belong to this category.

Yet, not all mass-occurring pathological symptoms are mass-hysteria-related. For example the mass poisoning of school children in the school canteen in London can be mentioned, manifested by gastrointestinal troubles shortly after the lunch. The complex microbiological, hygienic, and toxicological examination included a questionnaire for children, which showed a significant link between the symptoms and consumption of raw cucumber (relative risk 6.1). Microbiologically the cucumbers were safe but pesticide contamined, as proved by toxicology. In the discussion the authors warn against any overhasty diagnosis of mass hysteria.

Even when the concentration of toxicants fails to reach the risk values, other factors may be involved, e.g. ambient temperature, air humidity, etc., which have up to now not been included in our models but which are able to objectively influence the clinical course, morbidity and mortality rate [1]. There even may occur combination of the actual infection and mass hysteria. In some people evident hypersensitivity to some substances exists: their pathophysiological reaction then is capable of psychogenic effects on the environment.

Nevertheless, we presume the psychosocial aspects may be of basic importance in understanding the potential health risks. The more so, we can expect such problems when our knowledge of actual health effects of human exposure is incomplete or the intensity of exposure oscillates in levels raising doubts as to possible biological effects [4, 8].

Very serious problems, mostly in psychologically unstable patients, are neuropsychic and psychosomatic symptoms resisting to treatment. Despite the difficulty in objectivization they represent suffering that should not be underrated considering the quality of patient's life.

6.4 The Challenges for Prevention

The prevention of such conditions can either be systematic: early educational or popularisation campaigns, specific health education orientated to the development of industrial, transportation, or other types of constructions, and integration of the local civic activities in the program. The purpose of this should not be a cheap belittling of the risk but reasonable explaining of its acceptable rate, and also advantage likely to come from the realization of the structures. Any later efforts to inform the public about the true state of affairs is normally accepted with distrust or hate, in belief this information had been well-paid by the government, industry tycoon, army, or some other institution trying to camouflage the actual condition.

6.5 Conclusion

It is therefore recommended to carry out a relevant, competent epidemiological pilot study on potential incidence of some health problems (tumours, congenital malformations, etc.) still before starting the structures, to compare – using a set of reliable data, when the building had already been approved for use – the incident phenomenon with the previous conditions. Such a study, of course, is no alibi. In cases of positive findings the study could serve as basis for rational measures to minimize the health risk due to the operation of the particular facility. The concept of health risk minimalization must be included as a leitmotif in all stages of the design and realization, covering all potential risks for the environment and human health.

In medicine, until our days, the Hippocrates' statement still holds: Life is short, and Art is long; the occasion is fleeting, experience fallacious, and judgment difficult. The physician must not only be prepared to do what is right himself, but must also make the patient, the attendants, and externals to co-operate. If we honour this in therapy, we should do so in preventive medicine twice as much [5].

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Chapter 7
Enantioselectivity of Chiral Pesticides in the Environment

Camelia Drăghici, Elisabeta Chirila, and Mihaela Sica

Abstract The study is aiming to present the fate of chiral pesticides, the different behaviour of these optic active isomers in the environment and their ecotoxicological effects, as well as separation techniques available for the chiral pesticides studies. We conclude that there is a tremendous need to enlarge the studies related to the enantioselective behaviour of chiral pesticides in different contaminated environmental components, as well as their ecotoxicity to biota and humans.

Keywords Chiral pesticides • Enantioselectivity • Ecotoxicological effects • Chiral separation

7.1 Introduction

Chirality is a property of some organic molecules to behave like an object that is not superposable on its mirror image. The term comes from Greek language, where χειρ (kheir) means hand, as hands are nonsuperposable objects. The two molecules (Fig. 7.1) are referred as chiral isomers.

Chiral isomers (optic isomers or enantiomers) are organic compounds with identical structures, (same atoms, identically bonded) except for their molecular conformation (+/−, D/L, or R/S) due to an asymmetry element (centre, plane) which provides them identical physical-chemical properties in achiral

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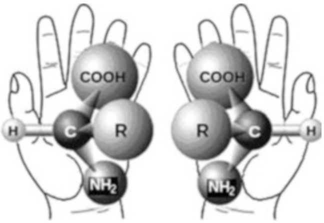

Fig. 7.1 Hands and chiral isomers of a generic amino acid

environments. Enantiomers placed in a chiral environment show different behaviours, both in the presence of a physical chiral media (polarised light plan) or of a chemical chiral one (solvent, reactant or catalyser). A racemate (racemic mixture) consists in an echimolar mixture of both enantiomers.

The biotic environment offers an excellent chiral media, due to the enzymes with protein structure, or to other chiral constituents. The presence of microorganisms in water and soil, or of proteins in plants, determines different behaviour of the chiral compounds, like some pesticides are. They will exhibit enantioselective phenomenon such as adsorption, degradation (biotic and abiotic) and leaching processes.

About 30 % of currently used pesticides are chiral being mostly used as racemates, due to economic reasons; therefore it is needed to define their enantioselectivity, to provide information for improved risk assessment [11]. Studies relieved that pesticide enantiomers have enantioselective bioactivity, toxicity, metabolism, bioaccumulation, and biodegradation behavior, thus altering the racemate, enantiomers ratio [22]. However, the research on enantioselective environmental fate and effects of chiral pesticides is still limited, particularly in the evaluation of enantioselectivity on their environmental impact, as well as their ecotoxicological and health risks [29].

This study will further present examples of studied chiral pesticides and their behavior in chiral media, as well as available methods and techniques for their determination in environmental, food and biological samples.

7.2 Chiral Pesticides and Their Enantioselectivity

Studies related to the environmental fate and biochemical transformations of the chiral organic pollutants, like chiral pesticides, chiral metabolites of polychlorinated biphenyls (PCB), synthetic chiral polycyclic musks, chiral hexabromocyclododecane (HBCDD), and chiral pharmaceuticals are, were reviewed [39]. The author underlined the need of understanding the role of stereochemistry in ecotoxicity, as well as the elucidation of factors controlling environmental fate of pollutants enantiomers.

Figure 7.2 gives the structure of the chiral herbicide dichlorprop, with the tow (*R*)- and (*S*)-enantiomers, where the asymmetric carbon atom is marked, while

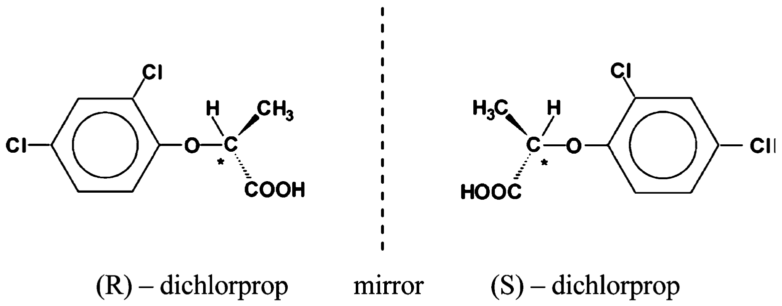

Fig. 7.2 Object and image in mirror of (*R*)- and (*S*)-enantiomers of dichlorprop

Table 7.1 Chiral pesticides from different classes and their use

Chiral pesticides	Organic class	Pesticide use
Metolachlor	Acetamide (acetanilide)	Fungicide, herbicides
Flamprop	Aminopropionic acid	Herbicides
Benalaxyl	Anilide	Fungicide
Diclofop, fenoxaprop, flamprop, fluazifop, haloxyfop, quizalofop-P-ethyl	Aryloxyphenoxypropionic	Herbicide
Simeconazole	Conazole	Fungicide
Imazapyr, imazaquin, imazethapyr	Imidazolinone	Herbicide
Imidacloprid	Neonicotinoid	Insecticide
Chlordane, dichlorodiphenyltrichloroethane (o,p′-DDT), heptachlor, hexachlorocyclohexane (α-HCH, α-lindane), toxaphene	Organochlorine	Insecticide
Bromocyclen	Organochlorine; cyclodiene	Acaricide; insecticides
Fenamiphos, malathion	Organophosphate	Insecticide
Indoxacarb	Oxadiazine	Insecticide
Dichlorprop, fenoprop, mecoprop	Phenoxyalkanoic acid	Herbicide
Metalaxyl, benalaxyl	Phenylamide	Fungicide
Fipronil	Phenylpyrazole	Insecticide
Bifenthrin, cyfluthrin, lambda-cyhalothrin, cypermethrin, fenvalerate	Pyrethroid	Insecticide
Permethrin	Pyrethroid	Acaricide; insecticide

Table 7.1 presents a selection of chiral pesticides, that will be further subject of discussions.

Similar to chiral drugs, only one of the two enantiomers of the chiral pesticide is active against the target pest, the other one is either inactive, or has a different active role, or even has adverse effects on some non-target species. However, few single-enantiomer pesticides are synthesized or produced [43]. Examples of different pest activity of the two enantiomers of chiral pesticides are given in Table 7.2.

Table 7.2 Chiral pesticides and their active enatiomeric forms

Chiral pesticides	Pesticide use	Active enantiomer	References
Mecoprop, dichlorprop	Herbicides	R-enantiomer	[35]
Imazapyr, imazethapyr, imazaquin	Herbicides	R(+) enantiomer	[34]
Metalaxyl	Fungicide	(R)-enantiomer	[32]
Indoxacarb	Insecticide	S-enantiomer	[22]
Metolachlor	Fungicide	R-enantiomer	[1]
	Herbicide	S-enantiomer	[18, 26]

For mecoprop, dichlorprop imazapyr, imazethapyr, imazaquin the herbicidal activities are exclusively related to the R- forms, as well as for metalaxyl the fungicidal use. For indoxacarb the S-form exhibit insecticidal activity. The optical activity is even more enantioselective in the case of metolachlor, S-metolachlor having herbicidal activity, while R-metolachlor is used as fungicide.

In their review on enantioselective chromatography techniques available for the study of transformation processes of chiral environmental pollutants [14] the authors presented a short history of the studies reported in this domain: in 1989, was published the enantiomer separation of α-HCH on a γ-cyclodextrin derivative as stationary phase, which may further be used to study the enantioselective transformation of α-HCH in the environment; in 1991, the enantioselective microbial transformation of α-HCH in the marine ecosystem have been proved for the first time; in 1991 as well, the enantioselective metabolisation of the same compound in Eider ducks was demonstrated; one year later, the photochemical transformation of α-HCH was studied. To conclude, the enantioselectivity of environmental chiral pesticides is based on their potential to discriminate by *microbial, enzymatic, or photochemical transformation processes* [14].

As enantioselective uptake indicators enantiomeric fractions (EF) or enantiomeric ratio (ER) (Eq. 7.1 and 7.2) are used.

$$EF_R = \frac{C_R}{C_R + C_S} \tag{7.1}$$

$$ER_R = \frac{C_R}{C_S} \tag{7.2}$$

where: C_R and C_S are the concentrations of the (R)- and (S)-enantiomers, respectively.

Enantiomeric enrichment of chiral pesticides in the environment was also subject of a reviewing article. The authors used EFs and ERs of chiral compounds to explain the mechanisms of enantiomer enrichment in air, soil, water and biota measured, over the past 10 years [13]. These differences of pesticides enantiomers behaviour in biotic environment, lead to further development of research studies of the pesticides uptake from different media (air, water, soil) by living organisms (plants, animals), transfers that can be or not enantioselective. Table 7.3 gives some examples of enantioselective behaviour of chiral pesticides in environment.

Table 7.3 Chiral pesticides and their enantioselective behaviour in environment

Chiral pesticides	Environment components	Enantioselective behaviour	References
Chlordane	Air-plant and soil-plant	Uptake and/or transfer	[20]
Chlordane	Atmospheric air and depositions, lake sediments, soils	Chiral signatures (shift from racemic to nonracemic)	[4]
α-HCH	Air and water	Air-water transfer	[15]
Chlordane	Carp tissues	Uptake and enantioselective elimination	[36]
Metolachlor	Soil and water	Biodegradation	[18]
Organochlorine pesticides	Air	Enantioselective depletion of the racemates	[38]
Organochlorine pesticides	Killer whale tissues	Bioaccumulation and enantiomeric profiling	[28]
Metolachlor	Constructed wetland	Stereoselective degradation	[1]
Fipronil	Chinese cabbage	Enantioselective degradation	[24]
Imidacloprid	Tomato roots, stem, fruits, soil	Translocation and persistence behaviour	[17]
Mecoprop, dichlorprop, metolachlor	Ontario streams	Enantioselective degradation and/or interconversion	[19]
Imazapyr, imazethapyr, imazaquin	Soils	Enantioselective degradation of R(+) enantiomer	[34]
Malathion	Chinese cabbage, rape	S-enantiomer degraded faster	[37]
	Sugarbeet	R-enantiomer degraded faster	
	Paddy rice, wheat	Non enantioselectivite degradation	

7.3 Ecotoxicological Effects of Chiral Pesticides

Pesticides enantiomers do not only exhibit enantioselectivity in the environment, but they also have different toxicological effects, subject that has become one of most challenging theme for researchers (Table 7.4).

It has been shown that the use of pesticides has increased the risk of intoxication of non-target species, such as birds and humans [25]. For example, synthetic pyrethroids (SPs) are among the most commonly used pesticides for agricultural and indoor pests control, especially in households to eradicate pests and insects, therefore also contributing to the exposure of humans to SPs [25].

More information about the enantioselectivity in environmental risk assessment of chiral pesticides is given in the reviewing article [43]. The co-authors are giving a survey on ecological fate and toxicology of chiral pesticides (synthetic pyrethroids, organophosphates, acylanilides, imidazolinones, phenoxypropanoic acids, organochlorines), also expressing their concern about pesticides that are not formulated as single- or enriched-enantiomer products.

Table 7.4 Chiral pesticides and their ecotoxicological effects

Pesticides	Toxicological effects	Test tissues	Enantioselectivity	References
Bromocyclen	Mammalian toxicity	Bream muscle	(−)-bromocyclen accumulated	[10]
		Rainbow trout	(−)-bromocyclen degraded	
(±)-*trans,* (±)-*cis* permethrins	Estrogenic activity	Zebrafish vitellogenin genes	(−)-*trans* permethrin greatest estrogenic activity	[16]
Bifenthrin	Enantioselective cytotoxicity	Human amnion epithelial (FL) cell	1S-cis-bifenthrin more toxic effects	[25]
Fenvalerate	Crooked body, yolk sac edema and pericardial edema	*Daphnia magna*, zebrafish embryo-larval	αS-2S- more toxic than αR-2R-FV	[27]
α-Cypermethrin	Embryo toxicity (yolk sac edema, pericardial edema)	Zebrafish embryos-larva	1*R*-*cis*-α*S*, 1R-*trans*-α*S* enantiomers more active	[40]
(Rac)-metolachlor, (S)-metolachlor	*In vivo* cellulase and catalase activities	Earthworms (*Eisenia foetida*)	(Rac)-metolachlor higher toxic effects	[41]
Metolachlor	Enantioselective phytotoxicity	Maize and rice roots	S-metolachlor stronger effects on roots growth	[26]
Malathion	Acute toxicity	Earthworm and honeybee	R-(+)-enantiomer more toxic	[37]

Abbreviations: *Rac* racemate

7.4 Chiral Separation of Optic Active Pesticides

Pesticides qualitative and quantitative analysis are mostly based on separation methods, different chromatographic and capillary electrophoresis techniques being developed, optimized and available. Gas chromatography technique (GC) is mostly used for volatile pesticides, with different detection modes: electron capture detection (ECD) especially for organochlorine pesticides, inductively coupled plasma detector (ICP), or coupled with mass spectrometry detection (MS). High performance liquid chromatography (HPLC), in its reversed phase mode, may also be used, especially for water soluble pesticides, using UV detectors, circular dichroism detection for the pure enantiomer discrimination (CD), of in tandem with MS detection. Capillary electrophoresis (CE) is restricted to charged compounds analysis, but its micellar electrokinetic chromatography technique (MEKC) is able to also separate the neutral pesticides. Usually CE utilizes the

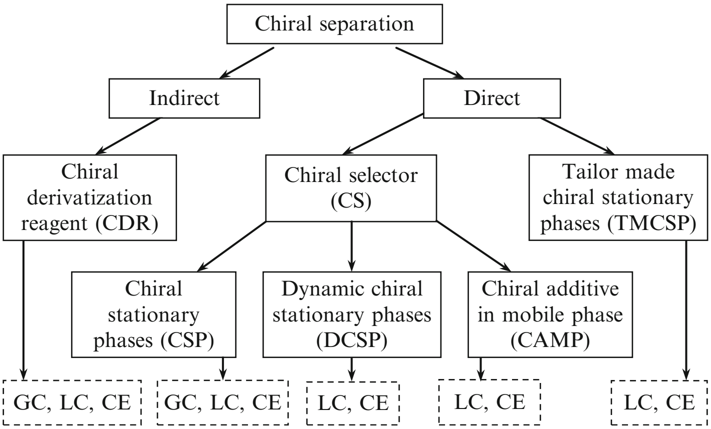

Fig. 7.3 Chiral separation modes

same detection type like HPLC, UV and MS being the mostly used. The separation techniques available for the enantioselective studies are subject of reviewing studies, for chromatographic techniques [14, 21, 30, 31, 42] or for capillary electrophoresis [2, 6, 12].

Chiral chromatographic separation techniques are based on chiral recognition mechanisms and were developed to also contribute to the studies of enantioselective behavior of the chiral pesticides in environment. Enantiomers can be separated using different approaches:

- indirect separation – is based on the racemate reaction with a chiral derivatization reagent (CDR) which is pure enantiomer, forming diastereomeric derivatives, separable in achiral system;
- direct separation based on the use of chiral selector (CS) – that will enantioselectively (preferentially) form a complex with one of the chiral isomers;
- direct separation based on tailor made chiral stationary phases (TMCSP).

Figure 7.3 presents chiral separation modes mostly developed for chromatographic and electrophoretic techniques (selection after Draghici C. [9]).

7.4.1 Chiral Selectors Used for Enantio Separations

Using direct enantioseparation by chromatographic techniques, the chiral selector may be introduces in either the stationary phase, forming a chiral stationary phase (CSP) or as chiral additive in the mobile phase (CAMP), in which case an achiral stationary phase is used. Sometimes, chiral selectors added in the mobile phase form a temporary dynamic chiral stationary phase (DCSP), like chiral micelles

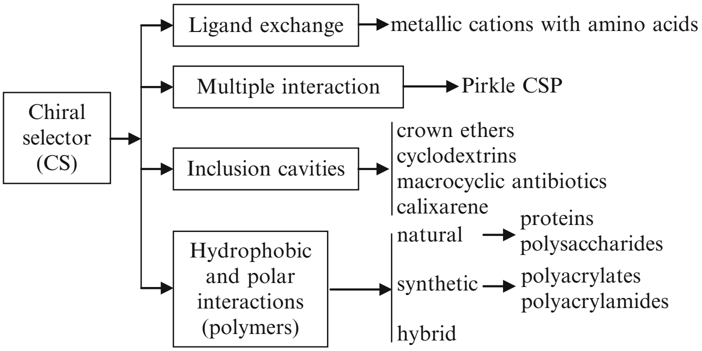

Fig. 7.4 Chiral stationary phases

form. For capillary electrophoresis techniques the CS is more commonly added in the background electrolyte, but CSP packed columns may also be used.

Very divers chiral stationary phases were developed, some of them are also commercially available. Different criteria were used for their classification in many publications, and one based on the selective interactions between the CSP and analyte, leading to chiral recognition is presented:

- ligand exchange mechanism;
- multiple attractive interaction (π-π, dipole-dipole, hydrogen bonding);
- inclusion into chiral cavities of low molecular mass
- hydrophobic and polar interactions favored by natural, hybrid or synthetic polymers (high molecular mass).

Figure 7.4 presents the mostly used chiral selectors utilised in chromatographic and electrophoretic techniques (adapted after Draghici C. [9]).

7.4.2 Applications of Chiral Separations of Optic Active Pesticides

Table 7.5 gives a selection of examples of chiral pesticides separation, with the indication of the chiral stationary phase (CSP) or the available commercial chiral columns that were used for enatioselective discrimination of the racemates.

The interest for enantioselectivity and separation of chiral pesticides is also reflected in studies related to the commercially available chiral pesticide standards, as agricultural reference materials [44]. The study relieved that certification of chiral organic reference materials should be performed with respect to both chemical and enantiomeric purity.

Table 7.5 Enantioseparation of chiral pesticides

Pesticides racemates	Matrix	Separation technique	Chiral selectors/chiral stationary phase (commercial column)	References
Acid herbicides: mecoprop, fenoprop dichlorprop, flamprop, haloxyfop, fluazifop, diclofop, fenoxaprop	Soil	CZE-UV	Vancomycin	[8]
Metolachlor	Soil and water	LC–MS	CDMPC	[18]
Toxaphene	Commercial fish oil	GC × GC–μECD	α-CD based capillary columns	[5]
Diclofop, fluazifop, quizalofop-P-ethyl, fenoxaprop	Standard solutions	HPLC-UV	Self-prepared CDMPC CSP; (S,S)-Whelk-O 1 columns	[7]
Benalaxyl	Soil and water	HPLC-UV, HPLC-CD	(R,R) Whelk-O 1 column	[23]
Chlordane, heptachlor	Standard solutions	GC–MS	2,3-DE-6TBDMS-β-CD	[3]
Bromocyclen	Fish tissue	GC-ECD, GC–ICP–MS	CP-Chirasil-Dex CB column	[10]
Metolachlor	Constructed wetland	HPLC-UV	(R,R) Whelk-O-1 column	[1]
Permethrin	Zebrafish	HPLC-UV	Chiralcel OJ-H (column)	[16]
Fipronil	Chinese cabbage	HPLC-UV, HPLC-CD	(R,R) Whelk-O 1 column	[24]
Bifenthrin	Human amnion epithelial (FL) cell	HPLC-UV, HPLC-CD, GC-ECD	Chiralcel OJ-H column	[25]
Fenvalerate	*Daphnia magna*, zebrafish, zebrafish embryo-larval	HPLC-UV, HPLC-CD	Chiralcel OJ-H column	[27]
α-cypermethrin	Zebrafish	HPLC-UV, HPLC-CD	Chiralcel OD and Chiralpak AD columns	[40]
Indoxacarb, lambda-cyhalothrin, simeconazole	Standard solutions	HPLC-UV	Chiralcel OD-H column	[22]
Metalaxyl, benalaxyl	Commercial fungicide formulations	EKC-DAD	Eight anionic CDs	[32]
16 pesticides (herbicides, insecticides, fungicides)	Commercial fungicide, soil, tap water	nano-LC-UV; CEC-DAD	Sepapak-2, Sepapak-4	[33]

Abbreviations: *CD* circular dichroism, *β-CD* β-cyclodextrin, *γ-CD* γ-cyclodextrin, *CDMPC* Tris (3,5-dimethylphenylcarbamate), *CEC* capillary electrochromatography, *Chiralcel OD-H column* cellulose tris-(3,5-dimethylphenyl-carbamate), *Chiralcel OJ-H* cellulose tris(4-methyl benzoate), *Chiralpak AD columns* cellulose derivateive, *CP-Chirasil-Dex CB column* column with chemically bonded β-cyclodextrin phase, *CSP* chiral stationary phase, *DAD* diode array detector, *2,3-DE-6TBDMS-β-CD* 2,3-di-O-ethyl-6-O-tertbutyldimethylsilyl-β-CD, *ECD* electron capture detector, *EKC* electrokinetic chromatography, *GC* gas chromatography, *HPLC* high performance liquid chromatography, *ICP* inductively coupled plasma, *LC* liquid chromatography, *MEKC* micellar electrokinetic chromatography, *MS* mass spectrometry, *nano-LC* nano-liquid chromatography, *Sepapak-2* cellulose tris(3-chloro-4-methylphenylcarbamate), *Sepapak-4* cellulose tris(4-chloro-3-methylphenylcarbamate), *Whelk-O 1column* 1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene bonded CSP

7.5 Conclusion

This study gives a presentation of the pesticides chirality and their enantioselective behavior in environment, air, water, soil, sediments and biota. Starting with the fact that chiral pesticides exhibit enantioselective uptake, from air-water-soil to plants and animals, and continuing with presentation of studies on their enantioselective toxicity, the study finally presents the separation techniques, as tools to investigate the chiral pesticides fate and behavior in the contaminated environment. More studies are required in order to detailed elucidate the different uptake, transfer or biodegradation mechanisms, results that will provide the manufacturers valuable information on the chiral (agro)chemicals production, as single or enriched-enantiomer, that might provide the expected benefit with minimum environmental impact.

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Chapter 8
Health Effects Associated with Acute and Chronic Exposure to Pesticides

Alina Farcas, Andreea Valceanu Matei, Cristina Florian, Mihaela Badea, and Gheorghe Coman

Abstract All living organisms are dynamic systems, functioning as a result of interdependent (bio) chemical reactions that are permanently maintained in an equilibrium state. Human exposure over a period of time to a complex mixture of pollutants at low level and consumption of polluted water and/or foods, is likely to significantly contribute to the human health status. Considering the time of exposure to toxicants, we can distinguish two different types of toxicity: acute toxicity (observed soon after short-time exposure to the pollutant) and chronic toxicity (resulted after long-term and/or repeated periods of exposure to lower doses of the chemical). Most of the acute effects are temporary, but may also cause coma and/or death. The chronic health effects have a latency period, which corresponds to the period of time between the first exposure and the development of the disorder. Most of the time, pesticide use involves their deliberate release into the environment for repelling, attracting, preventing, or killing any pests (target organisms) and may affect other organisms from the environment because they have relatively limited selectivity. Living organisms as well as humans are concurrently exposed to pesticides via the environment, which induces combined toxicological effects. The degree of health hazard depends on the quantity of pesticide and on the length of exposure (the dose of the pollutant). In this paper are presented general aspects on toxicology and clinical toxicology in view of the relationship exposure to pesticides – effects on human health.

Keywords Pesticide • Pollutant • Health effect • Acute toxicity • Chronic toxicity • Pest

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8.1 Introduction

All living organisms are dynamic systems, functioning as a result of interdependent (bio) chemical reactions that are permanently maintained in an equilibrium state. The presence of xenobiotic substances in a living system can easily disrupt this balance, by interacting with different components either through inhibition or activation [1, 4].

Human health is a very complex mixture of elements such as hereditary factors, lifestyle choices (eating habits, exercise, smoking, alcohol, drugs), socio-economic status, access to medical services, and most certainly the environment.

Research has indicated that interaction between the environment and human health is far more complex than is commonly understood.

Pollution may be defined as an undesirable change in the physical, chemical or biological characteristics of air, water, soil or food that can adversely affect the health, survival and activities of humans or other living organism [10].

Human exposure over a period of time to a complex mixture of pollutants at low level and consumption of polluted water and/or foods, is likely to significantly contribute to the human health status [29].

The role of toxicology and clinical toxicology is to study the toxic effects of chemical compounds on the human body, and recent developments in biochemistry, clinical biochemistry, biology and genetics can afford us the understanding of the processes at work in the human body in the presence of xenobiotics. In the human body, most of the biomolecules that interact with xenobiotics are proteins, which are structurally and functionally genetically conditioned [13].

Exposure to xenobiotic substances can produce toxicokinetic effects (enzymatic induction of metabolic pathway) and toxicodynamic effects (change in gene expression) in the human body.

It is useful to reconsider some commonly used definitions for toxic substances in order to facilitate the understanding of the processes, because these are often used interchangeably in literature. Therefore, a poison is legally, a substance that is fatal at a dose of less than 50 mg/kg body weight; a toxicant is a substance that may produce effects on plants, animals or humans; a toxin is a toxicant, produced during the metabolism of invading living organisms (microorganisms, plants, insects) which act on the host (in common usage, the word toxin is used as a synonym for toxicant – which is not correct); and a xenobiotic is a chemical that is foreign (not synthesized) into the body of the animal which is exposed to it.

Apart from the expected contributions of lifestyle choices, dietary factors and genetic state, many xenobiotics increase the risk of appearance of some biological or health effects [9, 21].

There are several factors that govern the biological effects of the pollutants and their metabolites in any living organism [11]. First, they need to enter the organism and be transported to the target sites where they need to bind to and interact with their biological receptors or be stored and resist the action of the degradative enzymes (biotransformation).

Solubility is a major determinant for the penetration of pollutants through cell membranes. Water and other biological media solubility influences the mobility of toxins, as blood and lymph serve as pollutants means of transport and liver, fat tissues, kidney and bone serve as pollutants storage.

8.2 Relationship Exposure to Pesticides – Effects on Human Health

Exposure to a pollutant is the presence of a certain concentration of pollutant material in the air, water or soil to which an animal is exposed in contrast with the dose received by an organism where we need to take into account the time of exposure and the quantity of pesticide [12]. Doses are often expressed in weight or molecular units per kilogram of body weight or per square meter of body surface area while exposure is a concentration of the compound in the air, water or soil to which an animal is exposed.

Considering the time of exposure to toxicants, we can distinguish two different types of toxicity:

- acute toxicity – observed soon after a short-time or one-time exposure to the chemical;
- chronic toxicity – resulted after long-term and/or repeated periods of exposure to lower doses of the chemical.

Pesticides are environmental pollutants [3, 7] and may be described as any physical, chemical, or biological agent developed to control or kill certain organisms (undesirable plants, animals or microorganisms). As a consequence, they have the potential to cause adverse effects to non-target organisms (associations with health effects with different latency periods for the different pesticide classes). Pesticide [8, 20] is the generic name for a substance or a mixture of substances classified on the basis of the organism killed (pest control) and the pattern of use [31]: insecticides, herbicides, fungicides, rodenticides, acaricides, molluscides, larvacides, scabicides, miticides, pediculicides, defoliants, repellants, desiccants, plant growth regulators, attractants (pheromones).

Plant extracts containing pyrethrin or nicotine and secondary plant metabolites such as phenols, terpenes, alkaloids, tannins, sterols, gums, and sugars were used in agriculture before the discovery of synthetic pesticides, as plant defenses against microbial pathogens or invertebrate pests [28].

Most of the pesticides are hydrophobic compounds and therefore tend to accumulate in human (mammalian) fatty tissue and induce many pathologic states [16, 19, 30].

For humans exposure to pesticide or pesticide residues can follow one or more of a number of pathways: through consumption of contaminated foods or drinking contaminated water, through residential application of pesticides, and in the course of occupational handling of pesticides.

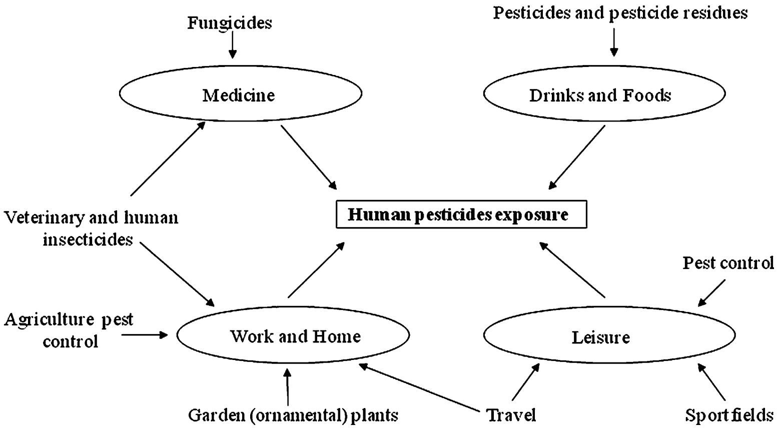

Fig. 8.1 Human sources and pathways of pesticides exposure

Routes of exposure to pesticides can be oral, inhalation and dermal, depending on pollutants physical and chemical properties [24]. The sources of pesticide exposure for humans and their pathways are shown in Fig. 8.1.

In the EU there is a significant presence of pesticides or pesticide residues in foods and consumers are exposed to more than one pesticide at the same time or in a short span of time [5, 17]:

- in 53–64 % of foods pesticides were not detectable;
- 32–42 % of foods contained detectable pesticide levels (below the maximum residue levels-MRL);
- 3–5, 5 % of foods contained levels above the MRL;
- 14–23 % of foods contained more than one pesticide;
- more than 50 % of streams contained five or more pesticides.

Most of the time, pesticide use involves their deliberate release into the environment for repelling, attracting, preventing, or killing any pests (target organisms) and may affect other organisms from the environment because they have relatively limited selectivity [18, 25].

Pesticides are known to be a major environmental hazard because only 5 % of the used pesticides reach the targeted pests, while more than 95 % of the used pesticides dispersed in the environment reach non-target organisms.

Because the use of pesticides induces environmental pollution, depollution strategies are necessary at the local level: chemical oxidation, photodegradation and phytoremediation [2, 11].

Living organisms in general, as well as humans in particular are concurrently exposed to two or more pesticides in their environment, situation that leads to the development of combined toxicological effects [29]. These emerging effects are:

- simple effect or independent action of the individual pesticide where the effect of one pesticide is the same whether the other one is present or not and the combined effects will be the sum of the individual effects.

- dose addition effect or agonist effect, which refers to mixtures of individual pesticides with the same mode of action and the same toxicological effects, which differ only in their potencies.
- interaction effect which is referred to as the joint action, where the combined effects of more pesticides may be greater (synergistic) or less (antagonistic) than the predicted effects.

Most of the pesticides are hydrophobic compounds and tend to accumulate in human (mammalian) fatty tissue and may induce many pathologic states [9, 22].

The degree of health hazard depends on the quantity of the pesticide and on the length of exposure (the dose of the pollutant).

In the dose–response relationship it is important for the response (effect) to be readily quantified in a reproducible way, relevant to the toxic processes.

The estimation of the dose–effect or concentration–effect relationship for pollutants (pesticides) is using some expressions: LD_{50} – lethal dose; LC_{50} – lethal concentration and IC_{50} – inhibited concentration.

LD_{50} is the dose which corresponds to a 50 % mortality of the exposed organisms and it is a measure for acute toxicity.

LC_{50} is the concentration in air or water which corresponds to a 50 % mortality of the exposed organisms, in a given amount of time.

IC_{50} is the concentration which corresponds to a 50 % inhibition of growth or activity.

Low toxicant concentrations may produce no observable effects, but as the concentration increases towards the critical level, symptoms will appear that will range from the lowest observable effect to severe affectation and even death.

Figure 8.2 shows the cumulative dose–response relationship as a sigmoid curve, where the no observable effect concentrations (NOEC) and lowest observable effect concentrations (LOEC) are represented alongside LC_{50} (LD_{50}).

Acceptable limits of pesticides or pesticide residues in foods or in the environment are designed based on this dose–response relationship curve, and the usage of pesticides requires authorization [32].

The degree of health hazard depends on the quantity of the pesticide used and on the length of exposure (the dose of the pollutant).

Most of the acute effects are temporary, but may also cause coma and even death [23].

The chronic health effects [26] have a latency period which corresponds to the period of time between the first exposure and the development of the disorder: leukemia, cancer, cirrhosis, lung diseases and asthma, obesity and diabetes, kidney and urinary tract diseases, cardiovascular and hematologic diseases, gastrointestinal disorders, genetic alterations, and pathologic states of the bone marrow, the central nervous system, the blood cells [6, 14, 15, 27].

Today, in our society, the debate over pesticide use is a social issue and continues at all levels, the controversy resting in the opposition between the rationale for pesticides and the need to minimize the health risks.

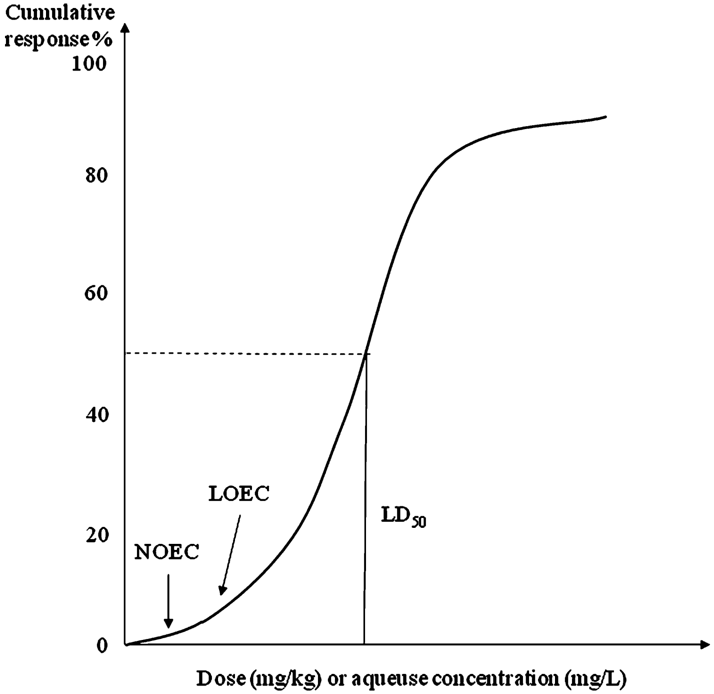

Fig. 8.2 Cumulative dose–response curve

8.3 Conclusion

The human body can be intermittently or continuously exposed to the environmental pesticides, which can easily enter it using various routes. The presence of certain pesticides in the human body can induce combined toxicological effects (altered cell function) and can lead to various diseases.

Our review presented general aspects on toxicology and clinical toxicology, through the point of view of the relationship between the exposure to pesticides and its effects on human health.

Today the purpose of a pest management system is to discourage the excessive proliferation of pests as well as to switch toward using the newest and least damaging pesticides in the attempt to maintain the agricultural productivity and the level of control over vectors of disease while decreasing the impact on health and the environment.

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Chapter 9
Pollutants Biotransformation

Andreea Valceanu Matei, Alina Farcas, Cristina Florian, Monica Florescu, and Gheorghe Coman

Abstract All living organisms are exposed to large amounts of xenobiotics, many of which may be toxics. The presence of xenobiotics in a living organism can unbalance the living body by inhibiting its growth or interfering with one or more components or chemical reaction on which it is dependent. The sum of the processes by which a xenobiotic (pesticide) is subject to chemical changes in living organisms is named biotransformation. Biotransformation reactions (phase I or phase II) are important in understanding the metabolism of endogenous molecules (endobiotics), or of the exogenous ones (xenobiotics) and their purpose is to increase the protective mechanisms developed in relation to cells or biological fluids. The equilibrium among the concentration of parent pesticides, biotransformation intermediates and conjugates, is responsible for the cellular, tissue or organism toxicity.

Keywords Pesticide • Biotransformation • Toxicity • Xenobiotic • Health

9.1 Introduction

The living organisms are dynamic systems which function as entities, being the result of interdependent chemical reactions and processes that take place continuously and are maintained in a steady state [5]: lipid peroxidation, oxidative stress, inflammation, genotoxicity, cytotoxicity.

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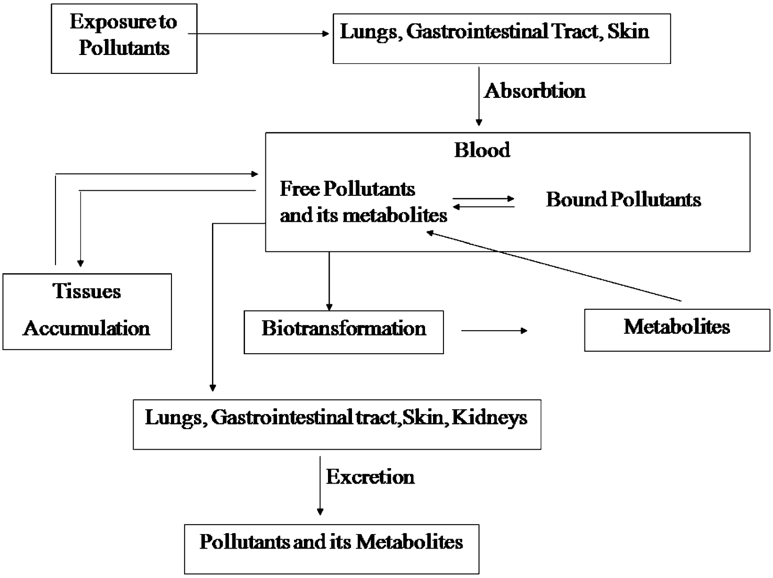

Fig. 9.1 The circuit of xenobiotics (pesticides) in human body

All living organisms are exposed to large amounts of xenobiotics, many of which may be toxics. The penetration of some xenobiotics in the human body may alter cell function and may induce various pathologic states [19].

The presence of xenobiotics in a living organism can unbalance the body by inhibition of its growth or interaction with one or more components or chemical reactions.

Among xenobiotics, pesticides have a great potential to induce biological changes in target organisms (pests) as well as in non-target organisms, thanks to their small specificity [13].

Pesticides (xenobiotics), in human (mammalian) organisms, suffer metabolic alterations in various organs and tissues [2, 4] such as liver, skin, kidney, plasma, intestine, brain and placenta (Fig. 9.1).

The sum of the processes by which a xenobiotic (pesticide) is subject to chemical changes in living organisms is named biotransformation.

9.2 Phase I Biotransformation

Biotransformation reactions (phase I or phase II) are important in understanding the metabolism of the endogenous molecules (endobiotics) or of the exogenous ones (xenobiotics). Their purpose is to increase protective mechanisms developed in relation to cells or biological fluids.

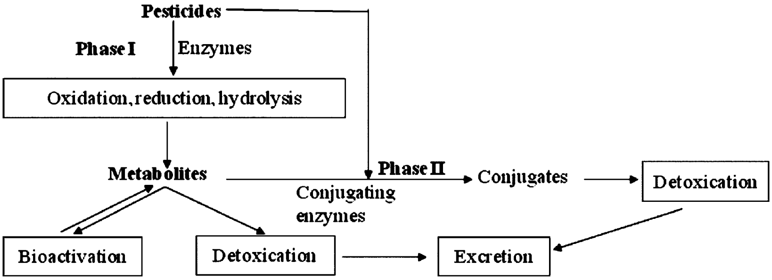

Fig. 9.2 General scheme of biotransformation

The parent pesticides or the pesticide metabolites resulted from phase I biotransformation, may act on functional compounds and induce pathologic states, or may be eliminated after the transport through body fluids, via bile or urine [5].

In a number of cases, the products of pesticide phase I biotransformation (metabolites) are more toxic than the parent pesticides, thanks to the hydrophilic compounds resulted from enzymatic biotransformation of initial hydrophobic compounds [8]. In this situation the term bioactivation is used [6, 7] because hydrophilic metabolites may easily interact especially with endogenous functional molecules (proteins, enzymes, nucleic acids) leading to an increase in toxicity.

Phase I biotransformation has not achieved spectacular results in terms of molecular mass and solubility changes in relation to the xenobiotic metabolite, but the new functional groups introduced by oxidation reactions will facilitate phase II biotransformation (conjugation) in the presence of phase II enzymes. Increases in molecular mass and solubility of xenobiotic metabolites are achieved through the process of conjugation [3, 17].

Many pesticides or their reactive more hydrophilic metabolites, formed in phase I biotransformation mostly by cytochrome P-450 enzymes, are detoxified by phase II biotransformation [21] in the presence of conjugating enzymes (glutathione S-transferases, UDP-glucuronosyltransferases, N-acetyltransferases, sulfotransferases (Fig. 9.2)).

Cytochromes P-450 (CYP) are the major phase I biotransformation enzymes, which consist of a family of enzymes that catalyze several reductive or oxidative reactions of xenobiotics or endobiotics to form the intermediates which can be substrates for phase II biotransformation enzymes [10, 12].

Other enzymes also act in Phase I biotransformation alongside of CYP, and these are: monooxigenases, peroxidases, hidrolases, dehidrogenases, aminooxidases, xantinoxidase (Fig. 9.3).

The basic reactions from phase I biotransformation, which introduce an oxygen atom on the substrate molecule are catalyzed by cytochromes P450, (monooxygenase activity).

The Phase I biotransformation of chlorpyrifos (an organophosphate pesticide), in the presence of CYP, leads to the corresponding oxon analog by desulfurization (Fig. 9.4). The metabolic desulfurization process of organophosphate pesticides is

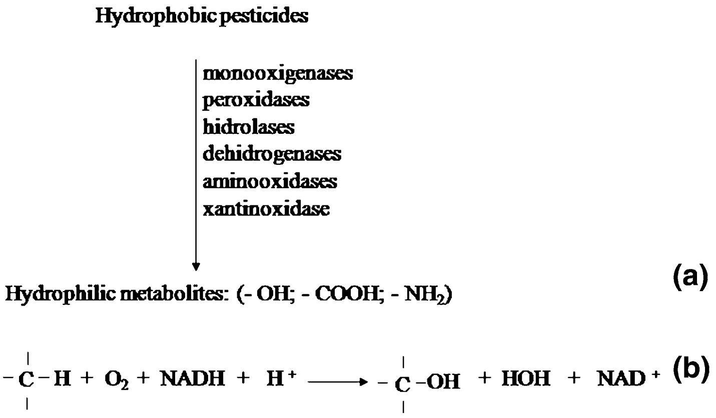

Fig. 9.3 Phase I Biotransformation, (**a**) general scheme; (**b**) scheme of monooxigenase action

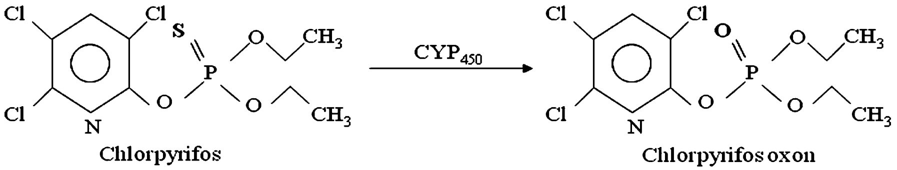

Fig. 9.4 Enzymatic transformation of chlorpyrifos in chlorpyrifos oxon in living organisms

considered bioactivation [1, 16], because oxon compounds are up to 1,000 times stronger inhibitors of acetylcholinesterase, than the parent compounds.

The CYP family of enzymes can lead to either the activation or detoxification of pesticides in living organisms. The relative rates at which pesticides are activated or detoxified can be essential for toxicology. For CYP activation of pesticides, the phase II biotransformation is essential in the detoxification process.

9.3 Phase II Biotransformation

Phase II biotransformation enzymes, engaged in catalyzing conjugation, have been less studied than the phase I biotransformation enzymes, engaged in catalyzing global biotransformation processes [11].

These species can be considered cellular incinerator enzymes, because they have the ability to catalyze the conversion of many xenobiotic substances (drugs, pesticides, organic compounds, carcinogens) or of their metabolites into compounds that can be excreted or degraded for excretion [20].

The Phase II biotransformation is generally seen as a way for detoxifying the living body, and the inhibition of the enzymes that catalyze these chemical changes may lead to increased toxicity of xenobiotics or of their metabolites [9].

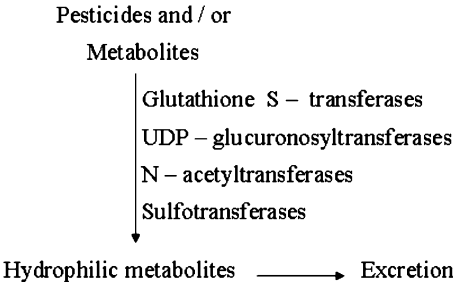

Fig. 9.5 General scheme of phase II biotransformation processes

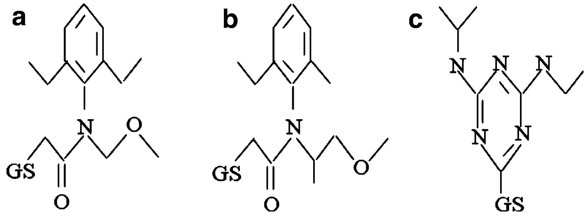

Fig. 9.6 Conjugate structures of some herbicides with glutathione (GS), (**a**) alachlor; (**b**) metalachlor; (**c**) atrazine

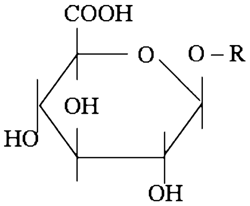

Fig. 9.7 General structures of β-D-glucuronides

A family of phase II enzymes, which includes (UDP-glucuronosyltransferases (UGTs), sulfotransferases (ST), Glutathione-S-transferases (GSTs), N-acetyltransferases) catalyzes the chemical conjugation reactions (Fig. 9.5).

Phase II biotransformation enzymes catalyze reactions that add hydrophilic inner cosubstrates (glutathione, UDP-glucuronic acid, 3′-phosphoadenozin-5′-phosphosulphate) to pesticides (Fig. 9.6) or their phase I biotransformation metabolites [14, 15].

Glucuronidation is a major detoxification pathway in all vertebrates, and the reaction is catalyzed by a multigene family of isoenzymes, the UDP-glucuronosyltransferases (E.C. 2.4.1.17).

UDP-glucuronosyltransferases catalyse the transfer of a glucuronic acid residue, from UDP-glucuronic acid to compounds possessing carboxyl-, hydroxyl-, amino-, or sulfhydryl groups [12]. Mammalian UGTs used UDP-glucuronic acid as glycosyl donor and the reaction products are water soluble β-D-glucuronides, which circulate in biological fluids and are excreted in urine (Fig. 9.7).

The detoxifying reaction catalyzed by UDP-glucuronosyltransferases can be inhibited by a large variety of compounds, other than environmental pollutants (pesticides):

- natural or synthetic toxic substances (food ingredients);
- endogenous compounds (bile acids, long chain acyl-CoAs);
- drugs.

The disturbance of the glucuronidation reactions, leads to an increase in the human pathologic potential and to the development of pesticide toxicities or death [18].

Glutathione S-transferases (E.C. 2.5.1.18) act as detoxifying enzymes, and catalyze conjugation of inner glutathione with electrophilic compounds [14, 15].

As UGTs, ST, GSTs are found in all aerobic organisms and also in plants, GSTs are responsible for their tolerance to the triazine pesticides metalachlor and alachlor (Fig. 9.6).

GSTs are able to interact with several classes of herbicides (triazines, diphenylethers, thiocarbamates, chloroacetamides) or their phase I metabolites, to detoxify the mammalian body [15].

The level of GSTs can be considered an important protective factor against xenobiotics (pesticides) and oxidative stress.

The sulfotransferases are a family of enzymes that catalyze the conjugation by transfer of a sulfonyl group, from a sulfate donor to amino or hydroxyl groups of pesticides or pesticide metabolites.

ST form a group of cytosolic enzymes, that have a higher affinity for substrates (xenobiotics) but lower reaction capacity than UGTs [10].

9.4 Conclusion

The sum of the processes by which a xenobiotic (pesticide) is subject to chemical changes in living organisms is named biotransformation.

The biotransformation processes in mammalian bodies are running in the presence of some specific or non-specific enzymes. At molecular level these enzymes transform the xenobiotics into metabolites that have electrophilic functional groups which can increase their solubility in biological fluids in order facilitate their elimination from the body, or can increase their reactivity compared to the parent pesticide, leading to a increase in toxicity.

Pesticides pose a public health risk for humans and induce numerous problems for the environment that are hard to solve.

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Chapter 10
The History, Toxicity and Adverse Human Health and Environmental Effects Related to the Use of Agent Orange

Vladimir Bencko and Florence Yan Li Foong

Abstract Agent Orange consist of 50 % *n*-butyl esters of 2,4,5-trichlorophenoxy acetic acid and 50 % 2,4-dichlorophenoxyacetic acid. These compounds are chemical plant growth regulators, which mimic the effect of plant hormones, provoking plants into frantic growth before they wither and die. The toxicity of Agent Orange is attributed to the contamination with dioxin, which was perhaps the most toxic molecule ever synthesized by man. Agent Orange's actual effect on human health remained controversial because even though dioxin at certain levels was clearly capable of causing serious diseases, those same diseases could also result from other causes. Dioxin is a persistent organic pollutant that will accumulate in animal fat and plant tissues and therefore can enter the food chain. The US National Toxicology Program has classified dioxin as "known to be a human carcinogen", causing namely, chronic lymphocytic leukemia, soft tissue sarcoma, non-Hodgkin lymphoma and Hodgkin disease with sufficient evidence of an association. Severe acute intoxication of dioxin caused chloracne, porphyria, transient hepatotoxicity, and neurotoxicity. Chronic persistence of dioxin in the human body may contribute to development of atherosclerosis, hypertension, diabetes, vascular changes, and neuropsychological impairment several decades after massive exposure. However, such chronic effects are nonspecific and multifactorial. This paper elaborates the aforementioned and other health effects of Agent Orange.

Keywords Agent Orange • 2,4,5-trichlorophenoxyacetic acid • 2,4-dichlorophenoxy acetic acid • Dioxin • Chronic lymphocytic leukemia • Chloracne

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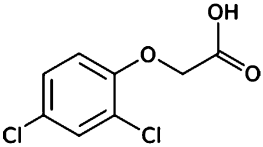

Fig. 10.1 2,4-D (http://en.wikipedia.org/wiki/File:2,4-dichlorophenoxyacetic_acid_structure.svg)

10.1 Introduction and History

In the 1930s, British research and testing paved the way for the discovery of chemical plant growth regulators that mimic the effect of plant hormones, and their implementation as a tactic in war to destroy enemy crops. Agent Orange contains two of these compounds – 2,4-D and 2,4,5-T – and works by provoking plants into frantic growth before they wither and die [47].

Oil- and water-based formulations of herbicides were used in Vietnam and several other places during wartimes. The herbicides were stored and shipped in 208-l barrels, and named after the colored band painted on each barrel [57]. These herbicides were procured by U.S. military from companies such as Dow Chemical, Monsanto, Hercules Inc., Diamond Alkali/Shamrock, and Ansul Chemical [68].

During the Vietnam War (1960–1971), Agent Orange and other herbicides were sprayed by U.S. military forces at a rate of more than an order of magnitude greater than amounts used for domestic weed control [57]. These herbicides were mostly sprayed over South Vietnam forests to kill crops to deprive Vietcong and North Vietnamese troops of food and to remove the vegetation cover used for concealment, making ambushes more difficult. Herbicides were also sprayed in Laos to remove the jungle canopy and expose the infiltrating men and supplies to attack from the air. This cleared large forest areas hiding sanctuaries and bases, forcing the Vietcong and North Vietnamese troops to move or risk discovery and attack [11]. Operation Ranch Hand involved about 8,930 spraying missions and sprayed about 72 million liters of herbicides [70, 71], 11 million of which consisted of Agent Orange [11].

10.2 Chemistry Involved

Agent Orange is an oil-based herbicide that was used to target broadleaf plants and woody shrubs and trees, including mangroves. Agent Orange is a 1:1 mixture of the *n*-butyl esters of 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) the molecular structure of which is presented on Figs. 10.1 and 10.2 [56, 68].

A memo from 1952 concerning an agreement between the U.S. Public Health Service and Monsanto about labeling requirements revealed an astonishing use of one of the company's most toxic PCBs. Monsanto referred to this as "the prize

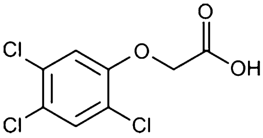

Fig. 10.2 2,4,5-T (http://en.wikipedia.org/wiki/File:2,4,5-T.svg)

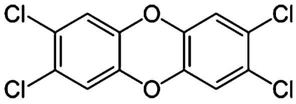

Fig. 10.3 2,3,7,8-TCDD (http://en.wikipedia.org/wiki/File: Dioxin-2D-skeletal.svg)

application" and also referenced cases of chloracne, deaths associated with exposure to PCBs among workers, and cases of workers' wives developing acne and dermatitis which was traced back to the halogenated hydrocarbon compounds on clothing of their husbands [8]. In 1966, Monsanto defended themselves and stated that when they learned that PCBs could possibly be in the environment, they acted promptly to carry out an investigation to gather information and acted responsibly, and continue to do so [63].

In more recent news, IBAMA, Brazil's environmental agency, investigated and discovered around four tons of Agent Orange which were awaiting to be dispensed to Brazilian ranchers to clear the Amazon rainforest to make way for agricultural land. The herbicide would be enough to wipe out 7,500 acres of the Amazon rainforest [34].

10.3 The Toxicity of Agent Orange

The 2,4,5-T used to produce Agent Orange was unintentionally contaminated during the manufacturing process with small amounts of an extremely toxic dioxin compound, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) [1].

TCDD (see Fig. 10.3) which is assigned a toxic equivalency factor (TEF) of 1 [65], has been described as "perhaps the most toxic molecule ever synthesized by man". Arthur Galston, a biologist in Yale who specializes in herbicide research reported that "vanishingly small" quantities of dioxin in the diet produced adverse health effects in test animals. A daily dose of dioxin concentrations as low as 5 parts per trillion (ppt) can induce a cancerous condition in rats. Concentrations about 1 part per billion (ppb) result in premature death from more acute causes; concentrations of 50 ppb produce rapid signs of acute toxicity and early death. Lower concentration of TCDD seem to produce the same effects as higher concentrations, but merely take longer to do so. Even the purest 2,4,5-T currently available commercially contains about 0.05 parts per million (ppm) (mg/kg) of TCDD [52].

The toxicity of dioxin (2,3,7,8-TCDD) results from its interaction with the Aryl hydrocarbon receptor (Ahr), a genetically expressed cytosolic ligand-activated transcription factor that controls the expression of a diverse set of genes [7]. TCDD-Ahr ligand-receptor binding is necessary, but it is not sufficient to cause the chain of events leading to various toxic actions [9]. The TCDD-Ahr complex followed by the dimerization of the Ahr with an Ahr nuclear transport protein (Arnt) translocates to the nucleus and interacts with specific DNA sequences (dioxin-responsive elements, or DREs) [23]. This leads to an inappropriate modulation of gene expression encoding various xenobiotic metabolizing enzymes and molecules that participate in cell-cycle regulation, apoptosis, inflammation and other important processes in the human body [36], for example, the induction of the transcription of cytochrome (CYP) CYP1A1 and 1B1 genes [22, 66]. Ahr also mediates the production of reactive oxygen species [59], which is associated with tumor-promotion activities of peroxisome proliferators [48]. Another possible mechanism of TCDD toxicity is disruption of the normal endocrine functions [39].

Polychlorinated dibenzo-p-dioxins, dibenzofurans, hexachlorobenzene and polychlorinated biphenyls are classified as persistent organic pollutants [58] that “endure in the environment, bioaccumulate through the food chain (animal fat and plant tissues), possess toxic properties, and resist degradation” [46]. Airborne dioxin can travel great distances and eventually settle onto soil, plants, and water. Dioxin dissolves readily in oils, fats, and organic solvents but poorly in water and it does not evaporate readily. Since dioxin does not react with oxygen or water and is not broken down by bacteria, it persists in the environment for long periods of time [61].

The main source of exposure in humans is the food chain [46]. Traces of Agent Orange have been found in food in Vietnam [55]. In the vicinity of former U.S. military bases such as Bien Hoa, where Agent Orange was stored in large quantities, soil sampled had TCDD levels that were 180 million times above the safe level set by the U.S. environmental protection agency [17]. Thus, exposure to dioxins includes both acute exposure by inhalation and skin contact, as was in the case of exposure of military personnel to Agent Orange in Vietnam; and chronic exposure by food and drink, and skin contact from accumulated dioxins in the environment [3]. Half-life of TCDD in humans was estimated to be in the range of 7–10 years. Today, background TCDD levels in human fat have a decreasing trend [46].

However, another study has concluded that “the prospect of exposure to TCDD from Agent Orange in ground troops in Vietnam seems unlikely in light of the environmental dissipation of TCDD, little bioavailability, and the properties of the herbicides and circumstances of application that occurred. Photochemical degradation of TCDD and limited bioavailability of any residual TCDD present in soil or on vegetation suggest that dioxin concentrations in ground troops in Vietnam would have been small and indistinguishable from background levels even if they had been in recently treated areas. Appreciable accumulation of TCDD in veterans would have required repeated long-term direct skin contact of the type experienced by U.S. Air Force Ranch Hand and U.S. Army Chemical Corps personnel who had handled or otherwise had direct contact with liquid herbicide, not from incidental exposure under field conditions where Agent Orange had been sprayed” [70, 71].

Table 10.1 Approximate exposures estimates in selected groups of subjects with the heaviest exposure, using a half-life of 7–10 years [46]

Place of exposure	Years	TCDD level in plasma (pg/g fat)[a]
USA, veterans [25]	1962–1971	50
Vietnam – population [15]	1962–1971	120–260
New Zealand [53]	Late 1960s	300
Seveso, Italy [31]	1976	390 zone A; 78 zone B
BASF Ludwigshaven, Germany [20]	1953	400
Netherlands [25]	1963	1,434
USA, 12 chemical companies [18]	1950s–1970s	2,000
Linz, Austria [37]	1973	2,682
Spolana, Czech Republic [42–45, 72]	1965–1968	6,100
Population level [4]	2000	2

[a] Maximum mean back-calculated TCDD level in plasma to the date of exposure (pg/g fat)

Despite all these findings, Agent Orange's actual toll on human health remain controversial. First, its potential toxicity was a function of the quantity of dioxin it contained, among other things, but the exact quantity of dioxin applied in any area is not known. Second, the dioxin levels in the Agent Orange to which American veterans were exposed are even less reliable. There is information on which areas aerial spraying missions were conducted, but the herbicide may have been blown away from target ground, so we cannot be sure about how much exposure the Vietnamese victims and U.S. veterans had received, individually. Third, the serious diseases caused by dioxin could also be the result of other causes. Nevertheless, biologist Arthur Galston concluded that "American service-men who worked with Agent Orange or who saw duty in the heavily defoliated zones of Vietnam have a legitimate basis for asking the government to look into the state of their health" [52].

Next Table 10.1 demonstrates approximate exposure estimates in selected groups of subjects with the heaviest exposure scenarios calculated on using of the half-life of persistence of TCDD in humans of 7–10 years based on its concentration in plasma expressed in pg/g fat comparing with a general population. The extreme cases were observed in occupational settings in accidents or due to neglecting principles of occupational safety.

In the 1970s, the level of TCDD exposure was first detected in breast milk and fish exposed to Agent Orange in Vietnam by congener-specific high-resolution gas chromatography high-resolution mass spectrometry (HRGC-HRMS) [5]. In the 1980s, adipose tissue and blood were also used as samples [50, 51]. Currently, CDC, the WHO and the U.S. Air Force use HRGC-HRMS to assess dioxin exposure [18, 35, 67].

10.4 Adverse Human Health Effects of Agent Orange

"Millions of Vietnamese people were exposed to Agent Orange during the war. The Vietnamese Red Cross says local studies have shown that as many as a million people now have disabilities or other health problems associated with Agent

Orange – approximately 100,000 of them being disabled children. Every year, particularly in the areas heavily sprayed with Agent Orange, thousands of children are born with illnesses and birth deformities, some of them very severe, while thousands of adults develop cancer or other health problems, according to the VNRC" [26].

Officials from the U.S. Department of Veteran Affairs (VA) estimate that about 2.6 million military personnel who served in Vietnam were affected by Agent Orange [14]. Since 1994, the U.S. government has directed the Institute of Medicine (IOM) to issue reports every 2 years on the health effects (both cancerous and non-cancerous) of Agent Orange and similar herbicides, titled "Veterans and Agent Orange," to provide a basis for government policy decisions. Each health effect is categorized as having one of the following: sufficient evidence of an association; limited/suggestive evidence of an association; inadequate/insufficient evidence to determine whether an association exists; or limited/suggestive evidence of no association [1].

Severe acute intoxication of dioxin may cause nausea, headaches, vertigo, vomiting, irritation of eyes, skin and respiratory tract, profuse sweating with extensive dehydration and weight loss, temperature dysregulation, severe breathing difficulties, cyanosis, and a deteriorating general status, followed after several days to weeks by chloracne, porphyria, transient hepatotoxicity, and peripheral and central neurotoxicity [24, 46].

Chronic persistence of TCDD in the human body can be present several decades after massive exposure, and may lead to atherosclerosis, diabetes, hypertension, vascular ocular changes, and signs of neural system damage, including neuropsychological impairment. Such chronic effects are nonspecific, multifactorial, and may be causally linked only to severely intoxicated subjects. This opinion is supported by the dose-dependent effect of TCDD found in exposed workers and by experimental animal studies [46]. Another study supported this, and added that long-term exposures to dioxins may cause disruption of the immune, reproductive, and endocrine system, and that the most sensitive population to dioxin exposure are foetuses and infants [32].

The International Agency for Research on Cancer (IARC) has classified TCDD as a group 1 carcinogen [25]. Studies involving thousands of Vietnam veterans, which potentially provide the most direct evidence of the health effects of Agent Orange exposure, and studies of three other groups, have provided important information about the carcinogenicity of Agent Orange. Each of these groups differs in the characteristics of the people exposed, the nature of the dioxin exposures, and other factors such as diet and other chemical exposures. The three other groups are: first, Vietnamese soldiers and civilians, often exposed for longer periods; second, workers exposed to herbicides in other settings, such as herbicide manufacturing workers, herbicide applicators, farmers, lumberjacks, and forest and soil conservationists, who often had higher blood dioxin levels than Vietnam veterans; third, people exposed to dioxins after industrial accidents in Germany, Seveso (Italy), and California, and after chronic exposures at work and in the environment [1]. Table 10.2 summarize links between exposure to herbicides including Agent Orange and risk of cancer published IOM.

Table 10.2 IOM: Links between herbicides (including Agent Orange) and cancer [1]

Sufficient evidence of an association
Soft tissue sarcoma
Non-Hodgkin lymphoma (NHL)
Hodgkin disease
Chronic lymphocytic leukemia (CLL), including hairy cell leukemia and other chronic B-cell leukemias
Limited/suggestive evidence of an association
Respiratory cancers (lung, trachea, bronchus, larynx)
Prostate cancer
Multiple myeloma
Inadequate/insufficient evidence to determine whether an association exists
Mouth, throat, and sinus cancers
Gastrointestinal cancers (esophagus, stomach, pancreas, colon, rectum)
Liver, gallbladder, and bile duct cancers
Bone and joint cancers
Skin cancers
Breast cancer
Female reproductive cancers (cervical, ovarian, endometrial, uterine sarcoma)
Testicular and penile cancers
Bladder cancer
Kidney cancer
Brain tumors
Cancers of endocrine glands (thyroid, thymus, etc.)
Leukemia (other than CLL and hairy cell leukemia)
Cancers at all other sites
Cancer (including AML) in the children of veterans

Recent epidemiological evidence does not conclusively demonstrate a causal link between TCDD exposure and cancer risk in humans. An updated cohort of U.S. herbicide producers generated negative results overall; the internal analysis provided evidence of an increased "all-cancer" risk in the highest exposure category, with a statistically significant exposure-response association in some of the many analyses performed. The updated surveillance of the Seveso population also provided evidence of increased all-cancer mortality 15–20 years after exposure among those living in the most contaminated area. Updated results from cohort studies of Vietnam veterans potentially exposed to TCDD did not consistently suggest an increased risk of cancer. However, this is not a reason for ignoring the weaknesses of the available evidence [10].

Other diseases or medical conditions that have been attributed to Agent Orange exposure (see Table 10.3) include developmental abnormalities [21], thyroid dysfunction [41], elevated serum cholesterol and triglycerides, liver damage, skin rashes, hypertrichosis, gum pigmentation, eyelid pathology, nausea, vomiting, and loss of appetite [27], death from cardiovascular disease and ischemic heart disease [19], enamel hypomineralization of permanent first molars in children [2],

Table 10.3 IOM: Links between herbicides (including Agent Orange) and other health effects [1]

Sufficient evidence of an association
Chloracne
Limited/suggestive evidence of an association
Amyloidosis
High blood pressure
Ischemic heart disease
Transient peripheral neuropathy
Type 2 diabetes
Parkinson disease
Porphyria cutanea tarda
Spina bifida in children of veterans

increased levels of serum luteinizing and follicle-stimulating hormones, and decreased levels of serum testosterone [16].

Chloracne is the "hallmark of dioxin toxicity" and is the most consistent manifestation of dioxin intoxication [60], but not all persons exposed to dioxin developed chloracne [64]. Symptoms include a hyperproliferative reaction of the cutaneous epithelium with squamous metaplasia of the cells lining the ducts of skin glands, resulting in comedones, cysts, and, in severe cases, pustules [40]. The skin areas most frequently affected are those that are usually in contact with hands contaminated by TCDD: under the eyes, behind the ears, neck, back and genital regions [37]. Chloracne may appear days or weeks after the initial exposure, and severe cases last for up to 15 years [40]. Chloracne in veterans is presumed to be related to their exposure to Agent Orange or other herbicides during military service when the disease appears within 1 year of exposure to a degree of at least 10 % disabling by VA's rating regulations [64]. The most politically popular victim of chloracne was Viktor Andriyovych Yushchenko, former Ukrainian President, whose face was greatly disfigured after the illness, and claimed that he had been poisoned [6]. The TCDD level in his blood serum (108,000 pg/g lipid weight) was 50,000-fold higher than the general population [54]. Initially, many physicians and scientists suggested rosacea and stress as the diagnosis instead of dioxin poisoning [6]. Indeed, mild forms of chloracne may resemble teenage acne, and this complicates the diagnosis [64].

TCDD exposure might be a risk factor for diabetes because Ahr antagonizes the functions of peroxisome proliferator-activated receptor (PPAR), a key regulator of systemic insulin sensitivity that delays the onset of type two diabetes [49]. Severely-exposed individuals have a high prevalence of diabetes [13]. Neurological and neuropsychologic findings due to TCDD exposure are inconsistent. However, there were some effects reported in Ranch Hand and Seveso, namely polyneuropathies and abnormal co-ordination [29]. There was a mild toxic effect on the peripheral nervous system manifesting as mild sensory neuropathy of the legs in a minority of the most severely exposed persons [62]. Some individuals who have been exposed to TCDD acquired acute porphyria cutanea tarda [33]. They had fragile skin, dark pigmentation, hypertrichosis, hepatomegaly, reddish-colored urine and urinary excretion of porphyrins [46]. However, this association has been debated by others [12].

There were inconsistent findings regarding reproductive outcomes such as spontaneous abortions, birth weight or birth defects in epidemiological studies of industrial populations, the population of Seveso and the Ranch Hand cohort. Parental exposure to Agent Orange may be associated with an increased risk of birth defects [38]. Greater dioxin exposure through breastfeeding at 7 months had a significantly adverse effect on the psychomotor outcome among breastfeeders [30]. Prenatal exposure to PCB might be related to adverse effects on the neurodevelopment and behavior of children. However, data on dioxin exposure is still limited [29].

10.5 Conclusion

Man-made Agent Orange has impacted the worldwide populations that it has had contact with. Its effects – whether proven or suspected – cause suffering and desolation to many. There is no antidote yet to decrease TCDD levels and treatment of effects are only symptomatic. Thus, it is applaudable that the U.S. has begun efforts to start addressing these remnant wounds of the Vietnam War. CNN reports that the U.S. government is working with the Vietnamese government and partnering organizations to treat a contaminated zone at the Danang airport. Workers will dig up soil, stockpile it, and treat it with high temperatures that will break down the dioxin [28]. Various organizations have been founded to help victims of Agent Orange, such as the Vietnam Agent Orange Relief & Responsibility Campaign, the Vietnam Association of Victims of Agent Orange/Dioxin, the Children of Viet Nam, the Danang/Quang Nam Fund, Inc., the Agent Orange Working Group under the VUFO-NGO Resource Centre, and many others. The authors hope that these efforts would be the first of many in helping to rebuild and restore the lives that Agent Orange has damaged.

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Chapter 11
The History of Organic Arsenical Pesticides and Health Risks Related to the Use of Agent Blue

Vladimir Bencko and Florence Yan Li Foong

Abstract Arsenicals in agriculture: Beginning in the 1970s, the use of arsenic compounds, such as for wood preservatives, began to grow. By 1980, in the USA, 70 % of arsenic had been consumed for production of wood preservatives. This practice was later stopped, due to the US Environmental Protection Agency (EPA) ban of the arsenic-and chromium-based wood preservative chromated copper arsenate. In the past, arsenical herbicides containing cacodylic acid as an active ingredient have been used extensively in the USA from golf courses to cotton fields, drying out the plants before harvesting. The original commercial form of Agent Blue was among ten toxic insecticides, fungicides and herbicides partially deregulated by the US Environmental Protection Agency (EPA) in February 2004, and specific limits on toxic residues in meat, milk, poultry, and eggs were removed. Today, however, they are not used as weed-killers anymore, with one exception of monosodium methanearsonate (MSMA), a broadleaf weed herbicide for use on cotton. Severe poisonings from cacodylic acid caused headache, dizziness, vomiting, profuse and watery diarrhea, followed by dehydration, gradual fall in blood pressure, stupor, convulsions, general paralysis and possible risk of death within 3–14 days. The relatively frequent use of arsenic and its compounds, in both industry and agriculture, points to a wide spectrum of opportunities for human exposure; this exposure can be via inhalation of airborne arsenic, contaminated drinking water, beverages, or from food and drugs. Today, acute organic arsenical poisonings are mostly accidental. Considerable concern has developed surrounding its delayed effects, for its genotoxic and carcinogenic potential, which has been demonstrated in epidemiological studies and subsequent animal experiments. The Conclusion is that there is substantial epidemiological evidence for an excessive

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risk, mostly for skin and lung cancer, among humans exposed to organic arsenicals in occupational and environmental settings. Furthermore, the genotoxic and carcinogenic effects have only been observed at relatively high exposure rates. Current epidemiological and experimental studies are trying to elucidate the mechanism of this action, pointing to the question of whether arsenic is actually the true genotoxic or rather an epigenetic carcinogen. Due to the complexity of its effects both options remain plausible. Its interactions with other toxic substances still represent another important field of interest.

Keywords Arsenicals in agriculture • Organic arsenicals use • Farmers' health • Arsenical pesticides • Carcinogenicity • Agent Blue toxicity

11.1 Introduction and History

During the Vietnam War (1960–1971), Agent Orange and other herbicides were sprayed by U.S. military forces at a rate of more than an order of magnitude greater than amounts used for domestic weed control [29]. The herbicides were stored and shipped in 208-L barrels, and named after the colored band painted on each barrel [37]. They were mostly sprayed over South Vietnam forests to kill crops to deprive Vietcong and North Vietnamese troops of food and to remove the vegetation cover used for concealment, making ambushes more difficult [5].

US soldiers attempted to blow up rice paddies and rice stocks by using mortars and grenades. But grains of rice were very durable, and were not easily destroyed – every grain that survived was a seed, to be collected and planted again. Soon, the "rice-killing operations" became more sophisticated. Rubber or plastic bladders were dropped directly into rice paddies, exploding on impact and releasing toxic herbicides. Barrels of herbicides were also dropped into the water irrigating rice paddies, polluting rivers and poisoning the soil and people for many years [11].

Agent Blue was used as a contact herbicide in South Vietnam for rapid defoliation, grassy plant control and rice destruction. Blue was the agent of choice for destruction of rice crops. More than four million liters of Agent Blue, also known as Phytar 560-G, was dispensed in the Department of Defense (DOD) herbicide program, according to miliary herbicide records [15].

Agent Blue works by rapidly defoliating or desiccating a wide variety of plant species of grasses and grains [13]. It works by uncoupling phosphorylation in plants [10]. It was used in situations requiring rapid defoliation, causing browning or discoloration within 1 day, with maximum desiccation and leaf fall occurring within 2–4 weeks [6]. By starving rice plants of moisture, the enemy (including millions of rice-growing villagers) would be starved of their most basic food. This formed an essential part of the U.S. government's "rice-killing operations" [21].

The term "Agent Blue" was first applied to cacodylic acid in a powder form that was mixed with water in the field. Cacodylic acid is a highly-soluble organic arsenic compound that readily breaks down in soil [15]. It is considered to have very low toxicity to mammals [13]. The original commercial form of Agent Blue was so common and so profitable that it was among ten toxic insecticides, fungicides and herbicides partially deregulated by the US Environmental Protection Agency (EPA) in February 2004, and specific limits on toxic residues in meat, milk, poultry, and eggs were removed [11].

However, in 2009, the U.S. Environmental Protection Agency issued a cancellation order to eliminate and phase out the use of organic arsenical pesticides by 2013, with one exception of monosodium methanearsonate (MSMA), a broadleaf weed herbicide for use on cotton. Small amounts of cacodylic acid (or disodium methanearsonate) were historically applied as herbicides in cotton fields, golf courses, backyards and other areas, but its use is now prohibited under the U.S. EPA 2009 organic arsenical product cancellation [7, 11]. Other organic arsenicals (e.g. roxarsone, arsanilic acid and its derivatives) are used as feed additives for poultry and swine to increase the rate of weight gain, to improve feed efficiencies, pigmentation, and to treat and prevent disease [8, 9].

The World Health Organization guidelines for safety limit of arsenic is at 10 μg/L in drinking water [35]. In Vietnam, the legal arsenic concentration limit is five times higher than in the WHO guidelines [4].

The groundwaters of the Red River alluvial tract in Hanoi are anoxic and rich in iron due to naturally occurring organic matter in the sediments [4]. The problems are caused largely from "tubewells," which pull water from depths of between approximately 30 and 120 ft. The wells, designed to provide safe drinking water by avoiding polluted surface waters, inadvertently tapped into arsenic-contaminated underground aquifers [2]. The use of Agent Blue during the Vietnam War and other industrial developments caused the levels of bio-available arsenicals to spike dangerously [20].

The arsenic contamination levels varied from 1 to 3,050 μg/L with an average arsenic concentration of 159 μg/L in rural groundwater samples from private small-scale tubewells. In a highly affected rural area, the groundwater used directly as drinking water had an average concentration of 430 μg/L. Analysis of raw groundwater pumped from the lower aquifer for the Hanoi water supply yielded arsenic levels of 240–320 μg/L in three of eight treatment plants and 37–82 μg/L in another five plants. Aeration and sand filtration that were applied in the treatment plants to remove iron lowered the arsenic concentrations to 25–91 μg/L, but 50 % remained above the Vietnamese standard of 50 μg/L. The arsenic in the sediments may be associated with iron oxyhydroxides and released to the groundwater by reductive dissolution of iron. Oxidation of sulfide phases could also release arsenic to the groundwater, but sulfur concentrations in sediments were below 1 mg/g. The high arsenic concentrations found in the tubewells (48 % above 50 μg/L and 20 % above 150 μg/L) indicate that several million people consuming untreated groundwater in rural areas might be at a considerable risk of chronic arsenic poisoning [3, 4]. According to Berg, arsenic contamination levels in aquifers of Vietnam are of the same order magnitude as in Bangladesh, where tainted groundwater contributed to what a World Health Organization report called the "largest poisoning of a population in history" [2].

11.2 The Toxicity of Agent Blue

Agent Blue contained 4.7 % cacodylic acid (also known as hydroxydimethyarsinine oxide or dimethylarsenic acid, DMAA), and 26.4 % sodium cacodylate as the active ingredients [13, 37]. Cacodylic acid (see its molecular structure on Fig. 11.1) is a colorless, odorless, and hygroscopic crystalline solid. It was also commercially available in the form of soluble concentrates. Water solutions of cacodylic acid are sometimes dyed blue [24]. Cacodylic acid is 54 % arsenic [27]. Besides DMAA, another organic arsenical compound which form the active ingredients of pesticides and herbicides used mainly for weed control is monomethylarsonic acid (MMAA). MMAA and DMAA are also metabolites of inorganic arsenic formed intracellularly by most living organisms (animals, plants and bacteria) [16].

The routes of entry of cacodylic acid are by ingestion, inhalation, skin and eye contact (irritant), and absorption through the skin [24, 28]. Cacodylic acid is more readily absorbed into the bloodstream when inhaled [10]. It is metabolized by the liver and bioaccumulates in and is excreted by skin, nails and hair [12].

Unlike inorganic arsenicals, DMAA and MMAA do not bind strongly to molecules in humans. Hence, their acute toxicity seem to be less than that of the inorganic arsenicals [1]. However, recent studies indicate that trivalent organic arsenicals [e.g., MMA(III) and DMA(III)] that are metabolic products of inorganic arsenic could possibly be more toxic than the parent compound [25, 30]. The mechanism of toxicity of arsenic include enzyme inhibition and oxidative stress as well as immune, endocrine, and epigenetic effects [32].

Analytical determinations of arsenic poisoning may be made by examining arsenic levels in urine, hair and toenails. Communities and individuals relying on groundwater sources for drinking water need to measure arsenic levels to ensure that their supplies are safe, and communities with arsenic levels greater than 5 μg/L in drinking water should consider a program to document arsenic levels in the population [17].

11.3 Acute Adverse Human Health Effects of Cacodylic Acid

Acute poisoning symptoms and signs usually appear within 1 h after ingestion. In individuals who were severely exposed to organic arsenicals, garlicky odor of the breath and feces are experienced, and there may be a salty, metallic taste in the mouth, along with abdominal discomfort [10]. There may also be vomiting, profuse and watery diarrhea, followed by dehydration, electrolyte imbalance, and gradual fall in blood pressure [11].

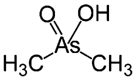

Fig. 11.1 Cacodylic acid (http://en.wikipedia.org/wiki/File: Cacodylic-acid-tetrahedral.png)

Acute effects on the central nervous system start as dizziness, headache, drowsiness and confusion, and may progress to weakened muscles, spasms, convulsions, stupor, general paralysis, coma, and possible death within 3–14 days. Death is usually the result of circulatory failure and possibly, renal injury [10, 11]. The oral lethal dose fifty, or LD50 for cacodylic acid in rats is 644 mg/kg [28].

Skin contact may cause irritation, burns, rash and loss of pigment. Eye contact may cause irritation, conjunctivitis and burns. Inhalation of cacodylic acid may irritate the nose and throat and cause ulcers to form on the nasal septum as well as perforations [23].

A study concluded that the inhibition of glucose uptake may contribute to the acute toxicity, especially of organic arsenicals, by further aggravating the depletion of intracellular carbohydrates [19].

11.4 Chronic Adverse Human Heath Effects of Cacodylic Acid

Because arsenic poisoning of humans can occur by gradual accumulation of small doses until lethal levels are reached, the use of Agent Blue and other organic arsenicals may pose a long-term danger [27].

Neurological symptoms are usually more frequent than gastrointestinal effects over prolonged exposure of organic arsenicals [10]. Cacodylic acid may cause paresthesias and/or weakness in the hands and feet [23].

The IARC classifies DMAA and MMAA as possibly carcinogenic to humans (Group 2B). Arsenobetaine and other organic arsenic compounds not metabolized in humans are not classifiable as to their carcinogenicity to humans (Group 3) [14]. DMAA induces single strand breaks in DNA, an organ-specific lesion, in the lungs of both mice and rats and in human lung cells in vitro. This damage may be mainly due to the peroxyl radical of DMA and production of reactive oxygen species by lung tissues [18].

Multi-organ initiation-promotion studies have demonstrated that DMAA acts as a promotor of urinary bladder, kidney, liver and thyroid gland cancers in rats and as a promotor of lung tumors in mice [18]. Lifetime exposure to DMA in diet or drinking water also causes a dose-dependent increase in urinary bladder tumors in rats [18, 34]. DMAA also has the potential to promote rat liver carcinogenesis, possibly via a mechanism involving stimulation of cell proliferation and DNA damage caused by oxygen radicals [33]. Besides this, DMAA has a promoting effect on skin tumorigenesis in K6/ODC transgenic mice [22]. Furthermore, DMAA strongly promotes tumorigenesis initiated by 4-nitroquinoline 1-oxide in the lungs of mice [36]. It has been proposed that cacodylic acid may cause lung carcinomas [12]. Pulmonary carcinogenesis may result from high-dose exposure to cacodylic acid because both inorganic arsenic and cacodylic acid share dimethyl- and tri-methylarsine as metabolites; the former has been associated with DNA damage in both rat and mouse lung tissue after high-dose oral acute exposures. Excess lung cancer has been reported in epidemiologic studies of smelter workers who were occupationally exposed primarily to pentavalent arsenic [31]. These and many more studies suggest that DMAA may play a role in the carcinogenesis of inorganic arsenic [18]. However, much of the carcinogenicity effect of organic arsenicals in humans still remain unknown.

A study led by Irvine concluded that margins of exposure estimated based on conservative estimates of daily intakes of arsenic in all of its forms indicate that exposure to MMAA or DMAA at environmentally relevant exposure levels, by the oral route is unlikely to pose a risk to pregnant women and their offspring [16]. In another study involving animals, cacodylic acid was administered to time-pregnant albino CD rats and CD-1 mice on days 7–16 of gestation, by gastric intubation as a solution in distilled water. Fetal and maternal toxicity was observed in both species. In the mouse, maternal toxicity was evident at the lowest dose, while teratogenic response was confined to cleft palate at 400 and 600 mg/kg/day. The effective maternal toxic dose in the rat was 40 mg/kg/day. In this species, incidence of irregular palatine rugae, i.e., ridges that were discontinuous and/or not lying in apposition at the palatal raphe, was significantly dose-related. The results suggest an “apparent no effect level” for this anomaly below 30 mg/kg/day [26].

Repeated skin contact may cause hyperpigmentation and keratosis. Malnourished people have been shown to be more predisposed to arsenic-related skin lesions [17]. White bands may form across the nails [10].

11.5 Conclusion

The acute and chronic effects of commercial organic arsenical herbicides which were used in a less toxic formulation than Agent Blue were described. Thus, we can expect a worse impact on those who were directly exposed to or directly handling Agent Blue in rice paddies during the Vietnam War [11]. Although the acute and chronic effects of organic arsenicals are not as many as that of the inorganic arsenicals [3], organic arsenicals still have a potential impact on human health which would be greater still as more studies in the future may uncover more of its currently unproven or unknown health effects.

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Chapter 12
Neurodevelopmental and Neurobehavioral Effects of Organophosphate Pesticides Exposure in Newborns and Children

**Cristina Florian, Alina Farcas, Andreea Valceanu Matei,
and Gheorghe Coman**

Abstract Pesticide use is an integral part of our modern society, whether we consider the developed or the developing nations, and their impact on human health is an important research subject. We chose to focus on the organophosphate (OP) pesticides because of their continued use and also because they are potent toxicants and neurological disruptors. Children and the newborn are more susceptible to the toxic effects of pesticides than the adult population. This paper summarizes the findings about neurodevelopmental and neurobehavioral effects of OP pesticides exposure in children from birth up to 7 years old. The major problems identified in the literature as the result of long term or sustained pesticide exposure of children pre- and postnatally are the presence of abnormal reflexes in newborns, mental and developmental delays, as well as the impact on behavior, memory and intelligence quotient (IQ) in older children and suggest the existence of a dose dependent inverse correlation between these effects and OP pesticide toxicity. It is important to understand and consider the seriousness of the pesticide problem in our society in order to find ways to mitigate and combat it.

Keywords Organophosphate • Pesticides • Exposure • Newborns • Children • Neurodevelopmental • Neurobehavioral

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12.1 Introduction

One of the most important public health problems in the prenatal and early life is the exposure to disruptive environmental agents with teratogenic and toxic effects. Some of the most prevalent disruptive agents are pesticides due to their continued use in agriculture, public health and medicine, these being the reasons why we can find them in appreciable quantities in water, air, soil and biota. From all of the different pesticide classes we chose to focus on the OP pesticides for their use as a substitute for the more toxic organochlorine pesticides and also because of the emerging evidence that they are, in their turn, potent toxins and neuroendocrine disruptors [5, 9, 10].

Humans become victims of these agents, but children are the most vulnerable to their hazardous effects because they are exposed both directly via the food and water they ingest and the air they breathe and indirectly, through their parents (Fig. 12.1), and because of the particularities of their metabolism. It has been shown that younger organisms have lower rates of metabolism for the enzymatic systems that handle toxins in general and pesticides in particular. It is believed that this is due to an age dependent variation in the maturation of these enzymatic systems, whether we look at the cytochromes, paraoxonase or carboxylesterase enzymes, which are the systems most active in OP pesticides metabolism [1].

The main mechanism of action of OP consists in the inhibition of acetylcholinesterase enzyme (hydrolase), leading to accumulation of acetylcholine in the synaptic cleft and the subsequent cholinergic syndrome [15]. Increasing new evidence suggests however, that there are other mechanisms that act on other enzyme systems that could be responsible for the neurodevelopmental and neurobehavioral effects of these compounds. The literature mainly refers to the activity of the biotransformation enzymes – cytochrome P and paraoxonase enzymes. These are responsible for the in vivo detoxification or activation of the various organophosphate pesticide substrates. Based on their level of expression and enzymatic efficiency, we can encounter degrees of organic manifestation in human children [1, 5].

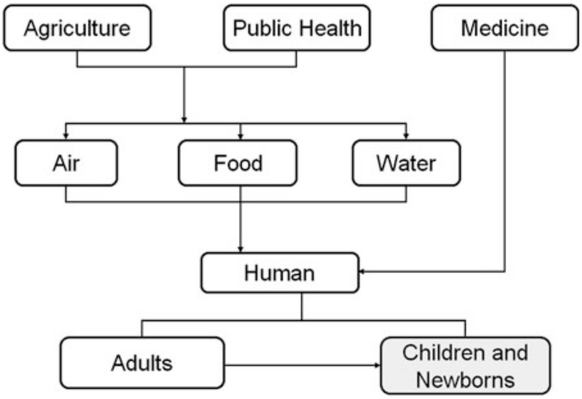

Fig. 12.1 Different pathways of children exposed

In this review we will discuss the neurodevelopmental and neurobehavioral implications of OP pesticides exposure in children and newborns. We aim to emphasize the seriousness and magnitude of this problem, the neurological effects of organophosphate pesticides poisoning in children being an important public health concern.

12.2 Findings and Results

In the studies that we considered, the characterization of the neurodevelopmental and neurobehavioral effects of OP pesticides [7] can be done according to at least three criteria:

- the age at exposure – pre – and postnatal [3];
- the level of exposure – low, chronic, acute or occupational [1, 11];
- the age (from birth up to 7 years old) at which the effects are quantified [12, 18].

Pesticides exposure was quantified by measuring either the concentrations of the pesticide itself (for example chlorpyrifos (CFP) as a model for OP pesticides), or the concentration of dialkyl phosphate (DAP) which is a metabolite and general biomarker for OP pesticide exposure, in blood or urine specimens [3, 13].

When considering the route of exposure or the environment that conditions this exposure, children of agricultural workers in particular have an increased risk of secondary pesticides exposure, during pesticides pulverization periods [14], and longer cohabitation with agricultural workers who handle organophosphate pesticides may be associated with lower acetylcholinesterase activity measured in maternal or children's blood, which may induce some neuropathological states in children or fetuses [16]. At the same time just as perilous has been proven to be the exposure that children face in urban environments, where the uses of OP pesticides for pest control significantly alters the air quality.

In these cases OP or OP residues may be presents in placental blood, children's blood or mother's milk, as circulating compounds.

The quantification of the effects of OP pesticides in children varies according to the age at which the evaluation is done, the level of exposure identified and the nature of the effect under investigation (Table 12.1). The findings suggest the existence of a dose dependent inverse correlation between OP pesticides exposure of children or their mothers as quantified by measurements of the chemicals in blood samples or of their metabolites in urine, and the indices for mental, psycho-motor and neurobehavioral development [8, 11, 17].

Young et al. demonstrated that newborns (less than 2 months old) exposed to OP pesticides in utero and postnatally exhibited abnormal reflexes (clinically relevant) on the Brazelton Neonatal Behavioral Assessment Scale [18]. These children were born to mothers from an agricultural community and exposure was quantified by urinary levels of DAP, dimethylphosphate and diethylphosphate metabolites. Also, according to other authors, children exposed in utero to indoor OP pesticides

Table 12.1 Neurodevelopmental and neurobehavioral effects of OP pesticides on children at different ages and exposures

Age[a]	Occupational exposure	Low-level chronic exposure
≤2 months	Absent or diminished reflexes	Absent or diminished reflexes
6 months 12 months	No statistically significant correlations	No statistically significant correlations
24 months	Mental development delays (which may be associated with PON1$_{\_108T}$[b] genotype) Increased risk of PDD[c]	Mental development delays
36 months	ADHD[d] Attention problems by maternal report	Mental development delays Psychomotor development delays PDD Attention problems ADHD problems
5 years	ADHD Attention problems by maternal report Especially in boys	No study/no results
7 years	No study/no results	Lower IQ Poorer working memory (cognitive deficit)

[a] Age of evaluation
[b] 108T allele – paraoxonase 1 enzyme
[c] Pervasive developmental disorder
[d] Attention deficit hyperactivity disorder

(New York City) exhibited abnormal reflexes on the same Brazelton Neonatal Behavioral Assessment Scale [2]. The exposure was quantified by analyzing maternal urine samples, and the higher levels of diethylphosphate and its metabolites were found to be correlated with a higher incidence of abnormal reflexes in newborns.

The neurological reflexes of newborn children characterize their current neurological status and indicate the direction of their future neurological development, hence, it is very important to discover early the existence and identify the causes of these abnormal reflexes (absent or diminished reflexes) in order to minimize the impact that OP pesticide toxicity has on them.

For children aged 6–12 months there are some existing studies but they didn't find statistically significant correlations between OP pesticides exposure and the appearance of neurodevelopmental and neurobehavioral symptoms [3, 11]

In regards to older children, at 24 months, a study on farm-worker families found a negative association of prenatal exposure to OP pesticides with mental developmental delays and pervasive developmental disorder. They used Bayley Scales of Infant Development (Mental Development indices) and measured six DAP metabolites in maternal and child urine [3].

In a more recent study researchers reported that the negative associations between DAP and mental development indices was strongest in children with PON1$_{\_108T}$ (108T allele – paraoxonase 1 enzyme). It has been identified in the literature that the enzymatic systems responsible for pesticide detoxification present a certain genetic polymorphism. Based on these findings, the effects of pesticide exposure have been quantified, and the observation was made that certain alleles render the carrier more or less susceptible to the toxic effects of OP pesticides. The presence of variant of $PON1_{\_108T}$ the paraoxonase enzyme has been linked to an increased susceptibility of children to these effects and a higher incidence of mental, developmental and behavioural problems [4].

According to Rauh, children at 36 months had an association between CPF (chlorpyrifos) levels in umbilical cord plasma and the following neurodevelopmental and neurobehavioral problems: mental development delays, psychomotor development delays, pervasive developmental disorder, attention problems, ADHD (Attention deficit hyperactivity disorder) problems. They reported that the higher the level of CPF measured in umbilical cord plasma the more important were the neurological symptoms identified. Measurements were made using the Bayley Scale of Infant Development II and the Child Behavioral Checklist. The same article also found an association between CPF burden and mental developmental delays in 24 month old children [11].

One of the most important aspects for children in the 2–3 years old group is the continued remodeling of their neurological pathways. This process, which has started in utero and continues through childhood and adolescence, is at risk in the presence of neurotoxins such as OP pesticides. The continued exposure of children to these chemicals leads to an impaired modeling of the neuronal structure, which is reflected in the mental and behavioral development described. These children are therefore at risk for early school failure and more serious behavioral problems which can lead to an altered social and professional integration.

Another recent study of subjects in an agricultural area investigated the correlation between urinary DAP metabolites measured in mothers and in their children, and attention problems and ADHD symptoms in these children. Children tested positive using Conners' Kiddie Continuous Performance scale at 3.5 and 5 years old; also, for the same age ranges, there were maternal reports of attention problems (especially in boys) measured using the Child Behavior Checklist [8].

ADHD is a more and more pervasive problem in the child population in the recent years. It is important for preschool aged children who could be suffering from ADHD to be identified and treated properly in order to mitigate its lifelong effects. In order to forestall the appearance of learning and behavioral problems, to insure an appropriate social integration and a healthy parent–child relationship, families of these children need to receive parenting advice and support [6].

In terms of school-aged children at 7 years old, there was evidence of a positive association between prenatal CPF exposure from air pollution and a measurable decline in IQ and deficits in working memory (cognitive deficits). Researchers used the Weshsler Intelligence Scale and the CPF toxicity was quantified by testing the umbilical cord blood plasma [12].

These problems (IQ and working memory deficits) have an important negative influence on children's life, conditioning their scholar achievements and professional future on one hand, as well as impacting their family and social lives.

12.3 Conclusions

The main route of exposure was ingestion of contaminated food and water for children in all of the studied environments. There were also significant contributions to intoxication from the quality of the air inhaled (indoor or outdoor), whether in agricultural settings where pesticides are used regularly or in urban environments where they are used as part of the disease prevention strategies.

The presence of pesticides in children's bodies alters their normal development at the mental, psychomotor and behavioral levels, depending on quantity of OP pesticides, method of exposure (directly, indirectly), period of exposure, ages of exposure (exposure time) etc. compromising their proper functioning and integration in society.

It is important to consider these and other similar findings as a warning signal for the seriousness of the pesticide problem in our society. This highlights the need for the dissemination of information regarding this issue and of implementing strategies for prevention and treatment.

Further research is needed in this area in order to verify the correlations described by these studies, to strengthen the statistical significance of some of the trends that emerged as well as to further characterize the mechanisms and causalities that underline these phenomena.

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Chapter 13
Organophosphate Pesticide Exposure in Humans – Is Prevention Possible?

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Abstract In the current context of economic development and population expansion the use of some form of pest control is essential for the maintenance of a high agricultural productivity, of a strict control over the potential vectors of disease and of efficient methods of intervention with least impact on the health of the humans and the environment. However, the current reality shows an alarming number of adverse events in connection with pesticide usage, some of them implicating humans, other affecting other living creatures and the ecosystem. Many of the adverse events are reported in rural situations and in developing countries, raising the question of information dissemination and education about the effects of pesticides on human health and also of the existence and implementation of safety methods and techniques. Also, at the local level, we can raise the issue of educating the public about the methods and behaviors that can reduce contact with environmental pollutants, promote health in the context of pesticide exposure and mitigate health effects of intoxication. The context of organophosphate pesticides use is a complex one, covering agricultural applications, pest management and medical uses, and the possibility of intervention in the direction of reduction and prevention of adverse health effects appears at every level of implementation and management of pesticide usage.

Keywords Pesticide • Organophosphate • Prevention • Human • Naturopathic model

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13.1 Introduction

Our human society has evolved in a manner that is more and more heavily dependent on chemical compounds for its continued survival. This arms race finds itself reflected in healthcare in several aspects, from the continuous battle between man and microbes to the struggle to overcome the adverse health effects of various chemical compounds that man has created.

Among the substances that constitute a serious threat to health we can list pesticides. Throughout time, humans have competed with other living creatures for the resources available, and from this paradigm aroused "the need" to gain the upper hand in this race. Combating the natural predators of the various food sources humans have, continues to be an issue to this day [4]. On the other hand, public health challenges have been addressed by using some of the same pesticides in combating parasites and pests that were a direct threat to human health in situations characterized by less than ideal economic and social situations [10].

The substances used historically have evolved, from basic compounds found in nature (of mineral or plant origin) to synthetic pesticides [3]. Of interest in the realm of synthetic pesticides are the organophosphate (OP) pesticides, which came to replace the more environmentally persistent, bioacumulating and biomagnifying organochlorine compounds. However, this class of pesticides, in spite of being less persistent in the environment, presents a high toxicity for humans [5]. We focused here on the possible methods of prevention and reduction of exposure to OP pesticides, which represent more than half of the insecticides currently in use [17].

Globally, the main activity areas where we use pesticides are in the agriculture, in the industry/community/governmental sector and finally the home and garden sector. From the total of 2,360,000 tons of pesticide used worldwide in 2007, about 17 % were insecticides, amounting to a rough estimate of 405,000 metric tons [16].

The use of this class of pesticides becomes in itself a public health concern not only because of the large impact that it has on human health [6], but also due to the varied and sometimes insidious routes through which humans come into contact with them. There are several situations where humans become exposed to these compounds in such high quantities: occupational exposure in agricultural workers who come in direct contact with the pesticides [7], chronic exposure to foods or materials contaminated with these pesticides [11], the use of pesticides for addressing public health problems, accidental exposure to significant quantities of pesticides and suicide attempts [8]. For the purpose of the present work, we will not include in discussion the intentional use of pesticides for self-poisoning, which in itself is a serious concern for public health and has made the subject of numerous studies.

13.2 Health Effects of Organophosphate Pesticide Exposure

Few people realize the real impact that these chemicals have on human health. The most obvious, is the induction of adverse health effects after acute high dose exposure, where the most prevalent is the cholinergic syndrome/crisis that could lead to death, the intermediate neurotoxic syndrome and later on the delayed polyneuropathy [2]. The chronic low-dose exposure is less studied, however there is mounting evidence of the adverse health effects that develop over time, with a certain latency period, after this type of exposure. The most common conditions that have been encountered after chronic human exposure to OP pesticides fall in one of the following categories:

- reproductive effects – such as birth defects, growth alteration, altered fertility;
- neurotoxic effects – seen in the array of neurodevelopmental, neurobehavioral and neurodegenerative diseases that have been linked to such exposure;
- genotoxic effects – mainly represented by chromosomal aberrations resulted after exposure, which may or may not lead to the development of various types of cancers [1]; and last but not least;
- dermatologic effects [13].

13.3 Management of Pesticides

Pesticide use is widespread across the globe, countries in the first world employing them just as much as third world countries do, in an attempt to increase agricultural productivity, stave off diseases, improve the standard of living and overall maintain and even improve the human quality of life [4].

However, there are differences from one country to another when it comes to the type of pesticide employed because different areas face different needs in pest control, countries face financial and public health problems of differing magnitudes from one area to another and some more researched chemicals which have proved too harmful for humans have been phased out or banned in first world countries where costs are easier to cover, while in other countries, with lower budgets and an even lower penetration of information they are still used.

Therefore at the global scale, the groups of population most at risk of harmful effects from pesticide exposure are:

- agrarian populations when compared to inhabitants in non-agrarian areas, and the trend is maintained across the globe without regard to the level of economic development;
- less educated individuals compared to more educated ones – due to the ability to become informed with respect to the adverse effects of such substances and to take the appropriate measures for protection for themselves and others;
- inhabitants of less developed nations compared to more developed ones – because of the trend to unload unwanted pesticides from the first towards the third world, the more permissive legislation in such areas, the pervasiveness of corruption and the lack of public information and transparency in these countries.

When we consider the management of pesticides, it is important to realize the need for a coherent action plan in order to mitigate adverse effects of pesticide use and set the stage for their responsible management from the phases of research and

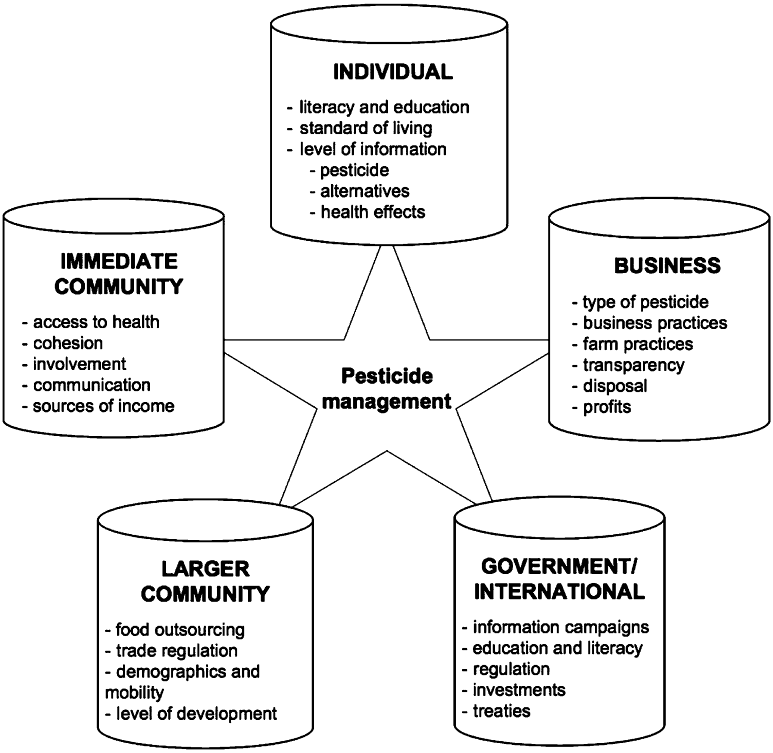

Fig. 13.1 Areas of pesticide management

development, through production, storage, dispensation and disposal. Every one of these stages implies human handling and human and environmental contact, and at every stage there are implications of the decision making process.

There are several areas of influence that are involved with the issue of responsible pesticide use, from the individual and his/her immediate community, to the larger community and its various forms of organization – businesses, governments and international groups (Fig. 13.1).

Each of these levels maintains a certain level of control and a particular degree of responsibility attached to their place in the network, an effective strategy only being possible when combining them all.

Transposing these areas of influence towards concrete actions that can become part of a coherent strategy, we can identify several levels of intervention (Fig. 13.2). The big picture is outlined by the methodology and regulation necessary at the local, regional and international levels, based on targets set through international negotiation and cooperation. These targets are informed by the new developments and findings provided by research and all of the information needs to be publicized, disseminated and emphasized at all of the levels of society.

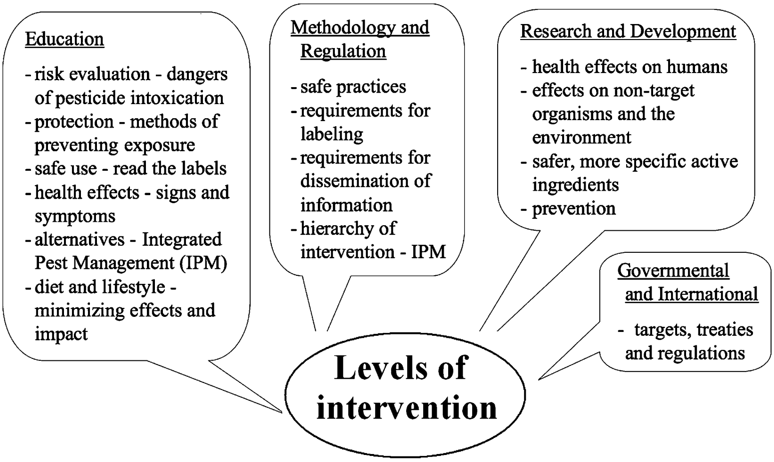

Fig. 13.2 Levels of intervention for pesticide management

Education is, in this respect, the most important vehicle for accomplishing this task. It has many facets and valences that need to be carefully considered and employed.

When considering prevention, it is very important that all the participants to the pesticide flow be aware of the nature of the chemicals they are handling, the proper measures for protection and the appropriate routes of dispensing and disposal.

Also, an important aspect alongside the knowledge about the nature of the pesticide is the accurate information regarding the health effects (symptomatology) of these chemicals, and the manner in which these can be mitigated – dietary choices (organic versus conventional foods, foods rich in antioxidants, food combinations); appropriate prevention measures concerning safety and exposure; timely treatments that are specifically tailored to the problem at hand [9].

13.4 Naturopathic Earth Care

In the recent years, the concept of integrated pest management has surfaced in connection with the attempt to reduce the impact of pesticides on the biosphere. It consists of a series of principles that guide the choices that are made in each and every pest problem encountered. It is based on thorough and intimate knowledge of the pest at hand (its habits, lifecycle and needs), of the available methods of intervention (from the least to the most invasive) and of the ecology of the loco-regional area where the problem exists. This complex knowledge informs the decision making process, helping to achieve the right amount of intervention for the problem at hand.

A very interesting parallel can be made here with the concepts postulated by naturopathic medicine. Naturopathy as a philosophy of life and health is based on

Table 13.1 Basic percepts of medicine. Naturopathic/medical percepts

PRIMUM NON NOCERE – First do no harm
Try to minimize harmful side effects and avoid suppression of symptoms
VIS MEDICATRIX NATURAE – The healing power of nature
Seek to identify and remove obstacles to the body's natural processes for maintaining and restoring health
TOLLE CAUSAM – Identify and treat the cause
Focus on the causes of a disease or condition, rather than its symptoms
DOCERE – Physician as teacher
Educate patients and encourage them to take responsibility for their own health (partnership for health)
HOLISM – Treat the whole person
Consider all factors – physical, mental, emotional, spiritual, genetic, social environmental when tailoring treatment to each patient
PREVENTION
Assess risk factors and susceptibility to disease, and make appropriate recommendations to maintain health and prevent illness

the same six percepts for health that govern medicine in general [15]. These have been coined centuries ago and medical practice is using them to a greater or a lesser extent (Table 13.1).

Based on these percepts, naturopathic medicine has drawn up a therapeutic order that aims to match the force of intervention with the magnitude of the problem. In this respect, each individual case will be met with the least force necessary to correct the problem and allow the organism to heal. The quintessential concept underlying this philosophy is that the physician's job is not to heal the patient but to aid the patient's own natural and inherent healing process [14].

Using the pattern of growing complexity from cells to organs to individuals, we can extrapolate further from individuals to groups/communities to ecosystems to the point where the whole biosphere becomes a self-regulating "organism". In this manner we can apply the same concepts of the therapeutic order to the biosphere in regards to pesticide usage and its effects, and health care becomes earth care.

At this point we become aware of the parallel between naturopathic medicine and integrated pest management (Table 13.2).

A core aspect of the naturopathic philosophy is that treatment must be individualized to meet the needs of a particular patient and the treatment of any disease is the treatment of the person. Similarly, every situation that requires the use of pesticides can be ranked on the scale of severity and matched with a solution that necessarily takes into account first the prevention, then the integrity of the system treated and the existing alternatives before synthetic pesticides are employed. Also of utmost importance is the rational use of pesticide, where just as in pharmacology we match the pest with the proper chemical in the right amount for the right length of treatment time.

In human health care, the concept of suppression is used for the situation where a treatment employed, which overmatches the magnitude of the problem, leads to a false cure of the patient, instead pushing the disease deeper into the system and masking the real symptoms. On the surface, the patient has been healed of the current

Table 13.2 Naturopathic therapeutic order versus integrated pest management

Naturopathic therapeutic order	Environmental therapeutic order/ *Integrated pest management*
Reestablish the basis for health	*Crop rotation*
Remove obstacles to cure by addressing the determinants of health	Maintain the health of the soils by rotating crops and reducing the likelihood of pest overgrowth/overpopulation and soil depletion due to monocultures
Stimulate the healing power of nature	*Pest resistant varieties*
Stimulating the healing power of nature by using nutritional, hydrotherapy, psycho-spiritual methods	Select those varieties of crops that can yield the most for the particular area
Tonify weakened systems	*Natural enemies*
Strengthen immune system, decrease toxicity, normalize inflammation, optimize metabolic function, balance regulatory systems, enhance regeneration, harmonize life force	Foster the natural ecosystem in the loco-regional area; in the local ecology each living organism has its own place in the pray-predator chain
Correct structural integrity	*Combined* cultures
Therapeutic exercise, manipulation, massage, physiotherapy	Intercalate crops on the same planting area, in order to use the natural repelling properties of some plants
Prescribe natural substances/modalities/ interventions	Use of *biopesticides* [12] and other *naturally derived* pesticides
Specific for pathology	
Prescribe pharmacological substances	Use of synthetic pesticides
Specific for pathology	*Responsible chemical use*
Use surgery, suppressive drugs, radiation, chemotherapy	
Specific for pathology	

ailment, but the real disease has been aggravated. Similarly, in earth care, we can observe situations that are met with a force of intervention which is too high or disproportionate compared to the problem at hand, and the ecological implications are loud, serious and long term.

13.5 Conclusion

The effects of pesticide use have to be recognized as a global issue, even though their use is regional or local. Recognition has to be given to the fact that our planet's ecosystem has checks and balances through which it is attempting to maintain its homeostasis, and just as the living organism circulates toxins using blood or sap, the biosphere has its recirculation cycles. This concept helps us understand how our actions at the local level will have global implications, and motivate us to look for alternatives and solutions for which the benefits outweigh the costs for all of the parties involved.

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Chapter 14
Degradation of Pesticides by TiO_2 Photocatalysis

Alina Dârjan, Camelia Drăghici, Dana Perniu, and Anca Duţă

Abstract Extensive research has been conducted on the removal of organic pollutants in water using heterogeneous photocatalysis. This study reviews some of the recent reports on TiO_2 photocatalysis applied for degradation of pesticides. The role of the operating parameters in the optimization of the process, the strategies used to develop visible active titania based photocatalysts and various ways to increase the cost-effectiveness of the photocatalytic processes are discussed. From the papers reviewed it can be concluded that under optimal conditions high efficiency in the photodegradation of pesticides can be reached.

Keywords Photocatalysis • Titanium dioxide • Pesticide

14.1 Introduction

Pesticides are widely used nowadays as a result of the intensive agricultural practices. Industrial effluents, agricultural runoff and chemical spills have led to widespread contamination of the environment with these bio-recalcitrant organic compounds [19]. Their removal from contaminated water bodies, soil and air represents a must, since most pesticides are toxic, chemically stable, low-biodegradable and can enter the food chain, undergoing accumulation in the body tissues of living organisms [4, 32]. Pollution with pesticides, despite being usually at low concentration, has raised substantial health concern, because it is known that toxicity occurs at very low amounts [23]. The removal of pesticides requires effective treatment measures. Advanced oxidation processes (AOPs) are considered promising methods for this purpose [37].

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AOPs rely on the in-situ generation of highly reactive and oxidizing radical species, which interact with organic pollutants leading to progressive degradation [33]. The main resulting species is the hydroxyl radical, a powerful oxidant and a highly reactive, non-selective reagent, which is easy to produce [24]. Among the different technologies proposed as AOPs, heterogeneous photocatalysis has been intensively studied and is considered an efficient technique to destroy organic pollutants in water, including pesticides, because the degradation runs at room conditions, it does not require expensive reagents and it has been reported to give, in optimized conditions, almost complete mineralization of organic substrates to carbon dioxide, water and inorganic acids [5].

Titanium dioxide is the most commonly used photocatalyst, because it is nontoxic, photochemically stable, has strong oxidizing power and relatively low cost [12, 19]. When TiO_2 is irradiated with photons with energy equal to or greater than its band gap energy ($E_g = 3.2$ eV [28]), electron–hole pairs are generated; holes, may subsequently lead mainly to the formation of hydroxyl radicals, but also superoxide and hydroxyperoxyl radicals. These oxidizing species attack the pesticides adsorbed on the surface of the photocatalyst or existent in the close vicinity [29].

The present study aims at highlighting the potential of titanium dioxide photocatalysis to degrade pesticides and offers a review of the most promising recent results, with emphasis on the measures applied to enhance the efficiency of the photocatalytic process.

14.2 Effects of Various Parameters on the Photocatalytic Degradation Process

14.2.1 Working pH

It has been widely reported that an important parameter in the photocatalytic degradation of organic compounds is the pH of the medium. This parameter is correlated with the photocatalyst surface charge, TiO_2 being amphoteric in aqueous solution [16]. Depending on the synthesis method, the point of zero charge (PZC) of titania varies from 4.5 to about 7 [23]. At pH below PZC, the surface of TiO_2 is protonated (Eq. 14.1), thus being positively charged and at pH above PZC, the surface is deprotonated (Eq. 14.2), being negatively charged [13]:

$$\mathrm{pH} < \mathrm{PZC} : \mathrm{TiOH} + \mathrm{H}^+ \rightarrow \mathrm{TiOH}_2^+ \tag{14.1}$$

$$\mathrm{pH} > \mathrm{PZC} : \ \mathrm{TiOH} + \mathrm{OH}^- \rightarrow \ \mathrm{TiO}^- + \mathrm{H}_2\mathrm{O} \tag{14.2}$$

The size of the aggregates formed by TiO_2 particles is also affected by pH. When the pH is close to PZC, the photocatalyst particles tend to agglomerate, forming large clusters [23]. This diminishes the surface area of TiO_2 and the amount of active sites and, so, the photodegradation rate is reduced.

The pH of the medium influences the ionization or speciation of organic compounds, which affects the electrostatic interactions between the photocatalyst and the pollutant molecules, [1]; if the adsorption is faster than photocatalysis, surface clogging occurs and the overall process efficiency is lowered down to zero.

The formation of hydroxyl radicals (generated through the reaction between hydroxide ions and holes) is also influenced by the pH of the medium. It was reported that in alkaline solutions, due to the presence of more hydroxide ions on titania surface, hydroxyl radicals are easier formed, which can increase the photodegradation efficiency [2]. Liu et al. [19] reported a two times higher degradation efficiency of methamidophos under alkaline conditions compared to acidic ones. Wu et al. [39] also observed an increase in the photodegradation rate of terbufos (unionizable compound) under alkaline pH. Similar results were reported in the degradation of thiram [15] and trichlorfon [20].

14.2.2 Photocatalyst Amount

Catalyst loading is an important parameter for an efficient photocatalytic degradation process. An increase in the amount of catalyst leads to an increase in the number of active sites available for the generation of highly reactive radicals. However, if the concentration of the catalyst exceeds an optimum value, catalyst particles may agglomerate, causing a reduction in the surface area, which means less available sites for photon absorption [2]. Moreover, at high photocatalyst concentration the degradation rate may decrease due to light scattering and screening effects [8]. Liu et al. [19] observed an increase in the photodegradation efficiency of methamidophos with the increase of TiO_2 concentration, but over a certain amount the efficiency slightly decreased. Similar results were reported for the photodegradation of terbufos [39], thiram [15], benomyl [25], bentazon [26], carbendazim [30]. The optimum amount of photocatalyst determined in these studies varied significantly, from 0.07 to 12 g/L, showing that the nature of the pesticide and the other operational parameters have important influences on the photocatalytic degradation efficiency.

14.2.3 Initial Pesticide Concentration

Another important parameter, whose influence should be studied, is the initial pollutant concentration. With the increase of the amount of the organic compound, more and more molecules adsorb on the photocatalyst surface, the photonic efficiency diminishes and the TiO_2 surface becomes saturated, causing the deactivation of the photocatalyst [13]. Park [25] investigated the effect of initial concentration of substrate on the photodegradation of benomyl. The degradation rate decreased with the increase of initial benomyl concentration, as result of possible UV-screening of the benomyl molecules. Similar results were obtained by Pourata et al. [26] in the photodegradation of bentazon and by Liu et al. [19] in the photodegradation of methamidophos. The degradation efficiency was reduced almost by half with the doubling of methamidophos concentration. Still, at concentrations close to, but above the standard discharge limits (ng/L), the real problem is diffusion, that may lead to very long degradation durations.

14.2.4 Irradiation Time

For a cost-effective process, the irradiation time should be as low as possible. Liu et al. [20] reported an increase in the photodegradation efficiency of trichlorfon from 33.8 to 96.5 % when the illumination time increased from 0.5 to 4 h. The photodegradation efficiency of methamidophos also increased substantially (from 19.6 to 87.7 %) with an increase in the irradiation time from 10 to 110 min [19]. Kaneco et al. [15] observed the complete disappearance of thiram after a short irradiation time- 30 min. However, the complete mineralization was observed only after 20 h of irradiation. This aspect is critical in pesticides degradation: oxidation without or with partial mineralization is to be avoided, since the by-products may have a toxic or pollutant potential even higher than the initial compound and they are much more stable.

The mineralization process can be monitored through reduction in total organic carbon (TOC) value or analysis of the chemical oxygen demand (COD) of the irradiated solution [23]. Yahiat et al. [40] reported a 90 % mineralization yield of cyproconazole within 50 h of UV irradiation. Mahmoodi et al. [22] reported a 90 % decrease in COD of fenitrothion solution after 4 h of UV irradiation. Some of the intermediates produced in the degradation process are considered more toxic than fenitrothion to aquatic organisms. Complete mineralization of propoxur was achieved within 8 h of UV irradiation using TiO_2/Hβ as photocatalyst [21]. Araña et al. [3] reported a complete mineralization of pyrimethanil and triadimenol after 7.5 h of irradiation and a slower mineralization rate of two commercial mixtures of pesticides (Folimat and Ronstar)- 82–83 % TOC reduction within 10 h of irradiation.

14.2.5 Electron Acceptors

The addition of electron acceptors to the photocatalytic system can enhance the photodegradation efficiency of organic compounds by preventing the charge carriers recombination and because more oxidizing species are produced [27]. Molecular oxygen is widely used as an electron acceptor [14], but hydrogen peroxide is considered to give better results [27], because it can also act as a direct source of hydroxyl radicals due to homolytic scission [14]. Liu et al. [19] studied the influence of H_2O_2, $K_2S_2O_8$ and $KBrO_3$ on the photocatalytic degradation of methamidophos under UV irradiation. The degradation efficiency reached 71.5, 78.4 and 81.4 % when adding optimum amounts of H_2O_2, $K_2S_2O_8$ and $KBrO_3$, respectively. Fenoll et al. [11] reported an enhancement of the photodegradation of cyprodinil and fludioxonil under solar radiation when adding an optimum concentration of $Na_2S_2O_8$. Metal ions, such as Fe^{3+} and Cu^{2+} can also act as electron acceptors in photocatalytic systems, as reported by Liu et al. [19, 20]. In both studies, the addition of a small amount of Fe^{3+} and Cu^{2+} caused the increase in the photodegradation efficiency of the two pesticides (methamidophos and trichlorfon).

14.2.6 Concurrent Species in the Wastewater

It has been reported that several species normally present in surface and ground water, such as HCO_3^-, CO_3^{2-}, Cl^-, or usually present in wastewaters can inhibit the photocatalytic degradation of the target compounds [10], as being usually in much larger amounts than the critical concentrations of pesticides. Wu et al. [39] observed a significant inhibition of the degradation rate of terbufos, in the presence of Cl^- and NO^{3-}, due the scavenging of holes and hydroxyl radicals by the adsorbed ions on the TiO_2 surface. Lhomme et al. [18] tested the efficiency of TiO_2 coated paper on the degradation of two commercial pesticide formulations. The results showed that the additives had a significant effect on the degradation of the active species (chlortoluron and cyproconazole), because they were probably degraded by photocatalysis in competition with the target pollutants.

14.3 Strategies to Enhance the Photoactivity of TiO_2 Under Visible Light Irradiation

TiO_2 (anatase) has a wide energy gap, of 3.2 eV, which is disadvantageous, because it can only be activated by UV radiation ($\lambda < 385$ nm). Ultraviolet radiation represents only a small fraction (4 %) of the incoming solar energy, whereas visible light corresponds to 43 % of it [41]. Various methods were used to shift the absorption threshold towards visible region: doping with cations or anions, metals coating, coupling with another small-band-gap semiconductor or dye sensitization [7]. Senthilnathan and Philip [31] examined the effectiveness of Ag^+, Fe^{3+}, Cr^{3+}, Cr^{3+}-N and N doped TiO_2 on the photodegradation of lindane under visible light irradiation. Nitrogen doped TiO_2 exhibited the highest photoactivity (complete degradation of lindane). Devi et al. [9] reported a high efficiency of Th^{4+}–TiO_2 in the photodegradation of chlorpyrifos under solar light irradiation, which was attributed mainly to the large red shift in the band gap (482 nm). Multi-walled carbon nanotubes (MWCNTs)/TiO_2 composite, developed by Wang et al. [38], was more efficient than pure TiO_2 in the degradation of 2,4-dinitrophenol, under solar irradiation. Kuo et al. [17] reported an enhanced degradation rate of carbaryl under solar irradiation when using a dye photosensitizer (methylene blue and rose Bengal).

14.4 Immobilized TiO_2

Photocatalytic processes can be performed with the photocatalyst suspended in aqueous media or immobilized on support materials. Suspensions of photocatalysts should be avoided if scaling-up is targeted, because they require particle-fluid separation after the treatment, which is a laborious, expensive process and because they have low quantum efficiency. Therefore, immobilized photocatalysts were developed, using various inert substrates: glass plates, films, glass fibre, ceramic, activated carbon, Rasching rings, nonwoven paper, fabrics, zeolites, molecular sieve materials etc. [6, 34, 42]. Suitable supports should be photochemically and mechanically stable, able to delocalize electrons and prevent leaching of the photocatalyst during irradiation [32]. Sharma et al. [32] reported a complete photodegradation of isoproturon, imidacloprid (commercial solution) and phosphamidon (commercial solution) using TiO_2 supported on SBA-15, a mesoporous silica sieve. Similar results were reported for the degradation of isoproturon using TiO_2 immobilized on H-mordenite (H-MOR) [33], Al-MCM-41 [34], zeolite HY [35] and porous nanosilica [36] as photocatalysts. In all cases, the higher efficiency observed using bare titania was attributed to the electrons delocalization and high adsorption capacities of the support material. TiO_2 supported on Hβ zeolite was reported by Mahalakshmi et al. [21] to exhibit a higher degradation rate of propoxur than bare TiO_2, due to a better adsorption capacity, charge carriers separation and inhibition of light scattering and particles aggregation.

14.5 Studies Upon Photocatalyst Recycling

To implement a cost-effective process, the photocatalyst must be available for a large number of working cycles, maintaining its photoactivity and stability. The possibility to reuse the catalyst TiO_2/SBA-15 was investigated by Sharma et al. [32] and the re-activation was found to be efficient by calcination. Similar results were reported for the photocatalysts TiO_2/H-MOR [33] and TiO_2/Al-MCM-41 [34]. The reproducibility of MWCNTs–TiO_2 catalyst in the degradation of dinitrophenol was studied by Wang et al. [38]. A catalysis efficiency of more than 96 % was reported even after the usage of the photocatalyst for five cycles, proving its stability.

14.6 Conclusions

Among various AOPs, heterogeneous photocatalysis using TiO_2 represents a viable solution for pesticides removal. Several problems need to be addressed: (1) the very low pollutant concentration, thus requiring highly efficient and long duration processes; (2) the need to effectively attend mineralization for avoiding toxic by-products, thus a careful oxidation control; (3) scaling–solutions, involving VIS-activated immobilized photocatalysts. Although encouraging results are reported at laboratory scale, there still is room for improvement in all three directions.

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Chapter 15
Safe Management and Disposal of Obsolete Pesticides (DDT) from the Institute of Public Health

Mihail Kochubovski

Abstract Institute of Public Health of the Republic of Macedonia as a public health and scientific-research facility as a result of performing its work, produce certain amount of medical waste, as well hazardous chemical waste. In the Waste Management Law, Official Gazette of the Republic of Macedonia No.68/04 and 107/07, in article 6 hazardous waste is defined as a waste that contain substances which have one or more hazardous properties, such as: explosively, reactivity (oxidants), flammable, irritation, toxicity, infectivity, carcinogenicity, mutagenicity, reproduction toxicity, eco-toxicity and properties of release poisonous gases under contact of water, air or acid, defined in accordance with this Law or other Regulative, and subsequently in the List of types of waste (Official Gazette of the Republic of Macedonia No.100/05) is noted and separately labelled as a hazardous waste, including each waste that is mixed with hazardous waste. Management with chemical waste, respectively collection, separation and its storage is properly solved in the frame of the Institute of Public Health of the Republic of Macedonia in context of the above mentioned Law. But, real problem is disposal of chemical waste produced in the laboratories of the Institute of Public Health. Related to the needs of solution of collection and transport of chemical waste produced in the former period, as well the possibility of eventual integral solution of the chemical waste problem, Institute of Public Health has conducted a situation analysis of quantities and content of hazardous chemical waste (as well DDT), which has remained in the chemical waste depots of the Institute of Public Health and at the regional Centers of Public Health. According to the Basel Convention that determines the rules and conditions for control of transboundary transport of hazardous waste and its storage, ratified by our side (Official Gazette No.49/97),

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there was a possibility for export of hazardous waste under conditions proscribed in the Convention and with an agreement with other side where exists conditions for treatment of it. The aim of the paper is to review challenges, experiences, and health-related risks linked to safe management and disposal of hazardous chemicals especially DDT stored in the Institute of Public Health and Centers of Public Health in the Republic of Macedonia. The final goal is to protect public health, especially Institute's staff and people that could be exposed to hazardous chemicals.

Keywords Public health • Safe management • Disposal • DDT

15.1 Introduction

The term "pesticide" originates from two Latin words: pest and cido. Pest – is an organism which causes destruction, disease or injury on plants and animals. Cido – means destroy or kill. Pesticides are mainly chemical compounds either artificially synthesized or extracted from plant products. Pesticides from biological agents have been developed lately. The term "pesticide" includes chemical substances, preparations or organisms used in pest control [8].

Prior to 1972 when its use was banned, DDT was a commonly used pesticide. Although it is no longer used or produced in the United States, we continue to find DDT in our environment. Other parts of the world continue to use DDT in agricultural practices and in disease-control programs. Even though DDT has been banned since 1972, it can take more than 15 years to break down in our environment.

Harmful effects of DDT are:

- Probable human carcinogen;
- Damages the liver;
- Temporarily damages the nervous system;
- Reduces reproductive success;
- Can cause liver cancer;
- Damages reproductive system.

Exposure to DDT is possible through:

- By eating contaminated fish and shellfish;
- Infants may be exposed through breast milk;
- By eating imported food directly exposed to DDT;
- By eating crops grown in contaminated soil (EPA [4]).

Chemicals affect our everyday lives. They are used to produce almost everything we use, from paper and plastics to medicines and food to gasoline, steel, and electronic equipment. More than 70,000 chemicals are used regularly around the world. Some occur naturally in the earth or atmosphere; others are synthetic, or human-made. When we use and dispose of them properly, they may enhance our quality of life. But when we use or dispose of them improperly, they can have harmful effects on humans, plants, and animals. Even when used properly, many chemicals can still harm human health and the environment. When these hazardous substances are thrown away, they become hazardous waste. Hazardous waste is most often a by-product of a manufacturing process – material left after products are made. Some hazardous wastes come from our homes: our garbage can include such hazardous wastes as old batteries, bug spray cans, and paint thinner. Regardless of the source, unless we dispose of hazardous waste properly, it can create health risks for people and damage the environment (EPA [5]).

The text of the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and their Disposal was adopted on 22 March 1989 and entered into force on the 90th day after the date of deposit of the 20th instrument of ratification, acceptance, formal confirmation, approval or accession by a country to the Convention, 5 May 1992. The overarching objective of the Basel Convention is to protect human health and the environment against the adverse effects of hazardous wastes. Its scope of application covers a wide range of wastes defined as "hazardous wastes" based on their origin and/or composition and their characteristics, as well as two types of wastes defined as "other wastes" – household waste and incinerator ash. Goals of the Basel Convention are:

- the reduction of hazardous waste generation and the promotion of environmentally sound management of hazardous wastes, wherever the place of disposal;
- the restriction of transboundary movements of hazardous wastes except where it is perceived to be in accordance with the principles of environmentally sound management;
- a regulatory system applying to cases where transboundary movements are permissible [6].

15.2 Material and Methods

It was used prospective environmental – epidemiological study to explore the quantity and content of hazardous waste stored in the Institute of Public Health and Centers of Public Health in the Republic of Macedonia. As well it was studied the manner of safe management of this hazardous waste. Study has been conducted from 2009 to 2011.

15.3 Aim

The aim of the paper is to review challenges, experiences, and health-related risks linked to safe management and disposal of hazardous chemicals especially DDT stored in the Institute of Public Health and Centers of Public Health in the Republic of Macedonia.

The specific goal is to protect public health, especially Institute's staff and people that could be exposed to hazardous chemicals.

15.4 Results and Discussion

Institute of Public Health of the Republic of Macedonia (IPH) as a public health and scientific-research facility as a result of performing its work, produce certain amount of medical waste, as well hazardous chemical waste.

In the Waste Management Law, in article 6 hazardous waste is defined as a waste that contain substances which have one or more hazardous properties, such as: explosively, reactivity (oxidants), flammable, irritation, toxicity, infectivity, carcinogenicity, mutagenicity, reproduction toxicity, eco-toxicity and properties of release poisonous gases under contact of water, air or acid, defined in accordance with this Law or other Regulative, and subsequently in the List of types of waste (Official Gazette of the Republic of Macedonia No.100/05) is noted and separately labelled as a hazardous waste, including each waste that is mixed with hazardous waste [13].

Management with chemical waste, respectively collection, separation and its storage is properly solved in the frame of the IPH in context of the above mentioned Law. But, real problem is disposal of chemical waste produced in the laboratories of the IPH.

Related to the needs of solution of collection and transport of chemical waste produced in the former period, as well the possibility of eventual integral solution of the chemical waste problem, IPH has conducted a situation analysis of quantities and content of hazardous chemical waste (as well DDT), which has remained in the chemical waste depots of the Institute of Public Health and at the regional Centers of Public Health.

According to the Basel Convention that determines the rules and conditions for control of transboundary transport of hazardous waste and its storage, ratified by our side (Official Gazette No.49/97), there was a possibility for export of hazardous waste under conditions proscribed in the Convention and with an agreement with other side where exists conditions for treatment of it.

Management with chemical waste, respectively collection, separation and its storage is properly solved in the frame of the IPH in context of the above mentioned Law.

But, real problem is disposal of chemical waste produced in the laboratories of the IPH.

Actual practice (until 2011) include its storage in the chemical safety depot in close vicinity of the Institute, with regard of secured regular surveillance, as well environmental protection measures and measures to protect human health.

Based upon article 32, paragraph 1 and 2 of the Waste Management Law, IPH needed final solution of the storage and final disposal of the hazardous chemical waste, up to article 33, paragraph 4 storage of hazardous waste cannot be longer than 1 year in case waste is planned for disposal.

IPH as hazardous waste producer has carried the evidence in accordance with the article 60, paragraph 1 of this Law.

In article 65, paragraph 1 is proscribed that legal and physical entities that are producing or posses hazardous waste can make an agreement for collection and transport of chemical waste with legal entities that have Licence for collection and transport of hazardous waste.

IPH during its work is continuously aiming on environmental protection and prevention of adverse health effects of the employees, as well population living nearby. In order to be able to solve this actual issue, particularly from the aspect of non-existing Hazardous Waste Landfill in the Republic of Macedonia, as well lack of the Hazardous Waste Treatment Facility, IPH as a health facility for tertiary preventive health care had a goal for proper solution of this actual environmental-health risk that exist in all country, in all legal entities that produce hazardous chemical waste. Related to the needs of solution of collection and transport of chemical waste produced in the former period, as well the possibility of eventual integral solution of the chemical waste problem, IPH has conducted a situation analysis of quantities and content of hazardous chemical waste (as well DDT), which was still in the chemical waste depots of the Institute of Public Health (1,126.5 l liquid and 5,888 kg solid chemical waste) and at the regional Centers of Public Health (1,22.65 l liquid and 90.95 kg solid chemical waste), or totally from the IPH and CPHs – 1,249.15 l liquid and 5,978.95 kg solid chemical waste.

Dunne et al. [2] in their paper have presented the results of a community health survey of people living near a hazardous chemical waste site in Kingston, Queensland. In comparison with a matched control group, people near the site were no more likely to report serious diseases, and reports of cancer and mortality rates did not differ in the two groups. Kingston residents reported higher rates of symptoms of general poor health, high levels of stress and anxiety and a higher incidence of miscarriages. The reports of poor physical health appear to be independent of proximity to the hazardous waste site and duration of residence in the area. Symptom prevalence and perceived recent decline in health correlate most strongly with the stress and anxiety measures. While long-term investigation is necessary, it appears at this stage that the chemical waste is not associated with an increase in major diseases as reported by those who were interviewed. When health in a broader sense is considered, however, it is clear that the situation has had a negative impact.

Increasing worldwide use of chemicals, including heavy metals used in industry and pesticides used in agriculture, may produce increases in chronic diseases in children unless steps are taken to manage the production, use, trade, and disposal of chemicals. In 2020 the developing world will account for 33 % of global chemical demand and 31 % of production, compared with 23 and 21 %, respectively, in 1995. Trasande et al. [12] in their paper have described present and potential costs of environmental exposures and discussed policy options to protect future generations of children in a sustainable development context. Specifically, they have described the principles of sound chemicals management. Authors recommended that industrializing nations in particular employ these principles to prevent disease among their populations while at the same time minimizing the risk to their own economic development.

The amount of waste produced is connected with the degree of a country's economic development; more developed countries produce more waste. The paper of Marinkovic et al. [11] reviews the quantities, manipulation and treatment methods of medical waste in Croatia, as well as hazardous potentials of medical waste for human health. They stressed that Croatian medical waste legislation is not being implemented. They recommended that it is very important to establish a medical waste management system that would implement the existing legislation in all waste management cycles from waste production to treatment and final disposal.

Gunson et al. [3] in their paper have elaborated a chemical exposure within the Mohs laboratory that can present a significant occupational hazard. Acutely toxic and potentially carcinogenic formaldehyde was found at high levels in a relatively standard laboratory configuration. They have recommended that a laboratory can be designed with a combination of physical environment and operational protocols that minimizes hazards and creates a safe working environment.

In the paper of Ma et al. [10] rates of hospitalization for asthma and respiratory infectious disease in children were modelled as a function of residence. After adjustment, living in a zip code containing a POPs waste site significantly increased the frequency of hospitalization for asthma and infectious respiratory disease. Living in a zip code with an 'other' waste site also increased hospitalization frequencies for both diseases. The association was strongest for zip codes whose residents were in the lowest quartile of medium family income. This evidence supports the hypothesis that living near a hazardous waste site increases risk of respiratory disease in children.

Approximately one in four Americans lives within 4 miles of a hazardous waste site according to the EPA. In light of this large proportion and the public's high level of concern that hazardous waste causes health problems, it is important for primary care physicians and other health care providers to know that residential proximity to some kinds of hazardous waste sites is associated with adverse reproductive effects. Findings from both state-based surveillance programs and studies of individual hazardous waste sites have shown increased risk of congenital malformations and reductions in birth weight among infants born to parents living near hazardous waste sites [7].

Material Safety Data Sheets (MSDS) must accompany all initial incoming shipments of all chemicals. MSDSs must be readily available to all personnel in the labs where the chemicals are stored and where they are used. MSDSs shall be kept in three-ring binders near the door so that personnel can familiarize themselves with new chemicals before getting them out and using them. Before ordering a new chemical, laboratory personnel should obtain information on proper handling, storage, and disposal methods for that chemical. The chemical storage area should be posted with an appropriate sign. Chemicals must be stored in appropriate containers and correctly labelled. Chemical compatibility must be determined to reduce the likelihood of hazardous reactions. All chemicals should be labelled before being transported. Containers used for the accumulation of hazardous waste must be in good condition, marked with the words "waste" or "spent" and its contents indicated, free of leaks and compatible with the waste being stored in them. All accidents, hazardous materials spills, or other dangerous incidents should be reported. All lab's staff must be trained how to use Personal Protective Equipment [1].

Kochubovski [9] in his paper has stressed the importance of organizing training for proper management of the health-care waste. In 2000, 2002, 2007 and 2008 have been provided training courses to responsible persons from the Health care Institutions, as well State Sanitary and Health Inspectorate.

15.5 Conclusion

It can be concluded that the way of managing and disposal of hazardous chemical waste from the Institute of Public Health and Centers of Public Health in the Republic of Macedonia has been made according to the rules established by the Basel Convention with the aim to safeguard the environment and human health.

15.6 Recommendation

All Health care Institutions that are producing chemical hazardous waste should have a chemical depot. Also they need to follow safe procedures to protect their staff's health, and to use personal protective equipment. Final disposal of hazardous chemical waste should be done on a Hazardous Landfill, or to be treated in High-Temperature Hazardous Waste Incinerators that fulfil emission standards according to the EU's requirements in order to protect the environment and human health.

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Chapter 16
Pesticides Mechanisms of Action in Living Organisms

Gheorghe Coman, Alina Farcas, Andreea Valceanu Matei, and Cristina Florian

Abstract Pollution is a phenomenon, which leads to ecological disequilibrium (alteration of biotic and abiotic) and may produce dangerous waste. Epidemiological studies, evaluate the relationship between the pollutants impacts over individual or collective risk and environmental factors. The rational use of pesticides in conjunction with other technologies may be justifiable in integrated pest management, the balance between benefits and effects being very complex. Pesticides are considered persistent pollutants, and may be classified according to chemical structure in the following main classes: organophosphates, carbamates, organochlorines, triazines, and pyrethroids. In this paper we present the mechanisms of action of the main pesticide classes in living organisms and especially in the human body. Organophosphate pesticides act on acetylcholinesterase, leading to development of cholinergic toxicity, because they decrease its enzymatic activity. The carbamate or phosphate pesticides inhibition of acetylcholinesterase, disrupts the equilibrium between acetylcholine synthesis and release on one hand and its hydrolysis on the other, and leads to its accumulation at synaptic level, with prolonged activation of cholinergic receptors. Organochlorine pesticides are highly lipophilic, and this property enhances their stability in living organisms and in the environment. They are largely stored in adipose tissue, a process called bioaccumulation, and this characteristic leads to the development of high toxicities in mammals. Triazines in high concentrations have been linked to increased cancer risk and incidence of birth defects. The pyrethroid insecticides acting on the sodium channels in the nerve membrane (neurotoxic), have high selectivity for insects, and do not have carcinogenic, mutagenic and teratogenic effects.

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Living organisms and humans are concurrently exposed to pesticides from more than one source, via the environment and food, and these may have a combined (synergistic or antagonistic) action, which can cause higher or lower toxic effects, in comparison with the situation of a single pesticide.

Keywords Organophosphate pesticides • Organochlorine pesticides • Triazine pesticides • Pyrethrin pesticides • Enzyme • Action mechanism

16.1 Introduction

Our present society is facing severe environmental problems, at the local, regional and global level. Maintaining or improving of the quality of the environment may be materialized through environmental monitoring.

The monitoring activities must identify and describe the evolution of the environmental qualitative and quantitative parameters in relationship with the affecting factors.

Pollution is a phenomenon, which leads to ecological disequilibrium (alteration of soil, air, water and biota) and consists of the following elements [17]: pollution sources, pollutants transport and pollutants target.

The pollution affects air (climatic changes, stratospheric ozone depletion, acidification), waters (eutrophication, oxygen reduction), foods (reduced quality and quantity of nutrients), biota (habitat depletion and deterioration, species threatening) and may produces dangerous wastes [8].

The human body is exposed to an enormous number of non-nutrient compounds from the environment, many of which could be toxic. Epidemiological studies, evaluate the relationship between pollutants impacts over individual or collective risk and environmental factors.

16.2 Pesticides

Pesticides are among the environmental pollutant agents, which kill unwanted living organisms (animals or plants). Usually a pest is considered any living organism, which is interfering in a negative way with the human activity.

The rational use of pesticides in conjunction with other technologies may be justifiable in integrated pest management, the balance between benefits and effects being very complex.

The intended effects of pesticide use are as follows [9]:

- the control of pests and vectors of diseases;
- the control of organisms that harm other human activities.

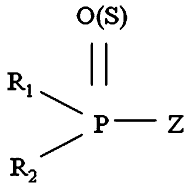


Fig. 16.1 General structure of organophosphate pesticides

On one hand, at community, national and global scales, there are three main benefit domains of pesticide use [10]:

- social: health and quality of life;
- economic: farm (agricultural) costs and profits;
- environmental: terrestrial, aquatic and air.

On the other hand, the presence of pesticides in non-target species (including humans) can induces some perturbations, dependent on their nature, and their interactions with functional molecules.

Pesticides are a public health risk, and their use is the subject of some controversies concerning “what is acceptable risk” or “how can we minimize the risk” [16].

Pesticides are considered persistent pollutants, and may be classified according to their chemical structure in the following classes: organophosphates, carbamates, organochlorines, triazines, and pyrethroids.

16.2.1 Organophosphate Pesticides

All organophosphate pesticides contain a central phosphorus (P) atom to which an oxygen or sulfur atom is doubly bound (Fig. 16.1). Two methoxy or ethoxy groups are also singly bonded to the central P atom, while a longer more complex structure (Z) is singly bonded to phosphorus, usually by an oxygen or sulphur atom [9, 11].

The toxicity of organophosphates is determined by the electrophilic magnitude of the central phosphorus atom, the steric nature of the substituents R_1 and R_2, and by the strength of the bond P – Z, and it is influenced by groups that increase the lability of the P – Z bond, which is broken during the inhibition process [32].

Organophosporus pesticides with p-CH_3–S–aromatic group or p-NO_2–aromatic group are the most toxic because these substituents enhance the electrophilicity of the P atom, by inductive and mesomeric effects. Mammals’ metabolic transformation (biotransformation) of these groups, leads to magnification of the electrophilic character of P atom and increases the pesticide toxicity (p-CH_3–S group can be oxidized to p-CH_3–SO group or p-CH_3–SO_2 group).

Also, the toxicity of organophosphate pesticides decreases, with increasing chain length of R_1 and R_2 groups, because it decreases the electrophilic magnitude of the central phosphorus atom.

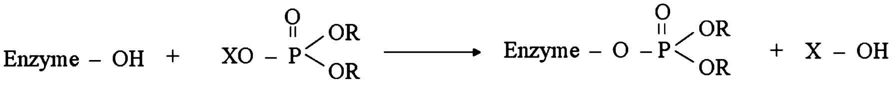
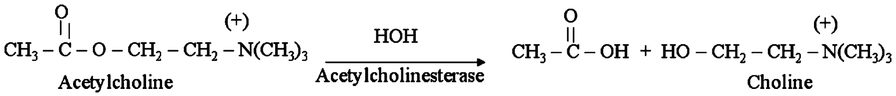


Fig. 16.2 Representation of biochemical interaction between organophosphates and acetylcholinesterase (Enzyme – OH)



$$CH_3-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-\overset{(+)}{N}(CH_3)_3 \xrightarrow[\text{Acetylcholinesterase}]{HOH} CH_3-\overset{O}{\overset{\|}{C}}-OH + HO-CH_2-CH_2-\overset{(+)}{N}(CH_3)_3$$

Fig. 16.3 Catalytical activity of acetylcholinesterase

The primary target of organophosphate pesticides is acetylcholinesterase leading to development of cholinergic toxicity, because they decrease its enzymatic activity [3, 31, 32].

Acetylcholinesterase is a serine enzyme with a hydroxymethyl group, present in the active site, which interacts with organophosphate pesticides to form an inactive complex (Fig. 16.2).

The physiological role of acetylcholinesterase is to catalyze the hydrolysis of acetylcholine, which is a major neurotransmitter in the central or peripheral nervous systems (Fig. 16.3). Acetylcholinesterase is a key enzyme responsible for terminating the nervous impulse.

Acetylcholinesterase inhibition causes acetylcholine accumulation in the synaptic cleft and the postsynaptic membrane is continuously stimulated. Lack of coordination of the neuromuscular system is a direct result of this mechanism of action, and the final response can be death [1, 30].

Acetylcholinesterase inhibition can also alter lymphocytic activity, and organophosphates have been associated with the development of immunological disorders [13, 20] or cancers [6].

Organophosphate pesticides are weak inhibitors of acetylcholinesterase when a sulfur atom is bound to phosphorus ($P = S$) in the general structure [29].

In phase I biotransformation processes, cytochromes P450 (CYP_{450}) act as monooxigenases on organophosphate pesticides ($P = S$) and they are transformed by desulfurization, in the corresponding oxon analogs ($P = O$). Metabolic desulfurization process of organophosphorus pesticides is considered bioactivation [3, 11], because oxon compounds are up to 1,000 times stronger inhibitors of acetylcholinesterase, than the parent compounds (Fig. 16.4).

Organophosphates may also influence homeostasis of liver, brain and muscle where are glycogenesis (glycogen synthesis from glucose), gluconeogenesis (synthesis of glucose from nonzaharidic organic molecules), glycogenolysis (breakdown of glycogen to glucose) and glycolysis (glucose transformation to two molecules of pyruvic acid or lactic acid) are taking place, through pancreas control of glucose homeostasis, and by pancreatic control of insulin and glucagon secretion [23].

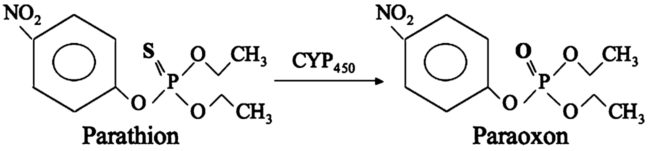


Fig. 16.4 Biotransformation of parathion to paraoxon (bioactivation)

H_2NCOOH (a) R – NH – COOH (b)

Fig. 16.5 General structure of carbamic acid (**a**) and carbamate pesticides (**b**)

Exposure to organophosphate pesticides (dimethoate, acephate, malathion, dichlorvos) induces hyperglycemia through decreased hexokinase activity in favor of glycogen storage activation (increase glycogen synthase activity) and in detriment of glycolysis [28].

The induction of stress (the response to every situation which threatens homeostasis) or oxidative stress (alteration of the enzymes associated with antioxidant defenses) are the molecular mechanisms activated in pesticide toxicity, especially in presence of organophosphate pesticides [24].

Organophosphate pesticides induce reactive oxygen species generation and reactive nitrogen species generation, which contribute to the pathogenesis of acute pancreatitis through destruction of β-cells in diabetes and development of autoimmune diabetes [23]. Organophosphates are the most commonly used pesticides and are heavily used in agriculture and in urban settings as insecticides. Because of this, most of the population has been exposed to organophosphate pesticides in homes, outdoors, in workplaces, or through vegetal and animal foods consumption and in most of the subchronic or chronic disease cases there is no clear etiology because physicians cannot establish a direct cause-effect relationship between those and the pesticide toxicity.

16.2.2 Carbamate Pesticides

The carbamate pesticides are derivatives of carbamic acid, where one of the hydrogen atoms attached to nitrogen is replaced by a longer organic substituent (Fig. 16.5).

The carbamate insecticides inhibit acetylcholinesterase, like the organophosphate insecticides (the same inhibition mechanism). Organophosphate and carbamate pesticides are primarily recognized by their acetylcholinesterase inhibition [2,14].

The carbamate or phosphate pesticides inhibition of acetylcholinesterase, disrupts the equilibrium between acetylcholine synthesis and release on one hand

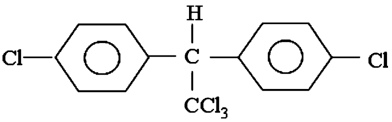


Fig. 16.6 Structure of DDT

and its hydrolysis on the other, and leads to its accumulation at synaptic level, with prolonged activation of cholinergic receptors [22].

Compared to organophosphate pesticides, the carbamate pesticides action is reversible, shorter in duration and milder in intensity.

16.2.3 Halogenated Pesticides

Halogenated compounds (organochlorine pesticides) are highly lipophilic, and this property enhances their stability in living organisms and in the environment. They are largely stored in adipose tissue, a process called bioaccumulation, and this characteristic leads to the development of high toxicities in mammals.

The toxicity of organochlorine pesticides is determined by their structure, the spatial arrangement of their atoms (symmetry and asymmetry), and the nature of the substituents [12, 19].

The main characteristics of organochlorine insecticides are:

- preponderantly absorbed via the digestive system;
- accumulate in lipid rich tissues (adipose tissue, liver, milk, brain);
- interact with membrane lipoproteins (staple of the nerve membranes) causing the distortion of the nerve impulse transmission;
- adverse health effects associated with organochlorine pesticide exposure may be: neurological deficiency, respiratory problems, dermal damage, memory disorders and cancer.

DDT (dichlorodiphenyltrichloroethane) is a penta chloro aromatic compound, which acts on enzyme systems or nervous tissue, and is highly insecticidal (Fig. 16.6).

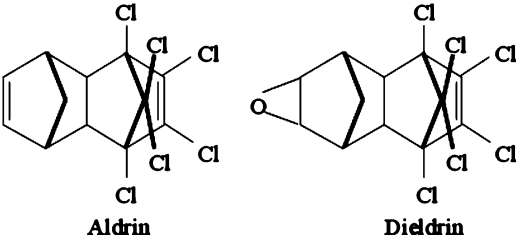
DDT was the first widely used synthetic pesticide and the first pesticide used as insecticide in 1939 as an organic compound with extreme persistence, stability and low cost [19].

The use of DDT has been credited for the eradication of malaria in Europe and the United States and for revolutionizing agricultural production, but it has been associated with numerous pathologies in many animal populations [4].

In the developed countries DDT use has been restricted and continues to be used only as vector control.

DDT exerts its toxicity by binding to lipoproteins in the nerve cell membrane, disturbing the ionic homeostasis, especially the sodium/potassium balance across this membrane.

Fig. 16.7 Structures of aldrin and dieldrin



Also, DDT and its analogues can act through other mechanisms in the human body:

- interact with the neuron membrane;
- alter the transmission of the nervous impulses;
- alter the mitochondrial phosphorylation (cell energy).

The highly chlorinated cyclic hydrocarbons (aldrin, dieldrin, heptachlor, endrin, alodan, chlordane) acts as lindane or DDT, to produce convulsivant actions (cyclodiene insecticides).

The presence of the double bonds in chlorinated cyclic hydrocarbons, increases pesticide toxicity, whereas presence of epoxide group in these compounds, decreased pesticide toxicity [18, 19].

Aldrin is a hexachlorinated cyclodiene and resembles the dieldrin structure, which possess a stable epoxide ring (Fig. 16.7). Dieldrin is a highly lipophilic compound and this property enhances its stability in the environment and in living organisms.

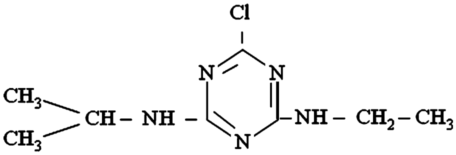
Dieldrin is an organochlorinated pesticide, and it is classified as chlorinated cyclodiene with an unusually stable epoxide ring compared to other compounds.

In living organisms, dieldrin induces reactive oxygen species (ROS) production and as a result can cause [18, 25]:

- a decrease of dopamine level, altering the function of dopamine transporters;
- perturbation of mitochondrial membrane potential, altering mitochondrial membrane perme-ability;
- the release of cytochrome c from inner mitochondrial membrane into the cytosol, where it activates caspases;
- caspase mediate hydrolytic cleavage and activation of protein kinase C, which acts on caspases by positive feedback (amplification of the apoptotic process);
- activated protein kinase C induced DNA fragmentation, which contributes to apoptotic cell death.

Exposure to chlorinated pesticides has been associated with some pathological states: non-Hodgkin's lymphoma, aplastic anemia, multiple myeloma, leukemia [6].

Fig. 16.8 Structure of atrazine



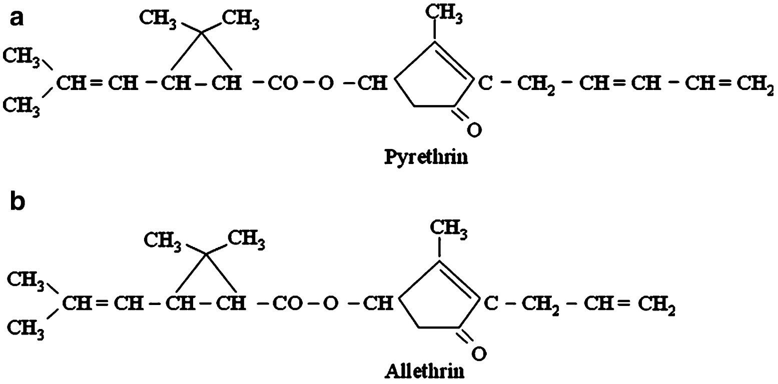


Fig. 16.9 Structures of some natural (**a**) and synthetic pyrethroids (**b**)

16.2.4 Triazine Pesticides

Triazines are moderately water-soluble herbicides (difficult to remove from potable water) and their chemical structure is based on an aromatic ring with three nitrogen atoms in alternating positions. Triazine derivatives possess some substituents (chlorine, amino groups) on the aromatic carbons (Fig. 16.8).

The best-known member of the triazines is atrazine, which is not very acutely toxic, but, in high concentrations, has been linked to increased risk of developing cancer and an increased incidence of birth defects [27].

16.2.5 Pyrethroid Pesticides

Pyrethroids are derivatives of natural pyrethrins and most of them contain cyclopropane carboxylic acid linked to alcohols through an ester or ether bond (Fig. 16.9).

Addition of an α-cyano group to general structure of pyrethrins, confers an increase of one order of magnitude of insecticidal activity for target or neurotoxic activity for non-target species [33].

Pyrethroids have 2–4 chiral carbons and the spatial conformation is determinant for their biological activity.

The pyrethroid insecticides acting on the sodium channels in the nerve membrane (neurotoxic), have high selectivity for insects, and do not have carcinogenic, mutagenic and teratogenic effects [5].

In humans the biotransformation of pyrethroids is rapid (ester hydrolysis or cytochrome P450 oxidation) and complete elimination from the body is realized in 2–8 days (detoxification).

A high dose of pyrethroids induces neurotoxicity in mammals (the non target species) in a similar manner to the effects it has in insects (the target species) for which pyrethroid pesticides possess a high selectivity. Compared to insects, mammals are three orders of magnitude less sensitive to pyrethroids and this characteristic supports the increasing substitution of other more toxic insecticide classes by these in order to decrease human occupational poisoning [26].

Target systems for pyrethroids in mammals are: protein phosphorylation (cell energy), noradrenaline release, membrane depolarization (permeability), nicotinic receptors, lymphocyte proliferation [7].

16.2.6 Action Mechanism Through the Reactive Oxygen Species

Paraquat (PQ) is a quaternary nitrogen herbicide, which is not absorbed from the gastrointestinal tract but is absorbed across the skin. Upon absorption, paraquat is little metabolized in the human body, and tends accumulates in the kidney and lung where it exerts its acute effects [15, 21].

In humans, chronic paraquat exposure is a possible etiological factor for Parkinson's disease, which is a pathological state manifested by abnormalities of motor control: tremors, rigidity, and loss of reflexes.

In the human body, paraquat induces oxidative stress (production of reactive oxygen species) and alteration of the mitochondrial metabolism, manifested through changes in cell energy levels (Fig. 16.10).

Living organisms and humans are concurrently exposed to pesticides, both via the environment and food, and these may have a combined (synergistic or antagonistic) action, which can causes higher or lower toxic effects, in comparison with the situation of single pesticide [27].

The Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA) in the USA, the Health Council of the Netherlands, the Committee on Toxicity of Chemicals in Food, the Danish Veterinary and Food Administration, and other national or international organizations, recommended the introduction of the physiologically based toxicokinetic modelling (PBTK) as a tool in the risk assessment of pesticide mixtures.

These models require a large amount of data for construction, and once constructed and evaluated, they can be used for predicting the level of pesticides at the target site [7, 34, 35].

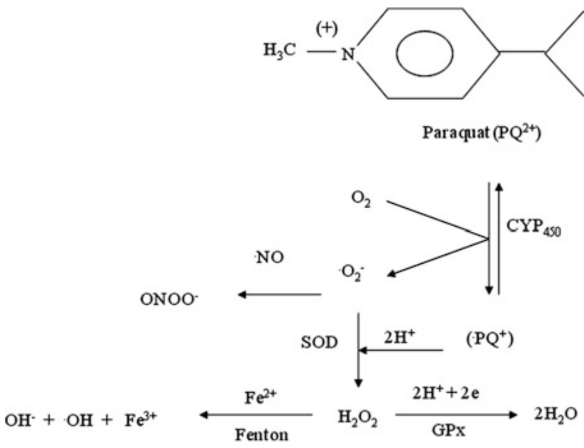


Fig. 16.10 Paraquat ROS generation and oxidative stress (*SOD* superoxide dismutase, *GPx* glutathione peroxidase)

16.3 Conclusions

This paper presents some theoretical aspects of the pollutants (pesticides) and their action mechanisms in living organisms (mammals).

The carbamate or phosphate pesticides acetylcholinesterase inhibition, disrupt the equilibrium between acetylcholine synthesis, release and hydrolysis, and leads to their accumulation at synaptic levels, with prolonged activation of cholinergic receptors.

Organochlorine pesticides are highly lipophilic, and this property enhances their stability in living organisms and in the environment. This characteristic leads to the development of high toxicities in mammals.

Triazines can induces in high concentrations some disturbing links to cancer risk and incidence of birth defects.

The pyrethroid insecticides are high selectivity for insects, and are not carcinogenetic, mutagenetic and teratogenetic effects.

Living organisms and humans are concurrently exposed to pesticides and may have a combined (synergistic or antagonistic) action, which can causes higher or lower toxic effects, in comparison with the situation of single pesticide.

Pesticides are heavily used in agriculture and in urban as insecticides, and most of population has been contaminated to it in homes, outdoors, workplaces, or through vegetal and animal foods and most of subchronic or chronic diseases are not diagnosed, because cannot be established a direct cause-effect by physicians.

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Chapter 17
Factors Influencing Pesticide Risks for Children

Thomas A. Lewandowski

Abstract Infants and young children constitute a particular population of concern in terms of exposure to pesticides (including obsolete pesticides). For various reasons (behavioral, anatomical and metabolic) children may have greater susceptibility to the adverse effects of pesticide exposure. This potential for increased susceptibility must be evaluated on a case-by-case basis however, because in some cases children (particularly older children) may have similar or even less susceptibility compared to adults. Research has also pointed out a number of toxicological modes of action that may be of particular relevance for children's health risks. These include effects on nervous system maturation, endocrine disruption and the influence of early life exposures on development of disease later in life. Although the importance of such modes of action is not yet fully understood, particularly at low levels of exposure, these are areas of expanding research and the data obtained are expected to be useful for improving health risk assessment in this population.

Keywords Children • Risk assessment • Susceptibility

17.1 Introduction

Society regards with exceptional unease concerns about the effects of pesticides and other chemicals on children's health [29]. While children have long been regarded as a key population at risk for certain chemicals (*e.g.*, lead, methylmercury), children are a population subgroup which increasingly drives regulations for

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a much broader set of chemicals. This increased emphasis may be traced to a 1993 US National Research Council (NRC) report, *Pesticides in the Diets of Infants and Children*, which considered the extent to which children differ from adults in terms the potential adverse health effects of pesticide exposures. The NRC report noted that while there is clear evidence of adult-child differences in susceptibility, children's susceptibility to pesticide exposure does not always lie in the same direction; in some cases, children may be less susceptible than adults [24]. Relative susceptibility must therefore be examined on a case-specific basis, considering the age group of concern, the chemical in question, and the types of health effects associated with the chemical [27].

Although it is common to discuss risks for "children" as a general category, this approach lacks precision because it encompasses a very diverse range of individuals, including premature infants, infants and neonates, juveniles from the ages of 1–12 years and teenagers. Individuals in this range exhibit an exceptionally diverse range of behaviors and physiology. Furthermore the age range of concern may be variable; in addressing soil lead exposures the USEPA focuses on children ages 0–6 years of age (because soil ingestion exposures in older children are similar to those in adults) whereas concerns for endocrine disruption might focus on children around the age of puberty. Thus, the first step in discussing children's risks from pesticide exposure is to recognize that risks will be age-specific and broad statements relating to "children" as a generic term may be incorrect in some cases. Given this caveat, it is correct to state that at specific ages, children may be more susceptible to certain chemical exposures than adults. This may be due to differences in behavior that contribute to chemical exposures (*e.g.*, soil ingestion), anatomical differences (*e.g.*, increased skin permeability) or metabolic differences (*e.g.*, differences in metabolic capacity). Each of these broad bases for increased susceptibility is discussed below.

17.2 Exposure-Related Susceptibilities

Exposure is a key factor in determining health risks; even the most inherently toxic chemical will pose no risk unless there is sufficient exposure. Many factors which result in an increased potential for exposure during childhood are readily apparent, particularly as regards the oral route. Children are more likely, particularly between the ages of 2 and 6, to crawl on the ground where they may pick up particles of soil, dust or chemical residues (*e.g.*, indoor applied insecticides). They may then ingest these particles or residues via hand-to-mouth behavior, something which occurs in all individuals but is particularly prominent in children. For example, studies conducted by Stanek, Calabrese and colleagues have estimated that on a typical day, the average child might ingest between 28 and 45 mg of soil or dust via hand-to-mouth activity [31]. The corresponding estimate for adults is 10 mg [30]. Furthermore, while all young children engage in putting non-food objects in their mouth, some children may do so to an extreme degree, an activity known as

Table 17.1 Age dependence of several key exposure variables

Parameter	Infant/ neonate	Young child 3–5 years	Older child 6–10 years	Teenager 12–18 years	Adult
Body weight (kg)	7	17.5	29	57	70
Water intake (L/kg-day)	0.04	0.05	ND	0.02	0.02
Breathing rate (m^3/kg-day)	0.64	0.47	0.34	0.25	0.19
Skin surface area (cm^2/kg)	ND	417	338	293	257
Total vegetable intake (g/kg-day)	6.8	7.125	5.55	3.8	3.6
Total dairy intake (g/kg-day)	62.7	21.15	13.3	6.3	3.4

Source: USEPA [36]

ND no data available

Values are approximate ratios of parameter to average body weight estimated for that age range; values were averaged for males and females where gender-specific values were provided separately

pica [7]. For such children, soil ingestion can be as high as 10–13 *g* of soil or dust per day [10]. The extent of hand-to-mouth activity is highly age dependent and primarily involves children between 1 and 6 years of age. Infants do not begin crawling until 6–10 months of age and therefore have very limited contact with soil or flooring surfaces. Children older than 6 years have generally very limited hand-to-mouth behavior and have soil/dust ingestion levels approaching that of an adult.

In addition to soil and dust ingestion, children also exhibit a greater intake of food and water (on a per kg-body weight basis) than adults. For example, water intakes of infants and young children are about double that of an adult on a body weight basis and intakes of particular food categories can be many times higher (Table 17.1). Children are also likely to have a more restricted and repetitive diet (*e.g*., juice rather than water, only fish sticks, only certain vegetables) which could lead to substantially different levels of chemical exposure via the diet compared to a typical adult. Infants and neonates also have a unique source of exposure during the early period of life – breast milk and/or baby formula – which they consume at a very high rate relative to their body weight (Table 17.2). Whether the child consumes formula-based milk or maternal milk can have an important bearing on exposure. While maternal milk may involve the transfer of lipophillic toxicants (*e.g*., chlordane, DDT) from mother to child, use of formula could be associated with increased exposure to a contaminated water supply.

Although the oral exposure pathway usually dominates children's exposure, other pathways may be important in certain cases. Children have an inhalation rate that is proportionally greater for their body size compared to an adult [36] and thus, on a body-weight basis will have higher exposures. The same applies to dermal exposure because the child has a larger surface area when scaled to total

Table 17.2 Age dependence of breast milk intake

	Infant age			
	1 month	2 months	3 months	4 months
Breast milk intake (g/kg-day)	159 ± 24	129 ± 19	117 ± 20	111 ± 17

Source: USEPA [36]

Data from infants that were exclusively breast fed during the first 4 months of life

body mass [36]. As shown in Table 17.1, the skin surface area per kg of body weight of a young child (ages 3–5 years) is approximately 60 % greater than that of an adult. Practically speaking, these differences result in only a slight increase in overall dose, a difference usually overwhelmed by the difference in the oral pathway. They may be important in cases where the oral route of exposure is absent.

17.3 Physiologically-Based Susceptibilities

The moment of birth initiates dramatic changes in physiology. From birth to the late teens, the child's body grows and matures becoming increasingly adult-like. It would be incorrect however to assume that the progression is strictly linear. The pattern of growth and development during childhood varies among different organ systems, and can be quite complex (Fig. 17.1). Examples include:

Organ Size Changes – Patterns of organ growth during childhood are highly organ specific. For example, human babies have brains that are much larger in size relative to their body compared to an adult's. This may have significant implications for chemical distribution; based on their larger relative brain size, children's brains may experience higher doses of a chemical of interest.

GI System Acidity – Stomach pH can have significant effects on the absorption of many chemicals, particularly metals and ionizable organic compounds. This would apply to pesticides that are weak acids or bases such as 2,4-D, MCPA, metasulfuron-methyl and glyphosate. The pH of the stomach is fairly neutral (pH 6–8) at birth, becomes acidic (pH 1–3) in the first few days of life, but then more basic during the neonatal period (pH >5). Stomach pH reaches adult levels (pH 1–3) by around 2 years of age [5].

Body Water/Body Fat – Body water and body fat percentages play an important role in determining how a chemical is distributed in the body. Tissue hydration decreases consistently with age, from about 74 % in the full-term neonate, to approximately 55–60 % in the adult [5]. Body fat generally follows a more complex pattern, being relatively low at birth (14 %), rising during the first few months of life, leveling off through childhood and then declining around puberty, particularly in males.

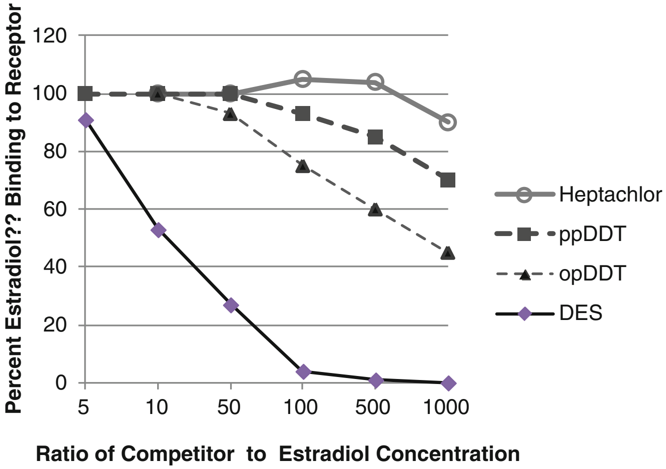

Fig. 17.1 Important physiological changes occurring during development

Skin permeability – In adults, the stratum corneum, a layer of dead highly keratinized cells on the skin surface, constitutes a barrier to dermal penetration of many compounds. Although not an absolute barrier (particularly for lipid soluble chemicals) the stratum corneum can substantially attenuate skin permeability. This surface layer is immature in newborns but rapidly develops and thickens during the first 4 months of life [20].

Blood Brain Barrier – The blood brain barrier is a multicomponent structure that prevents harmful substances from entering the brain from the blood supply. The blood-brain barrier in human infants is relatively undeveloped and displays greater permeability to drugs and other exogenous compounds until about 3–4 months of age [32]. Little data exist to quantify the function of the blood-brain barrier at earlier ages [39].

Developing Tissues – Besides the specific example of the blood-brain barrier, the cells of many other tissues in the infant and young child are undergoing rapid division and maturation. These include the cells of the central nervous system, the reproductive organs and the immune system. These highly active cells are susceptible to chemical insult and if eliminated or damaged early in life may leave the individual with diminished capacity later in life (discussed in greater detail in Sect. 17.5).

Elimination – Chemicals are cleared from the body primarily via either the urine or feces (biliary excretion). Other forms of elimination, such as the excretion of some metals in the hair, have a relatively minor impact on body burden. Kidney function is relatively immature at birth but rather quickly reaches adult levels: blood filtration ability at about 1 month of age and renal tubular function by about 1 year [5]. Maturation of biliary elimination is much slower and may only approximate adult levels when the child is several years old [5].

17.4 Metabolism-Based Susceptibilities

Another important component of children's potentially increased susceptibility relates to metabolic capacity. At birth, many, although not all, of the metabolic enzymes in the liver (the primary metabolizing organ) have much less capacity compared to the adult. Thus infants may metabolize many chemicals less efficiently than adults, meaning the chemicals are much more slowly eliminated and, consequently, may accumulate to higher levels. This may represent an adverse situation for chemicals which exert a direct toxic effect in the body but a less adverse situation for chemicals which must first be metabolized to a reactive intermediate (*e.g.*, some pyrethroid insecticides).

Metabolism of many compounds involves two primary phases. Phase I reactions typically involve either breaking the molecule into smaller parts or adding oxygen to the molecule to create a reactive site (*e.g.*, heptachlor is oxidized to heptachlor epoxide). Phase II reactions typically involve attaching a more water soluble ligand to the reactive site to enhance elimination via either the urine or bile.

Maturation of the ability to carry out Phase I reactions develops during the first few years of life. Many important Phase I reactions are carried out by a family of enzymes known as cytochrome P450s (CYP 450 s). The different members of this family (called isoforms) possess substrate specificity and metabolize different types of chemicals. For example, CYP1A2, CYP2C19 and CYP3A4 are responsible for metabolism of many pesticides and other xenobiotics whereas CYP2D6 is important in the metabolism of many pharmaceuticals [1]. Maturation of the CYP450 isoforms is age dependent, although many reach adult activity levels by the end of the first year of life, as indicated in Fig. 17.2 [4]. Some isoforms (*e.g.*, CYP3A7) are also present during the fetal period and then decline over the first few months of life as adult forms become more active.

The progression in enzyme maturation can also be illustrated with data obtained for a different Phase I enzyme, hepatic carboxylesterase. This enzyme carries out important Phase I reactions on a number of pesticides (the pyrethroids and pyrethrins) as well as a number of prodrugs (*e.g.*, the antiviral drug oseltamivir). Pharmaceutical metabolism studies used for evaluating drug safety and efficacy allow such enzyme maturation data to be collected where it could not be ethically collected in controlled studies with pesticides. As shown in Fig. 17.3, activity of the hCE-2 isoform of hepatic carboxylesterase is below adult levels for the first few years of life but appears to achieve adult levels (or near adult levels) for the remainder of the juvenile period. The hCE-1 isoform appears to mature somewhat later but nonetheless reaches 80 % of adult levels by age 6–9 [41]. The phenomenon of increased enzymatic capacity during later childhood is common to many Phase I enzymes.

The maturation of Phase II enzymes is typically a somewhat slower process. In adults, activated molecules (*i.e.*, Phase I reaction products) are most commonly combined with ligands such as glucuronic acid, glycine or bile salts prior to being eliminated from the body. For example, glucuronidation is important in the

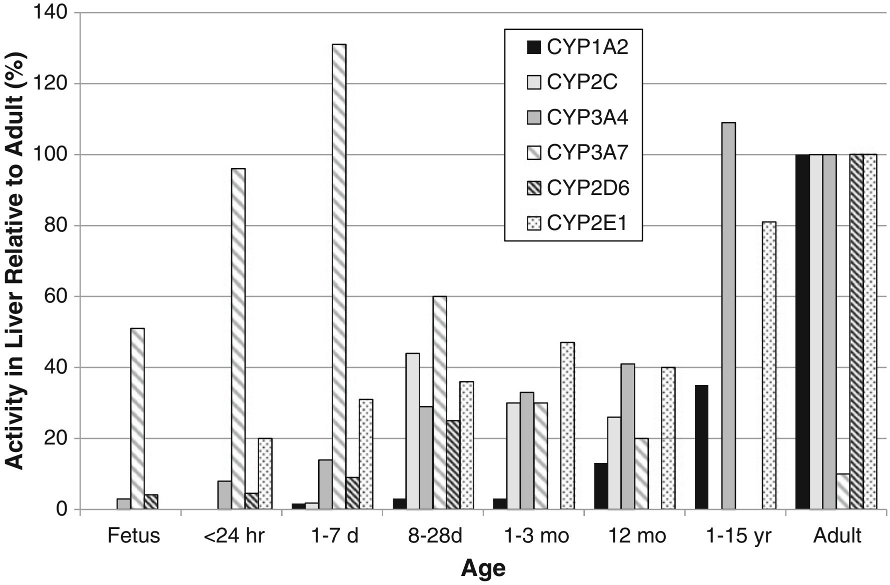

Fig. 17.2 Maturation pattern of several CYP enzymes during development (Adapted from Alcorn and McNamara [4])

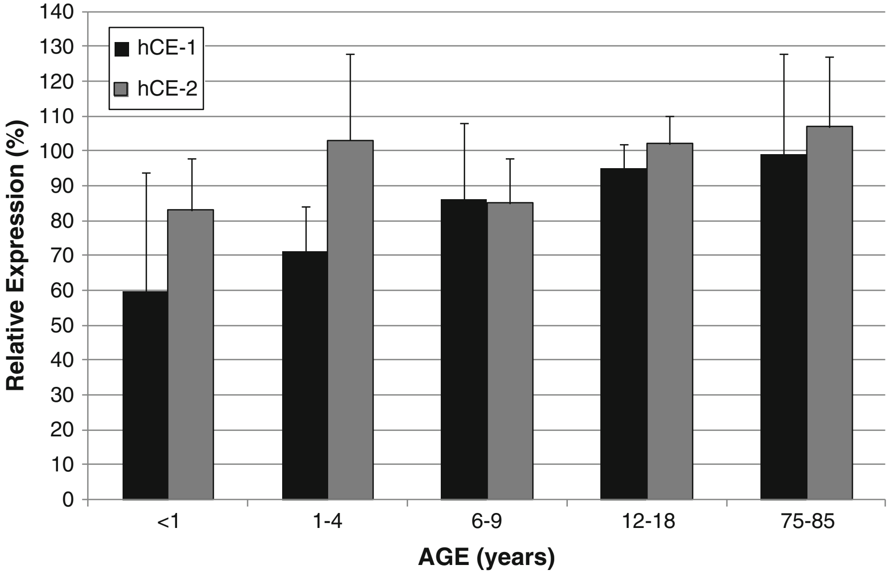

Fig. 17.3 Maturation pattern of human hepatic carboxylesterases (CE) during development. Relative expression of hCE isoforms in hepatic s9 fractions (Data from Zhu et al. [41]). Error bars = standard deviation

elimination of pyrethoids and also appears to be significant for DDT [2, 3]. The glucuronidation pathway matures rather slowly in children, not reaching adult levels until 6–30 months of age, depending on how competency is measured [4]. However, conjugation with sulfate is an alternative metabolic pathway that is very active in infants and which may partially compensate for the limited glucuronidation capacity [4, 12]. For example, the drug acetominophen is excreted primarily as a glucuronate conjugate in adults but primarily as a sulfate conjugate in infants [22]. The extent to which sulfation can be used for pesticide metabolism in infants is currently not well understood.

The examples given above indicate that, in general, the period of susceptibility in childhood may be relatively short, involving only the first few years of life. Consistent with the NRC's findings, case-specific factors may nonetheless be important. For example, a recent study has suggested that children's paraoxonase activity (an enzyme important in the toxicity of some organophosphates and carbamates) does not reach adult capacity until age 9 [14]. Situation-specific factors to consider include the dissociation constant (pKa), the lipid and water solubility, the distribution in the body and the specific enzymes responsible for the pesticide's metabolism. When such data are not available, they should be considered research priorities so that the potential risks for children can be reliably assessed.

17.5 Toxicological Modes of Action of Particular Concern for Children's Risks

In addition to behavioral or physiological factors of particular interest for assessing children's risks, there are also a number of toxicological modes of action which may be particularly important for children. Many of these are relatively new considerations that may not have been well investigated for obsolete pesticides, most of which underwent their primary toxicological evaluation in decades past. Recent data have suggested, however, that these critical modes of action should not be overlooked.

17.5.1 Effects on Nervous System Maturation

The development of the human brain is a remarkable and carefully coordinated process. Over the course of nervous system development, over one hundred billion neurons in the brain must find their proper location and form connections with their neighbors. Nerve fibers must become insulated with myelin in order to transmit signals with proper speed and neurons must develop the proper machinery for producing, secreting and recycling the appropriate neurotransmitters. Evidence suggests that neurotransmitter activity in early life is necessary for proper neuron connectivity and thus exposures to chemicals which perturb neurotransmitter activity such as insecticides may interfere with this process. Concerns have been raised that doses of insecticides that are too low to cause obvious clinical effects can, if experienced in early life, interfere with neuron conditioning and lead to subtle cognitive effects [26].

It is clear that pre-natal or post-natal insecticide exposure can have persistent effects on brain neurotransmitter activity. For example, Tang et al., [33] exposed neonatal rats to the insecticide methyl parathion at doses ranging from 0.3 to 0.9 mg/kg-day from day 1 to 21 after birth. These treatments produced observable clinical symptoms (tremor) in the pups after treatment although they resolved between treatments. The authors observed that rats tested at post-natal day 40 showed persistent decreases in acetylcholinesterase activity and acetycholine receptor binding at all doses tested. Although all groups showed some recovery by postnatal day 40, results for all treated animals remained statistically different from those in control animals. In another study, Gupta et al. [15] exposed pregnant rats to methyl parathion from day 6 to 20 of pregnancy. The two doses studied were selected as either producing no frank maternal toxicity (1.0 mg/kg-day) or causing minimal visible maternal toxicity (1.5 mg/kg-day). Activity of acetylcholinesterase and choline acetyl transferase (the key enzyme in acetylcholine synthesis) was assessed in offspring at postnatal days 1, 7, 14, 21, and 28. Both doses resulted in reduced acetylcholinesterase activity on postnatal day 1 but only 1.5 mg/kg-day resulted in persistent reduction in acetylcholinesterase as well as increases in choline acetyl transferase. In parallel experiments examining behavioral effects, Gupta et al. also reported that methyl parathion exposure was not associated with behavioral deficits in most tests conducted (*e.g.*, startle response, passive avoidance, rotarod performance) but that operant behavior (assessed as learning to press a bar in response to a reward), cage emergence and accommodated locomotor activity were impaired at 1.0 mg/kg-day but not at 1.5 mg/kg-day. Operant behavior was assessed at 3–6 months of age, well beyond the period of exposure. The authors could not explain the paradoxical dose-response pattern but noted that U shaped dose-response curves are commonly seen in dose response curves for psychoactive drugs.

While data from animal studies suggest an effect of pre-natal or postnatal pesticide exposure on brain physiology and function, data from human studies are less definitive. For example, Rauh et al. [25] conducted a study of 254 inner city children in the USA with potential exposures to chlorpyrifos from home application. The authors measured chlorpyrifos levels in cord plasma after birth, thus obtaining a surrogate measure of *in utero* exposure. To assess potential effects on cognitive development they used the Bailey Scales of Infant Development administered at 12, 24, and 36 months of age. Using the resulting data they developed multivariate regression models which included race, gender, maternal IQ and education, tobacco smoke exposure and home environment as co-variables. Chlorpyrifos cord blood was divided into two groups, high exposure (>6.17 pg/g) and low exposure (<6.17 pg/g). The overall range in cord blood values was from <1 to 63.3 pg/g. Rauh et al. reported that prenatal chlorpyrifos exposure was associated with increased odds of some adverse neurodevelopmental outcomes.

For example mental development index (MDI) and psychomotor development index (PDI) scores were significantly decreased in the high chlorpyrifos exposure group at 36 months of age, although this was not observed at 12 or 24 months. The authors also reported that the high category of gestational chlorpyrifos exposure was also associated with increased odds of developing attention problems, ADHD or pervasive developmental delay (PDD), although the confidence intervals for these outcomes were very wide (*e.g.*, odds ratio for diagnosis of ADHD: 6.50 [1.09–38.69]).

Another human study, Torres Sanchez et al. [34] evaluated prenatal DDT and DDE exposures and their potential effects on psychomotor development in 244 mother-child pairs living in an area of Mexico with endemic malaria. Mean serum DDE levels in the maternal population were approximately 7 ng/mL. Cognitive development was evaluated in children at different times during the first year of life using the MDI and PDI scores noted above. The authors reported that only 1st trimester DDE concentrations were associated with decreased PDI scores (-0.52 points, $p = 0.02$), MDI scores were not associated with estimates of pre-natal exposure. The authors noted their results were somewhat at odds with results of two earlier studies, one of which found no association between prenatal DDE levels and PDI and MDI scores, the other finding both scores affected. Torres-Sanchez *et al.* subsequently conducted a follow up study of this population, examining MDI and PDI scores at 12, 18, 24 and 30 months after birth. The researchers found that the previously observed effect on psychomotor development did not persist beyond 12 months of age, although it seems likely that the exposure would have continued after birth [35].

To date, the data indicating that low level insecticide exposures can exert subtle effects on nervous system development have generally been obtained from studies in laboratory rodents. Studies in humans are less definitive, due to the influence of co-exposures to other chemicals and other important covariables (*e.g.*, socioeconomic status). This remains an area of active research and considerable regulatory interest.

17.5.2 Endocine Disruption

The possibility that chemical exposures could disrupt the functioning of the endocrine system is another area of particular concern for children's health risks. Through the secretion of hormones (small molecules with very high affinity for specific cellular receptors), the endocrine system controls or influences many of the body's most important processes (*e.g.*, thermal regulation, energy production, blood sugar concentrations, reproductive function, immune response). Proper functioning of the endocrine system is also critical for both pre-natal and post-natal development [37]. The concept of endocrine disruption postulates that organic chemicals such as pesticides can mimic or interfere with the function of endogenous hormones, thus leading to improper signaling.

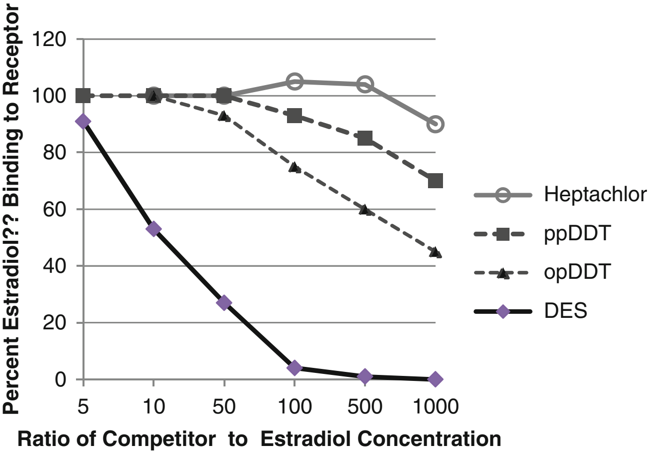

Fig. 17.4 Relative estrogen receptor binding affinity of several pesticides

One way to measure the ability of a chemical to cause endocrine disruption is via a competitive binding assay. Such an assay examines the ability of the chemical of interest to compete with a hormone for binding to the hormone's normal receptor. A chemical, which has the potential to dislodge a hormone like estrogen from its normal receptor at expected exposure concentrations would be an obvious endocrine disruptor. Studies have shown that pesticides are far less potent and have much lower affinity for the estrogen receptor compared to endogenous estrogen. For example, Blair et al. [9] demonstrated that while the non-pesticide diethylstilbestrol (DES) was able to substantially displace estrogen from its receptor at only a 10 times higher concentration, a 100-fold excess concentration of o,p′-DDT was required to have even a limited effect on estradiol-estrogen receptor binding (Fig. 17.4). p,p′-DDT and heptachlor were even less effective competitors for estradiol. Thus, at very low concentrations in plasma, these pesticides would not pose a concern as anti-estrogens. However, circulating levels of estrogen/estradiol are extremely low, in the tens to hundreds of pg/mL range, and at sufficient exposure, plasma concentrations of pesticide might be substantially higher. Thus it is not only the relative binding affinity but also the circulating concentrations that must be considered when examining potential risks.

Beyond receptor binding studies, studies conducted in whole animals have suggested that DDT has the potential to disrupt reproductive development. For example, Hojo et al. [17] reported delays in sexual maturation in male rats exposed *in utero* to 50 or 350 ppm DDT in the diet, although there were no deficits in reproductive function. These doses also caused alterations in serum estradiol and progesterone levels in female animals. However, both doses also caused systemic toxicity to the parental animals (tremors, liver toxicity, mortality), suggesting a limited relevance to potential human environmental exposures which would be expected to occur at levels below those causing such significant toxicity.

Studies of potential DDT effects on reproduction have also been conducted in human populations. For example, Bhatia et al. [8] conducted a case-control study of male reproductive tract abnormalities in the San Francisco area. They reported no association between maternal serum DDT/DDE concentrations and deformities of the male reproductive system. On the other hand, Cohn et al. [11] reported a decreased probability of pregnancy in women exposed to DDT as babies *in utero*. Maternal serum samples were drawn 1–3 days after delivery and analyzed for DDT and DDE. Time to pregnancy was then assessed 28–31 years later in 289 daughters of the women enrolled in the study. Median o,p′-DDT, p,p′-DDT and DDE concentrations in maternal blood were 0.49, 13.05, and 48.19 μg/L, respectively. Cohn et al. reported a decrease of 32 % in the probability of pregnancy per 10 μg/L p,p′-DDT in maternal serum (95 % CI: 11–48 %). However, they also observed a 16 % increase in probability of pregnancy per 10 μg/L p,p′-DDE in maternal serum (95 % CI: 6–27 %). This rather paradoxical finding (which the authors discuss could be due to an anti-androgenic effect of p,p′-DDE) illustrates the difficulty in understanding the complex nature of potential endocrine disruption.

As with the subtle neurological effects discussed earlier, the data indicating that insecticide exposures can cause endocrine disruption in human populations are limited, with many studies in both experimental animals and humans reporting results that are difficult to interpret. The effects that have been observed appear to be associated with fairly high levels of exposure so the relevance to typical human exposures is unclear. The data appear to be substantially stronger for effects on wildlife (*e.g.*, in amphibians, fish) which may be more sensitive to such effects [23].

17.5.3 Fetal Basis of Adult Disease

An emerging concept in both science and regulation is the idea that exposures *in utero* or in early childhood can predispose an individual to a disease that emerges later in life [16]. This has been described as a fetal basis for adult disease (FeBAD). Recent speculation has focused on early life exposures as causative agents in Parkison's Disease (PD) [6, 18, 19, 21]. It is known that the brain contains a certain number of non-renewable dopamine producing cells and that these cells are lost in PD. When a sufficient number of these cells are lost (typically later in life) the symptoms characteristic of PD (fine tremor, difficulties in gait) will become manifest. The new theory suggests that early life insults (*e.g.*, infections, chemicals, injuries) which destroy some of these cells may either cause an individual's PD to appear earlier or, when combined with normal age-related loss, may shift an individual into the symptomatic pool. The data supporting this hypothesis remain limited. A number of animal studies have shown an association between prenatal exposure to several herbicides (paraquat and maneb) and the emergence of PD-like symptoms in the animals at older ages [6]. Herbicide exposure has also been shown to be a risk factor for PD in a number of epidemiology studies (reviewed in [13]). However, these studies have also shown associations between PD and various infections, brain injury, and smoking [13]. The data are therefore somewhat muddled. Nonetheless, PD is one of most active areas of research concerning the long term consequences of early life pesticide exposures.

The case with PD involves destruction of cells. An alternative mechanism under consideration involves early life alterations in cellular components (usually DNA) which only manifest themselves late in life. As we learn more about how DNA is used in our cells it has become apparent that the genetic code itself is only part of the story. Epigenetic mechanisms such as the addition of methyl groups to DNA can alter gene expression and are used by normal cells to regulate their internal processes. Several recent studies have suggested that prenatal exposure to arsenic, which is known to interfere with DNA methylation, can predispose an individual to lung disease or cancer as an adult (reviewed in [38]). With specific reference to obsolete pesticides, a recent animal study conducted by Zama and Uzumcu [40] showed that prenatal and perinatal exposure (gestational day 19 to postnatal day 7) of rats to methoxychlor (20 or 100 μg/kg-day), resulted in persistent hypermethylation of ovarian genes later in adult life. A number of studies have also suggested the possibility of obesogens, chemicals which alter fat metabolism and predispose an individual to weight gain, obesity and a constellation of related diseases (*i.e*., diabetes, heart disease, certain cancers) later in life. A recent review by Slotkin [28] has suggested that organophosphate exposure could be linked to later life development of diabetes and obesity. Slotkin summarized studies which showed that 1 mg/kg-day of chlorpyrifos, administered on the first 4 days after birth, resulted in a pre-diabetic state in rats when tested as adults. Similar findings were reported by this research group in rodent studies of diazinon and parathion.

The FeBAD hypothesis remains largely speculative. Findings from animal studies are suggestive but difficult to extrapolate to humans. Conducting good epidemiology studies, which can be more informative, will be challenging. The long time interval between exposure and disease appearance will entail considerable expense, potential loss of subjects, and challenges in terms of controlling potential confounders (*e.g*., diet, genetics). What is very clear is that the FeBAD concept is proving to be particularly appealing to researchers and regulatory scientists, combining as it does two key populations of interest (children and the elderly).

17.6 Conclusions

Infants and young children constitute a particular population of concern in terms of exposure to pesticides. Children may engage in particular activities (crawling, hand-to-mouth activity) that result in increased exposures to chemicals in the environment. Children also have a higher respiration rate, greater relative skin surface area, and higher relative food and water intakes than adults, each of which contributes to a greater exposure potential. Furthermore, the developing physiology and metabolic capacity of infants and young children may also create an increased toxicological susceptibility. The potential for increased susceptibility must be evaluated on a case-by-case basis however, because in some cases certain age groups may have similar or even less susceptibility compared to adults. Research has also pointed out a number of toxicological modes of action that may be of particular concern for children's risks. These include subtle effects on nervous system development, endocrine disruption and the impact of pre- and perinatal exposures on development of disease later in life. Although the importance of such modes of action is not fully understood, particularly at low levels of exposure, further research in these areas is warranted.

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Chapter 18
Chromatographic Methods for Determination of Chlorophenols as Biomarkers of Exposure to Pesticides and Organochlorine Substances

Paulo de Morais, Teodor Stoichev, Maria Clara Basto, and Maria Teresa Vasconcelos

Abstract Chromatographic methods for chlorophenols (CPs) determination that can be applied to clinical (blood, urine) and breast milk samples are reviewed. Special emphasis is given to sample storage conditions and to the application of preconcentration techniques. Solid phase extraction, solid phase microextraction, stir-bar sorptive extraction, liquid phase microextraction and steam distillation methods are considered in view of the potential use of CPs as biomarkers of exposure to different pesticides.

Keywords Chlorophenols • Chromatography • Blood plasma • Urine • Breast milk

18.1 Introduction

Chlorophenols (CPs) are highly toxic chemicals, widespread in the environment, mainly used as pesticides, wood and leather impregnation agents and in chemical synthesis. They are derivatives of phenol having different degrees of substitution, with one to five chlorine atoms: monochlorophenols (MCPs), dichlorophenols (DCPs), trichlorophenols (TCPs), tetrachlorophenols (TeCPs) and pentachlorophenol (PCP). Their determination in liquid biological samples is an indicator of exposure to CPs or other chlorinated organic substances, including polychlorinated dioxins and furans [3].

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The CPs in human urine appear as a result of occupational or environmental (food, water, air) exposure to CPs or as metabolites of other chlorine-containing compounds [12, 16]. The excretion of PCP in the urine of the general population is related to its use, especially in the past, as wood and leather preservation agent [2, 7]. CPs in urine could also potentially be biomarkers of exposure to a wide range of other products, like insecticides, fungicides, herbicides and industrial chemicals [7]. However, even if there was a reason for such claim, the concentration of CPs in urine has been related to the exposure to a parent compound in a limited number of cases. For example, lindane is metabolized to 2,4,6-TCP and 2,4-DCP in mouse [15] and to TCPs and TeCPs in calf [17]. More information is available on the urinary excretion of CPs as metabolites of chlorobenzenes (CBs), used as solvents, lubricants and heat transfer fluids and as intermediates in chemical synthesis. Hexachlorobenzene is applied as fungicide and 1,4-dichlorobenzene (1,4-DCB) has a household use as moth repellent. It is the reason for the presence of 2,5-DCP, a metabolite of 1,4-DCB, in urine samples of professionally non-exposed individuals [2, 7]. Its concentrations have been positively correlated with the levels of 1,4-DCB in blood [8]. 2,5-DCP concentrations in urine have been also used as a biomarker for professional exposure to 1,4-DCB [9]. MCPs are excreted in urine as metabolites of monochlorobenzene [13] and 2,3-DCP and 3,4-DCP as metabolites of 1,2-DCB [14]. In both cases, the concentrations of CPs in urine were related to the airborne exposure to CBs.

The concentrations of free (non-conjugated) CPs in urine samples are usually less than 1 μg L^{-1}, or less than 0.1 μg L^{-1} for the case of PCP [16]. After de-conjugation of CPs in the urine samples, the maximum concentrations, depending on the compound and the level of exposition, were in the order of a few μg L^{-1} [1], from less than 1 μg L^{-1} to several tens μg L^{-1} [4, 10–12], from a few μg L^{-1} to hundreds μg L^{-1} [2] and from several tens to several thousands μg L^{-1} [7]. The median concentrations are usually from less than 1 μg L^{-1} to several tens μg L^{-1} [2, 7].

CPs are retained in blood and only to a lesser extent in adipose tissue [25]. Direct exposure to CPs can be proved by analyzing blood or blood plasma samples. However, there is a limited number of analytical methods, for example, to determine polychlorinated CPs. The concentrations of PCP found in the blood plasma were in the range <0.02–6.3 μg L^{-1} [25].

Babies are particularly vulnerable to chemical contamination. In breast milk, CPs were measured with maximum values in the order of tens to a hundred μg kg^{-1} but median concentrations were at or below 1 μg kg^{-1} [23]. The most frequently detected, and with highest levels, were 2,4-DCP, 2,4,5-TCP and PCP. PCP is of particular interest, since it has the highest toxicity of all CPs.

18.2 Methods for CPs Determination

18.2.1 Sample Storage

Care must be taken when storing samples before the analysis of CPs. There are discrepancies in the literature about the stability of CPs. The CPs were found to be stable in frozen urine samples [12, 17]. However, other authors previously reported decomposition of PCP at the same conditions [20].

Urine samples have been collected in sterilized containers and stored at −20 °C [4, 9, 12, 16] or −30 °C [13] or even at −80 °C [11] prior to use. If acetic acid (1 % v, v) is used for the preservation of urine, it can be stored at 4 °C [1]. Human plasma should be immediately frozen and stored up to 1 month [19]. Blood samples have been stabilized with ethylenediamine tetraacetic acid (EDTA), stored at 4 °C and analyzed within 24 h of collection [22]. Milk samples have been stored frozen at −20 °C [21, 23].

18.2.2 Sample Pretreatment and De-conjugation

CPs in urine are presented mainly as glucuronide and sulfate conjugates but free forms also exist and were found to be up to 20–30 % of the total [4]. When methods with low limits of detection (LODs) are going to be used, it is possible to proceed directly to the analysis of free forms of the CPs [9, 16]. Sometimes, it is not possible to measure the CPs without previous de-conjugation due to the low concentrations of free CPs in the urine of non-exposed individuals, especially when using methods with relatively high LODs. Different techniques for hydrolysis of the conjugates have been developed. Most of the researchers used acid hydrolysis with sulfuric [1, 12] or hydrochloric acid [4, 6, 13, 24]. Conditions (acid concentration, temperature and time) for acid hydrolysis were optimized [4]. Enzymatic hydrolysis was carried out with β-glucuronidase and sulfatase at 37 °C for 3 h [7, 10, 11]. The choice of hydrolysis procedure could depend sometimes on the determination method used. For example, alkaline hydrolysis for the urine samples could be preferred if the acid and enzymatic treatments show interferences with the analytical procedure [18]. Alkaline hydrolysis was also used for the case of breast milk samples [23] but the lack of variety of analytical methods for the determination of CPs in milk does not allow drawing general conclusions.

Blood plasma can be analyzed without any sample pretreatment [19]. However, sometimes long pretreatment procedures could be necessary. Precipitation of plasma proteins and the release of bound analytes from the matrix could be achieved by adding water-miscible solvents, like methanol and acetone [25]. That procedure required subsequent evaporation of the organic solvents.

18.2.3 Determination of Chlorophenols

Some of the methods used for water analysis, reviewed by [5], have been applied for CPs determination in biological fluids. The analytical methods for CPs determination in human milk, urine and blood plasma, the degree of chlorine substitution in the analytes and the LODs are summarized in Table 18.1. From the concentrations

Table 18.1 Methods for determination of CPs in liquid biological samples

Determination method/sample type	CPs[a]	LOD μg L^{-1}
LLE-Acetyl-GC-MS/urine[c]	2–5	3.1–5.4
LLE-BackE-ClPropyl-GC-MS/MS/urine[d]	2, 3, 5[b]	1–2
LLE-GC-FID/urine[e]	2	500
LLE-FLLabel-LC-FLD/urine[f]	1–3	3.1–9.9
LLE-LC-UV/urine[g]	1	100
SPE-GC-MS/urine[h]	1–3, 5[b]	0.3–0.6
SteamD-SPE-GC-ECD/urine[i]	3–5	0.01–0.03
SPME-GC-MS/urine[j]	1	8
SPME-GC-MS/urine[k]	1–5	0.001–0.04
HS-SPME-GC-ECD/urine[l]	2	0.55
Acetyl-SBSE-TD-CT-GC-MS/urine[m]	2–5	0.01–0.02
Acetyl-HF-LPME-GC-MS/urine[n]	2–5	0.1–0.2
SLME-LC-biosensor/plasma[o]	1[b]	43
Acetyl-HS-SPME-GC-MS/plasma[p]	5	0.02
HS-SPME-GC-ECD/blood[q]	3–5[b]	0.05–0.44
SteamD-LLE-Benzoyl-GC-ECD/milk[r]	2–5	0.1–1.0
HS-SPME-GC-ECD/milk[s]	3–5[b]	0.56–1.01

[a] Number of Cl atoms in the analytes; [b] Other compounds also analyzed; [c] Kontsas et al. [12]; [d] Hill et al. [7]; [e] Kumagai and Matsunaga [14]; [f] Wada et al.[24]; [g] Kumagai and Matsunaga [13]; [h] Crespín et al. [4]; [i] Bartels et al. [1]; [j] Guidotti et al. [6]; [k] Lee et al. [16]; [l] Hsiao et al. [9]; [m] Kawaguchi et al. [11]; [n] Ito et al. [10]; [o] Norberg et al. [19]; [p] Zhou et al. [25]; [q] Röhrig et al. [22]; [r] Veningerová et al. [23]; [s] Röhrig and Meisch [21]

of CPs found in real samples (see the introduction), target LODs for CPs can be established. The LODs of the analytical methods for CPs determination in urine (after de-conjugation) must be set to target values in the order of 1–10 μg L^{-1}. Similarly, for blood plasma and breast milk samples, the target LODs have to be less than 1 μg L^{-1}. The use of gas chromatography (GC) with flame ionization detector (FID) or liquid chromatography (LC) with ultraviolet (UV) detection leads to very high LODs (Table 18.1). Even the use of very efficient microextraction techniques, for example, supported liquid membrane extraction (SLME), could not lead to low LOD owing to the weak detector response to CPs [19]. The methods that match the target LODs include either GC with electron-capture detection (ECD), mass spectrometry (MS), tandem mass spectrometry (MS/MS) detection or LC equipped with fluorescence detection (FLD) after fluorescence labeling (FLLabel) of the CPs. Nevertheless, it is always necessary to preconcentrate the analytes.

The liquid-liquid extraction (LLE) is not currently preferred due to the use of large quantities of organic solvents and tedious, time-consuming procedures with relatively high LODs (Table 18.1). Lower LODs have been provided [7] when LLE was combined with back extraction (BackE) and the CPs were derivatized by chloropropylation (ClPropyl).

Styrene-divinylbenzene solid phase extraction (SPE) cartridges were used to preconcentrate CPs from acidified urine samples [4] or from the distillate after the removal of the CPs from the matrix by steam distillation (SteamD) [1]. Usually, preconcentration by SPE leads to much lower LODs (compared with LLE-based methods) but small quantities of organic solvents are still required. In another method, the SteamD was combined with LLE to extract the CPs from breast milk samples [23]. After derivatization, the pentafluorobenzoyl derivatives (Benzoyl-CPs) were analyzed by GC-ECD obtaining LODs from 0.1 to 1 μg L^{-1}.

Microextraction methods have been mainly applied to determine CPs in liquid samples since they provide the lowest LODs, usually in the order of ng L^{-1} to μg L^{-1} (Table 18.1). Owing to the low surface tension of the biological liquid samples, leading to drop instability, the use of liquid phase microextraction (LPME) is limited to the hollow fiber (HF) technique. [10] extracted acetylated derivatives of CPs (Acetyl-CPs) from urine samples by using HF-LPME and analyzed them by GC-MS. Urine samples were also analyzed without derivatization by direct solid phase microextraction (SPME) coupled to GC-MS [16] or with headspace (HS) SPME-GC-ECD [9]. The latter method has been also used for analysis of blood [22] and milk samples [21]. In another method [11], the Acetyl-CPs in urine samples were determined by stir-bar sorptive extraction (SBSE) coupled to thermal desorption (TD) GC-MS with cryogenic trapping (CT). The matrix effects were overcome using isotope-labeled surrogate standards.

18.3 Conclusion

Not all analytical methods used to determine CPs in liquid biological samples have sufficiently low LODs to study the behaviour of CPs and other chlorinated organic compounds in individuals non-exposed professionally to CPs. The choice of the detector and, sometimes, the application of derivatization procedure seem to be important for the development of sufficiently sensitive analytical methods. Microextraction techniques are preferable, because they are either solvent-free or require much lower amounts of organic solvents. Furthermore, they are very sensitive and require a small sample volume, which is very important in the analysis of liquid biological samples. The potential use of CPs as biomarkers of exposure to different pesticides is still a field that needs research.

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Chapter 19
Environmental and Health Situation with Obsolete Pesticides in Egypt

Amir El-Shahawy and Lubomir I. Simeonov

Abstract Obsolete pesticides are pesticides that are unfit for further use or for re-conditioning as they have been de-registered locally or banned internationally. More commonly, however, a stock of pesticides becomes obsolete because of long-term storage during which the product and/or its packaging degrade. The total quantity of potentially obsolete pesticides held in developing countries and countries with economies in transition is thought to be huge, on the order of tens or hundreds of thousands of tons. The amount can only be estimated, however, because many stocks have not been inventoried or even located. The problem of obsolete pesticides is very serious and action is urgently needed to identify and secure or eliminate existing stocks and prevent new accumulations. Stocks of obsolete pesticide that stay unmanaged can pose a serious health hazard, especially to people living near these stocks. Comprehensive information about the impact on public health is scarce and there has been no attempt to systematically document health effects. Egypt shares most of the environmental problems of developing countries. One of the most important health and environmental problems is the obsolete pesticides and this paper will shed the light on the current status of obsolete pesticides in Egypt, environmental and health impacts and efforts conducted by the country to mitigate both short-term and long term impacts.

Keywords Obsolete pesticides • Health • Egypt • North Africa • POPs • Organochlorines • DDT

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19.1 Introduction

Obsolete pesticides are defined by the Food and Agriculture Organization of the United Nations (FAO) as all pesticide products not in current use because they have been banned, have deteriorated or are damaged, have passed their expiration date, cannot be used for any other reason, or are not wanted by the current owner. Obsolete pesticides are chemically complex, given that nearly 1,000 active ingredients in many thousands of formulations are used to manufacture pesticides around the world. More than 20 % of obsolete stocks consist of Persistent Organic Pollutants (POPs): Chlorinated hydrocarbons (organochlorines) such as dichlorodiphenyl ethane (e.g., DDT, DDD and DDE), cyclodiene (e.g.: aldrin, dieldrin, heptachlor) that persist in the environment; bioaccumulate in humans, wildlife, and fish; and are highly toxic, [14, 28].

Obsolete pesticide stocks have been accumulating in many developing countries and economies in transition in recent decades. International organizations estimate that nearly 500,000 tons are stockpiled worldwide, about 50 % of which are located in countries of the former Soviet Union; while, obsolete stocks total about 50,000 tons across the African continent and 30,000 tons in Latin America [28].

These obsolete pesticides represent a major hazard on human health [20]. Low-level exposure to POPs such as DDT may cause endocrine disruption, lowering of disease resistance and affecting reproduction [17]. They can also cause more acute health impacts, including poisoning. Persistent organic pollutants cause a range of health hazards, even at low levels of exposure, including reproductive and developmental disorders, damage to the immune and nervous systems, and different types of cancers [13, 17]. Exposure during key phases of fetal development can also cause serious developmental hazards in children [15].

The main causes of accumulation of obsolete pesticides in developing countries are: inadequate storage and poor stock management, banning the products, unsuitable products or packaging, donation or purchase in excess of requirements, lack of coordination between donor agencies, and commercial interests of private sector and hidden factors [26].

The condition of the stocks is highly variable. Some stocks consist of unprotected and unidentifiable amounts of mixed products, corroding containers and contaminated soils into which pesticides have leaked from containers or production plants. Many stocks are located near farm fields, homes, or water sources, as the pesticides were stored close to where they were to be used. Many stocks are abandoned, unmanaged, have no labels, and have no clear “owner” who is responsible for them. In most cases, such stocks would be classified as hazardous waste under international laws and controlled by the Basel Convention if subject to transboundary movements [12].

Almost every country in Africa has stockpiles of obsolete pesticides that accumulated over the past several decades. Such countries often lack the training and resources to safely manage pesticide use, storage, and destruction, particularly at remote storage sites. Many warehouses are not well secured. Over time, containers and packages become deteriorated; hence, spills and leaks find their way into surface waters from runoff or leach through soil to pollute groundwater, resulting in environmental contamination and human exposure [26].

FAO, United Nations Environment Programme (UNEP), international donors, aid agencies, governments of countries with stocks, pesticide producers, and non-governmental organizations have taken on projects to locate, collect and eliminate existing stocks of obsolete pesticides and to prevent the accumulation of new ones. FAO has developed written guidelines and codes of conduct to help developing countries better manage pesticides, eliminate obsolete stocks, and avoid accumulating new ones. The OECD Development Assistance Committee (OECD - DAC) has written guidelines for aid agencies that describe the problem and show how aid should be directed to avoid it. But these efforts have been able to address only a small part of the very large problem of obsolete pesticides [12].

19.2 Obsolete Pesticides in North African Countries

Due to many causes, almost all of the African countries have accumulated huge quantities of obsolete organochlorine pesticides (OCPs) and other persistent organic pollutants that caused contamination of different environmental compartments, and thus required actions of remediation. These hazardous pesticides are contaminating soil, water, air, and food sources. They pose serious health threats to rural as well as urban populations and contribute to land and water degradation. Poor people often suffer a disproportionate burden because, in poor communities these dangers are compounded by a other factors including unsafe water supplies, poor working conditions, illiteracy, and lack of political empowerment [4].

Poor communities often live in closer proximity to obsolete pesticide stocks than wealthy people and children are at a higher risk of exposure than adults. The World Health Organization (WHO) estimates that pesticides may cause 20,000 unintentional deaths per year and that nearly three million people may suffer acute and chronic effects, mostly in developing countries. The risk on poor communities becomes maximized by inadequate access to healthcare systems; especially in farming communities [24].

North African countries could be classified under main categories based on quantity of stockpiles they hosted: More than 1,000 tons (Morocco and Sudan), 500–1,000 tons (Egypt and Tunisia), 100–500 tons (Mauritania), Less than 100 tons (Algeria) Countries of no available data (Libya), Table 19.1.

Table 19.1 Obsolete pesticides quantities in some North African countries

Country	Quantity (ton)
Algeria	20
Egypt	600
Libya	?
Mauritania	257
Morocco	2,265
Sudan	24,418
Tunisia	882

Source: Bernstorff and Stairs [8]

? no FAO survey conducted in Libya up to 1999

19.3 Obsolete Pesticides in Egypt

In Egypt, limited Egyptian cotton fields were treated with insecticides before 1950. Thereafter, the treated area expanded rapidly. During the period 1950–1955, some cotton fields were treated using DDT. The reported major obsolete pesticides used in Egypt during a 30-year period were toxaphene (1955–1961), endrin (1961–1981), DDT (1952–1971), and lindane (1952–1978) [18].

DDT and lindane have been officially prohibited from agricultural use in Egypt since 1980, and in 1996 a Ministerial Decree prohibited the import and use of multiple pesticides including aldrin, dieldrin, endrin, chlordane, heptachlor, DDT, toxaphene and lindane [23].

Obsolete pesticides are no longer in use in Egypt; however, they are still being found as residues and they are occurring in food now as a result of environmental contamination. Herbivores may ingest these residues in water, plants, and grasses, so, the residues can eventually find its way into human food [18].

19.3.1 Environmental Pollution by Obsolete Pesticides in Egypt

In many developing countries, storage sites have been located far from residential areas; however, they become now surrounded by fast-growing urban communities. Where pesticides are stored in the open, families that live and work in the vicinity may suffer acute or chronic exposure. Long-term exposure has been linked to a range of health hazards, from disorders of the nervous, immune, reproductive, and endocrine systems and various types of birth defects to injury of specific organs of the body and cancer. Nearby such storage sites, livestock may be found grazing and contaminated water is used for drinking and land irrigation for growing edible crops [26].

Table 19.2 Residues of obsolete pesticides (ng/L) in water samples collected from different locations along the River Nile[a]

Sampling site	Total DDTs	Total cyclodienes
Rosetta	98.51	32.39
Damietta	90.87	65.57
Al-Mansoura	102.67	48.43
Edfina	10.13	228.11
Dessouk	37.21	186.82
Kafr El-Zayat	29.75	415.37
Delta Barrage	2.65	29.75
Beni Suef	56.83	26.5
El-Menia	82.42	16.77
Assuit	100.56	75.28
Naga Hammady	297.72	40.65
Esna	586.3	75.37
Kom Ombo	1,035.25	41.27
Aswan	1,048.24	28.5
Lake Nasser	841.47	20.86

[a] Adapted from Wahaab and Badawy [25]

DDT dichlorodiphenyltrichloroethane

Cyclodienes, include aldrin, dieldrin, endrin

19.3.2 Air and Precipitation

The published data on the levels of obsolete pesticides in the atmospheric environment of Egypt is very limited probably due to the methodological difficulties [5].

19.3.3 Freshwaters

Previous studies on fresh aquatic environments were mainly focused on the Nile River and the four major delta lakes: Maryut, Edku, Burullus and Manzala [5], Table 19.2. Cyclodiene pesticides (aldrin, endrin and heptachlor) had been reported in water samples collected from the Nile river, with aldrin concentrations more than endrin [10]. Higher concentrations were reported at Kafr El Zayat city and at sites on the Rosetta than on Damietta branch. Total concentrations of cyclodienes were found in the range 8–21 ng/l in water samples collected from Lake Manzala [29].

Samples from ground water, Nile River and tap water were collected from Kafr El Zayat governorate, which has one of the largest pesticide factories in Egypt, and DDT residues were found at levels below the maximum allowable limits set by the WHO for drinking water [9]. In addition, the levels of DDTs detected in the groundwater underlying the sludge and solids disposal facility for the sludge, scum and screenings generated at the East and West Wastewater Treatment Plants in Alexandria, Egypt have been found at levels below the drinking water standards set by the U.S. Environmental Protection Agency (EPA) [19].

19.3.4 Seawater

In Egypt, El Rahawy drain is one of the most severely contaminated areas by OCPs because it has been used for a long time and it receives wastewater discharge from all sewage of Giza governorate in addition to local domestic of ElRahway village. The production of OCPs was officially banned in Egypt since late 1990; however, OCPs are still being used for agricultural and public health purposes because of their low cost, easy to use and versatility against various insects [16].

19.3.5 Sediments

It was reported that high concentrations of total DDT were encountered at locations within Alexandria inner harbor and at sites located near ship activities and storm sewer outfalls. The concentrations of DDT in the Inner Harbor were at levels suspected to cause detrimental biological effects for benthic organism and the ratios of DDTs/DDTs plus their metabolites in sediment samples from certain sites were 0.86 or higher, indicating recent input of DDT [6].

19.3.6 Food Stuff Pollution by Obsolete Pesticides in Egypt

During the past 20 years, Cyclodiene pesticides have been determined in a large number of fish in coastal areas in Egypt. Concentrations were lower than the acceptable tolerance levels for human consumption and temporal trends showed decreasing levels of cyclodiene pesticides in Abu-Quir Bay. Fish samples (Mugil cephalus) collected from Abu-Quir, Gamasa and Damietta exhibited concentrations in the range 4–9 ng/g ww that suggests a decline in DDTs concentrations during the last decade [1].

The following tables summarize the levels of obsolete pesticides reported in Egypt in food stuff samples collected from meats (Table 19.3), dairy milk products (Table 19.4), vegetables (Table 19.5) and medicinal plants (Table 19.6):

19.3.7 Levels of Obsolete Pesticides in Humans

Human milk samples that were collected from 20 governorates showed that DDTs were the most frequently found pesticide in human milk studied in Cairo area. Nearly 51 % of the samples had DDT levels below the detection limit. In none of the samples where DDT was detected, the WHO recommended limits were exceeded [21].

Table 19.3 Obsolete pesticides in meats (muscles, livers, kidneys) from camel, cattle, and sheep taken from Sharkia Province, Egypt[a]

Contaminant	% of contaminated samples
DDTs	54.4
Lindane	47.8
Aldrin	44.4
Dieldrin	33.3
Endrin	15.6
Toxaphene	<10.0

[a]Adapted from Sallam and Morshedy [23]

DDT dichlorodiphenyltrichloroethane

Table 19.4 Residue levels of obsolete pesticides in dairy products[a] (ng/g dw lipid) and in cattle meat[b] (mg/kg fat) reported in Egypt

Foodstuff	Cyclodienes	Total DDTs
Buffalo milk	140.03	1,014.19
Milk powder	8.56	38.83
Damietta cheese	10.00	80.5
Cattle meat	84.60	67.00

[a]Adapted from Barakat et al. [6]

[b]Adapted from El-Kady et al. [11]

Table 19.5 Residue of obsolete pesticides (mg/kg) in vegetables and soil from an organic farm at El-Sadat city, Egypt[a]

Pesticide	Root beet	Soil	Green onion	Soil	Potatoes	Soil
Total DDT	2.02	6.90	2.82	6.77	1.78	6.18
Lindane	0.61	1.35	0.84	1.27	0.60	1.27
Aldrin	ND	ND	ND	ND	ND	ND
Dieldrin	0.35	0.77	0.70	0.58	0.30	0.64
Endrin	ND	0.71	ND	0.85	ND	0.93
Endosulfan	0.27	0.83	0.66	1.03	0.23	0.64

[a]Adapted from Salim [22]

ND not detected

Table 19.6 Mean concentration levels (mg/kg) of obsolete pesticides in samples of some medicinal plants collected from Egyptian market[a]

Pesticides	Peppermint	Chamomile	Anise	Caraway	Tilio
Total DDT	0.384	0.039	0.575	0.352	0.241
Lindane	0.028	0.501	0.346	0.311	0.081
Aldrin + Dieldrin	0.072	0.006	0.255	0.196	0.048
Endrin	0.068	0.089	0.091	0.016	0.015

[a]Adapted from Abou-Arab and Abou-Donia [2]

DDT dichlorodiphenyltrichloroethane

In a following study, 292 mother's milk samples collected from 10 Egyptian governorates representing rural, metropolitan and newly reclaimed desert areas. The percentage of samples exceeding the acceptable daily intake for children

(ADIs) set by the FAO/WHO ranged from 7 to 48 % in all governorates. Higher percentages of unacceptable samples were recorded in Kaliobia and Menoufia governorates, which can be attributed to the higher agricultural activity in the Delta region. Nevertheless, the results suggest the absence of recent DDT sources, and prove the effectiveness of the restrictions imposed on the use of DDT in agriculture, vector control and in household since the early 1970s [5].

Average residues levels were recorded for aldrin, dieldrin, endrin in 292 mother's milk samples collected from 10 Egyptian governorates; while, Lindane was detected in 95 % of human milk samples. The higher levels of lindane were a reflection to its use in agriculture and in control of cattle ecto-parasites. The percentages of samples that exceeded theADIs were 15 % mostly from the Delta Nile area [21].

However, even with the observed decreasing trend of organochlorine pesticides in mother's milk, the current information on levels in food and humans of some obsolete pesticides appears to be scarce to make an evaluation of health risk assessment at the actual levels of contamination [5].

19.3.8 Recent Efforts to Manage Obsolete Pesticides in Egypt

The World Bank's current portfolio of projects to eliminate obsolete pesticides gives a special attention to Africa and to high priority countries with large amounts of obsolete pesticides as Egypt. The Bank is administering US$60 million in funds to support the first phase of the Africa Stockpiles Program (ASP). This continent-wide initiative aims to safely eliminate obsolete pesticide stocks from African countries and build capacities to prevent further buildup and the current challenge is to eliminate these pesticides from the continent in an environmentally sound and safe manner within 12–15 years [28].

Egypt's National Implementation Plan (NIP) has established national priorities for completing inventories of POPs materials, planning their disposal and launching a large-scale public awareness campaign. To assist Egypt in implementing the priority POPs activities identified in its NIP, the Canada POPs Trust Fund has supported a project to: (i) to identify obsolete pesticides in Egypt; (ii) safeguard high-risk stocks of obsolete pesticides [27].

Cement kilns are occasionally used to treat hazardous waste in a number of countries. The high temperatures of the kilns are very suitable for the total break-down of these pesticides and would also ensure no emission of dioxins and furans if it was efficiently implemented. The use of cement kilns offers a very competitive and cost-effective alternative for the elimination of obsolete pesticides, especially in countries like Egypt with few waste management facilities [3]. A pilot project funded by the Government of Finland is being conducted for assessment and monitoring the disposal by incineration of obsolete pesticides in cement rotary kilns in Egypt, (BCRC [7]).

19.4 Conclusions

Egypt has many ongoing efforts and projects to elaborate a comprehensive inventory for obsolete pesticides and to eliminate and secure the available amounts; however, those efforts aren't yet sufficient. The reported levels of contamination by obsolete pesticides in the environment, food stuff and samples from humans are not exceeding the maximum allowable levels; but, there are still health hazards that can occur on the long term and they are not yet well documented by the available literature on Egypt. One of the top priorities issues should be finding alternative non-combustion technologies for disposing obsolete pesticides

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Chapter 20
Analytical Method for Determination of Some Priority Substances in Surface and Ground Water Samples by Liquid Chromatography – Mass Spectrometry

Borislav Lazarov, Youlia Manova, and Anna Dimitrova

Abstract Chemical status of water bodies is determined through environmental quality standards, defined for priority pollutants (Directive 2008/105/EC, Off J Eur Commun, December 24, L 348/84, 2008), mostly represented by persistent organic compounds and pesticides. There is a need for precise and accurate analytical methods for reliable assessment of environmental status of surface and ground waters. The aim of this work was to develop an innovative multiresidue analytical method for simultaneous determination of some priority substances in surface and ground waters. Determined target compounds include the pesticides: Acetochlor, Alachlor, Chlorfenvinphos, Chlorpyrifos, Diuron, Isoproturon, Pendimethalin and Trifluralin. Analytical approach was based on a solid-phase extraction (SPE) as a sample pretreatment procedure and a liquid chromatography–mass spectrometry (LC–MS) as an instrumental method. Optimal chemical conditions for quantitative separation and preconcentration of the analytes in a single step extraction procedure are defined. LC–MS method performance is discussed in details. The achieved analytical recovery for all compounds was greater than 86 % and repeatability is up to 10 %. The established detection limits were ranged between 1 and 10 ng/L and the quantification limits were between 5 and 50 ng/L, respectively. The concentration levels of measured priority substances in main Bulgarian rivers were presented.

Keywords Pesticides • Surface water • Ground water • Multiresidue determination • Solid phase extraction (SPE) • Liquid chromatography mass spectrometry (LC–MS)

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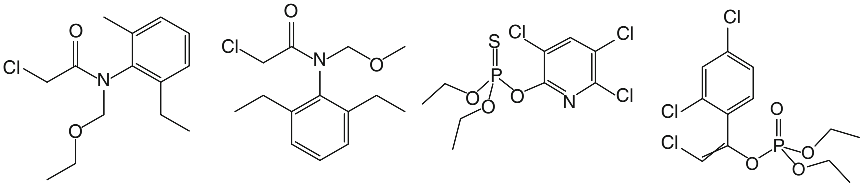
20.1 Introduction

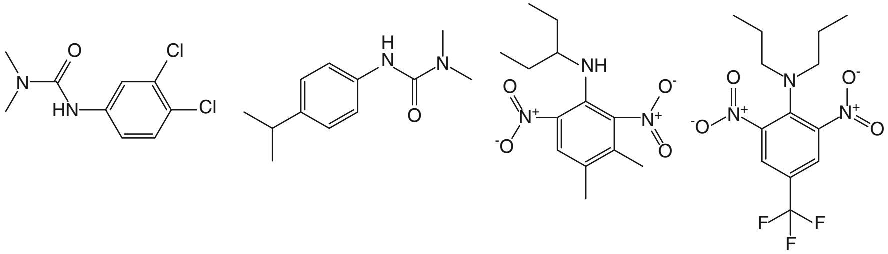
Organic contaminants in the environment are released from different anthropogenic activities such as industrial chemical production, agricultural application and domestic activities [6]. Many of these contaminants are stable and can present for a long period of time in the environment. So due to their extensive use and persistence, many of these compounds can be transferred in surface and ground waters and have to be considered as a potential risk for aquatic and soil ecosystems [1, 5]. In this context, strict regulations for the control of contaminants concentration levels in the environment have been established. In the field of water policy, the European Union (EU) adopted the Water Framework Directive (WFD) [3], which is probably the most significant international legislation in the field of water quality. The Directive takes a broad view of water management, setting the prevention of any further deterioration of water bodies and the protection and enhancement of the status of aquatic ecosystems as its key objectives. The overriding objective of the policy is the achievement of "good status" in all water bodies. Its implementation should result in an intensification of monitoring of aquatic ecosystems and increased control of contaminants. Article 16 of the WFD sets out EU strategy against pollution of water by chemical substances. According to the provisions of this article, a list of 33 priority substances, which represent a significant risk to or via the aquatic environment at the EU level, has been established and will have to be re-examined by the European Commission (EC) at least every 4 years [2]. Most of the current list are organic contaminants (hydrocarbons, organochlorine compounds, organic solvents, pesticides, and chlorophenols).

According to the WFD, good chemical status for a water body is obtained when the concentrations of the priority substances in water, sediment or biota are below the Environmental Quality Standards (EQSs): this is expressed as "compliance checking". In the next stage, the EC has proposed EQSs for water only. They are listed in a daughter Directive [4]. In order to reach the "good status" for all European water bodies, Member States have to implement river basin management plans, including water monitoring programs, and take measures when results do not comply with the EQSs. Under this Directive Member States are required to take actions to meet those quality standards by 2015. For this purpose a programme of measures are in place and should become operational by 2012.

Bulgaria, as a Member of the EU, must meet the WFD environmental standards in order to achieve "good chemical status" of its waters. Therefore precise and accurate analytical methods are needed for reliable assessment of environmental status of surface and ground waters. The objective of this work was the development and optimization of an innovative multiresidue analytical method, based on solid phase extraction (SPE) followed by liquid chromatography mass spectrometry (LC–MS) for a simultaneous determination of some of the priority substances in surface and ground waters. The target compounds are eight pesticides from the list of 33 priority substances: Acetochlor, Alachlor, Chlorfenvinphos, Chlorpyrifos, Diuron, Isoproturon, Pendimethalin and Trifluralin. Chemical structures of the selected analyzed pesticides are presented on a Fig. 20.1.



Acetochlor Alachlor Chlorpyrifos Chlorfenvinphos



Diuron Isoproturon Pendimethalin Trifluralin

Fig. 20.1 Chemical structures of the analyzed pesticides

20.2 Experimental Section

20.2.1 Chemicals and Reagents

Acetochlor (2-Chloro-2′-methyl-6′-ethyl-N-ethoxymethyl-acetanilide) and Pendimethalin (3,4-Xylidine, 2,6-dinitro-N-(1-ethylpropyl)-) were provided by-Sigma-Aldrich (St. Louis, MO, USA). Alachlor (2-chloro-2′,6′-diethyl-N-(methoxymethyl)acetanilide), chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioic acid), chlorfenvinphos (O,O-diethyl-O-1-(2′,4′-dichloro-phenyl)-2-chlorovinyl-phosphate), diuron (1,1-Dimethyl-3-(3,4-Dichloro-phenylurea), isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea) and trifluralin (2,6-Dinitro-N,N-dipropyl-4-(trifluoromethyl)-benzamine) were provided by Dr. Ehrenstorfer (Augsburg, Germany). Isoproturon-d6 (Dr. Ehrenstorfer, Augsburg, Germany) was used as internal standard. All analytical standards were of high purity (>97 %). The solvents methanol, acetonitrile and acetone (HPLC grade) and formic acid (for analysis) were provided form Merck (Darmstadt, Germany). The individual stock standard solutions were prepared in methanol at the concentration of 100 mg/L and stored in the dark at 4 °C. The composite working standard solution was prepared by mixing the appropriate amounts of the individual standard solutions and diluting with methanol to the final concentration range between 5 and 100 ng/mL. Recovery studies were performed in different matrix samples (surface water, ground water and distilled water) by appropriate dilution of composite standard solution, in order to obtain a final concentration for each compound in the samples of 0.05 μg/L. All aqueous solutions did not contain more than 0.1 % methanol.

Table 20.1 Extraction parameters – recoveries and residual standard deviation (n = 6) of the selected pesticides using two different SPE cartridges in combination with elution solvent methanol/acetone (3:2)

	Bond Elut C18 LRC (C18, 200 mg/10 mL)		Oasis HLB (hydrophilic–lipophilic balance, 200 mg/6 mL)	
Compound	Recovery, %	RSD, %	Recovery, %	RSD, %
Acetochlor	52	5.9	92	6.2
Alachlor	55	6.1	93	7.3
Chlorfenvinphos	78	7.7	89	9.2
Chlorpyrifos	82	13.8	95	6.6
Diuron	61	12.1	112	8.0
Isoproturon	58	10.4	86	12.7
Pendimethalin	42	15.6	89	10.7
Trifluralin	53	9.8	94	8.9

20.2.2 Sample Collection

All water samples were collected in 1 L glass amber bottles and stored in a dark place with temperature at 4 °C. All samples were transported and extracted within 24 h after the sampling. If it is necessary, the pH value of the samples were adjusted in the range from 5.0 to 7.5 with 0.1 M HCl.

20.2.3 Sample Extraction

The optimization of the sample preparation method is an important process to achieve the highest enrichment efficiency and the best recovery. The following parameters that may affect the SPE procedure efficiency were optimized: the sorbent type in combination with different elution solvents and the sample volume. The SPE procedure was performed with 24-ports SPE tubes vacuum manifold equipped with vacuum pump (Alltech, USA). Two different SPE cartridges were compared for extraction of selected pesticides: Bond Elut C18 LRC (C18, 200 mg/10 mL, Agilent Technologies, USA) and Oasis HLB (hydrophilic–lipophilic balance, 200 mg/6 mL; from Waters, Milford, MA, USA) (Table 20.1), using three different elution solvents: methanol, methanol–dichloromethane (1:1) and methanol-acetone (3:2). For this experiment, 1,000 mL of ultrapure water was spiked with the working standard solution in order to achieve a final concentration of 100 ng/mL for each analyte in the final extract. Spiked water samples were loaded at a flow rate of 10 mL/min followed by 15 mL of ultrapure water. The cartridges were then dried under air flow for about 1 h and analytes were eluted with 10 (4 + 4 + 2) mL of selected elution solvent methanol/acetone (3:2) at a flow rate of 2 mL/min. Extracts were evaporated to 1 mL under N_2 stream and water bath up to 30 °C. The final extracts were filtered through 0.20 μm polytetrafluoroethylene (PTFE) filters and analyzed.

Table 20.2 Gradient elution conditions

Step	Time, min	Flow, mL/min	% A	% B
0	0	0.250	60	40
1	20	0.250	20	80
2	25	0.250	5	95
3	35	0.250	5	95
4	35.1	0.250	60	40
5	38	0.250	60	40

After the selection of the cartridge type the sample volume was optimized. The tested volumes were: 250, 500 and 1,000 mL. Ultrapure water was spiked with working standard solution to produce concentration of 100 ng/mL for each pesticide in the final extract. Extracts were prepared in the same way as in the previous experiment. The developed method was validated by performing recovery studies using both ground water and surface water samples spiked with standard solution to produce concentrations of 30 ng/mL in the final extracts. The developed and optimized method was finally applied to the analysis of ground and surface water samples.

20.2.4 LC–MS/MS Analysis

The chromatographic analysis was performed on Agilent LC system 1200 Series (Agilent Technologies, USA) equipped with a binary pump, automatic injector and a thermostatted column compartment connected to a Agilent 6300 Series Ion Trap MS equipped with electrospray ionization source (Agilent Technologies, USA). The chromatographic separation was performed with Zorbax Eclipse® XDB-C18 (150 mm × 2.1 mm i.d. and 3.5 μm particle size) column from Agilent (Agilent Technologies, USA). The separation was achieved with the gradient elution conditions described in Table 20.2. Used mobile phase components are 0.05 % formic acid solution in ultra-pure water (A) and acetonitrile (B) with a constant flow rate of 0.25 mL/min. The column temperature was set at 30 °C and the injection volume was 20 μL. Chromatograms of a standard mixture of the studied compounds and the transitions used for quantification are shown on a Fig. 20.2.

Mass spectra were obtained by electrospray ionization (ESI) technique in the positive ionization mode and Multiple Reaction Monitoring (MRM) with a minimum of two precursor-product-ion transitions for each compound. All MRM transitions for each compound are shown in Table 20.3. The optimal source working parameters for monitoring all ions were as follow: spray voltage (3.5 kV), the nitrogen flow 40 PSI for the nebulizer gas (N_2), dry gas (N_2) flow (10 L/min) and heated capillary temperature (350 °C).

Analytical instrument control and data acquisition were performed with ChemStation and Ion Trap controlling software both provided from Agilent (Agilent Technologies, USA).

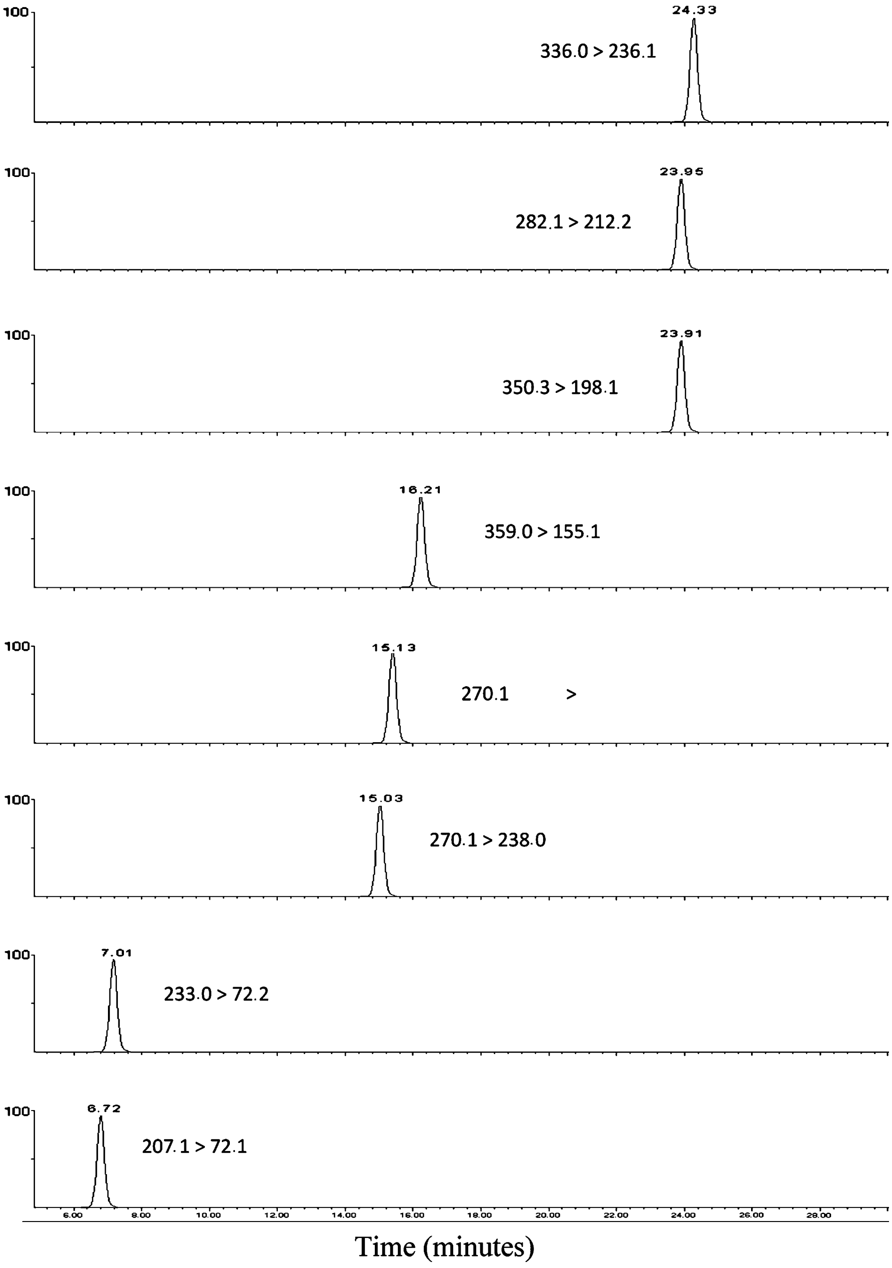


Fig. 20.2 Chromatograms of a standard mixture of studied compounds and the transitions used for quantification

Table 20.3 MRM transitions of the compounds (in bold are the transitions which have been used for quantification analysis)

Compound	RT, min	MRM transition
Acetochlor	15.1	**270.0 → 224.0 (1.0)**
		270.0 → 148.1 (1.0)
Alachlor	15.0	**270.0 → 238.0 (1.0)**
		270.0 → 162.2 (1.0)
Chlorfenvinphos	16.2	**359.0 → 155.0 (1.0)**
		359.0 → 127.0 (1.0)
Chlorpyrifos	23.9	**350.0 → 198.0 (1.0)**
		350.0 → 153.0 (1.0)
Diuron	7.0	**233.0 → 72.0 (0.6)**
		233.0 → 165.0 (0.6)
Isoproturon	6.7	**207.0 → 72.0 (0.9)**
		207.0 → 165.0 (0.9)
Pendimethalin	23.9	**282.0 → 212.0 (1.0)**
		282.0 → 194.0 (1.0)
Trifluralin	24.3	**336.0 → 236.0 (1.0)**
		336.0 → 185.0 (1.0)
d6-Isoproturon internal standard	6.5	**213.0 → 78.0 (0.8)**

20.2.5 Method Validation

Extraction recoveries of target compounds were determined using both ground water and surface water samples spiked with 30 ng/L. Prior to analysis, samples used as blanks were proven to be free from the pesticides considered.

The precision of the method was determined by repeated intraday and interday analyses (analyses of six replicated spiked samples in 1 day, and in 3 successive days, respectively), and expressed as the relative standard deviation (RSD).

Limits of detection (LODs) and quantification (LOQs) were determined from ground water and surface water samples spiked at the concentration levels of each compound giving a signal-to-noise ratio (S/N) of 3 and 10, respectively.

For estimation of the linear dynamic range, five point calibration curve was constructed in a calibration range between 10 and 100 ng/mL. Each calibration standard was analyzed using the described method. For each compound was plotted the correlation between the measured values of the signal for the bold MRM transition (Table 20.3) versus the concentration in the solution by using a least-square regression analysis. Correlation coefficients greater than 0.99 where observed (Table 20.4).

20.3 Application to Real Samples

The developed multiresidue analytical methodology was applied in a study carried out in Bulgaria in order to accomplish the Water Framework Directive [3] concerning the survey of the Directive target contaminants in surface water. Twenty

Table 20.4 Analytical parameters of the LC–MS/MS method

Compound	Correlation coefficient, r^2	LOD, ng/L	LOQ, ng/L	RSD, %	R, %
Acetochlor	0.9968	1	5	5.2	92
Alachlor	0.9995	5	10	3.5	93
Chlorfenvinphos	0.9989	5	10	5.3	96
Chlorpyrifos	0.9992	5	10	4.7	103
Diuron	0.9991	6	40	3.1	112
Isoproturon	0.9987	6	50	5.8	89
Pendimethalin	0.9984	10	50	6.6	86
Trifluralin	0.9997	5	10	4.1	94

Table 20.5 Concentration levels of analyzed pesticides in the water samples

Compound	Concentration level, ng/L
Acetochlor	<5 ÷ 70
Alachlor	<10 ÷ 50
Chlorfenvinphos	<10 ÷ 50
Chlorpyrifos	<10
Diuron	<40 ÷ 90
Isoproturon	<50
Pendimethalin	<50 ÷ 900
Trifluralin	<10 ÷ 80

samples were analyzed for the eight pesticides listed above. The samples of surface water were collected from several main rivers in Bulgaria. The obtained results are summarized in the next table (Table 20.5).

20.4 Conclusion

With the growing attention to contaminants in the environment, there is a need for fast and sensitive analytical method that can screen for a wide variety of compounds simultaneously. The analytical methodology described above provides a sensitive method for the determination of some of the priority organic substances, which are pesticides, included in the [3] – Acetochlor, Alachlor, Chlorfenvinphos, Chlorpyrifos, Diuron, Isoproturon, Pendimethalin and Trifluralin. The SPE followed by LC-MS/MS gives the sensitivity and selectivity necessary to the detection of these compounds at environmental relevant concentration in the nanogram per liter range. The development of a single extraction procedure for all samples and all analytes, sensitive and robust irrespective of concentration levels and water physico-chemical properties is a real analytical challenge and a real advance in environmental analysis. This analytical method will allow the monitoring launched by the EU on water quality, and will make possible to analyze temporal and/or spatial distributions of described pesticides in the aquatic environment.

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Chapter 21

Structural Changes of Biological Compartments in Danube Delta Systems Due to Persistent Organic Pollutants and Toxic Metals

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Abstract The main objective of this paper is to highlight the dynamics of biotic community in terms of spatial and temporal relationship. The biotic community dynamics in St. Gheorghe Branch were discussed based on existing data and the results of our own research during 2003–2009. Surface water and sediment samples have been collected monthly from two control sections, Murighiol and Uzlina. The analyzed parameters were according to the Norm concerning the reference objectives for the surface water quality classification (Romanian Order MEWM no. 161/2006) and also, to the EU Water Framework Directive 2000/60/EC which established a framework for community action in the field of water policy. The variation of parameters during the investigated period along St. Gheorghe Branch was strongly influenced by the climatic conditions (drought, flood). Further studies need to be carried out in this area in order to accomplish a sustainable management and to evaluate the occurrence of persistent organic pollutants and toxic metals in tissues, and to assess the risk of these contaminants on the ecosystem and human health.

Keywords Danube Delta • Aquatic ecosystems • Phytoplankton • Zooplankton • Macro invertebrates • Metals • Organic compounds

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21.1 Introduction

Rivers have always played an important role for life existence. The aquatic ecosystems are dynamics systems that keep their stability in conditions of permanent fluctuations of biotic and non-biotic parameters. The dynamic equilibrium of aquatic ecosystems is accomplished because of the connections between the species and environmental conditions and also because of the mutual connections between the existing populations [32].

The intervention of human activities on rivers has determined the instability of this equilibrium having as a last resort its damage. It is the case of Danube which has suffered alteration processes of the ecological balance for human development. The "interventions" has determined the instability of the equilibrium by: over-exploitation of the riparian resources (biotic and abiotic), planning the river course (embankment, course changing), establishment of the human settlements [25].

The implementation of the EU Water Framework Directive 2000/60/EC (WFD) [42] in Europe led to an extensive use of bio-monitoring programs in order to access the water quality of streams and rivers, and the correct measures to apply in case of a quality status below "Good". The aim of the WFD is focused on biotic indicators. Biotic indicators should reflect the impact of all stressors. However, transport and contamination of sediments, as well as the spread of invasive species, have not received enough attention.

From this point of view, the study of biotic – *phytoplankton, zooplankton and benthic invertebrates* components – from the position of the systemic method and conception was done in order to characterize the dynamics and role within the integrated aquatic ecosystems.

Phytoplankton is unicellular, microscopic, unattached plants found in freshwater and marine ecosystems. In comparison to many other biological organisms, phytoplankton is relatively homogeneously mixed throughout the water column. They optimize their residence in the photic zone by a number of mechanisms: controlling buoyancy using gas vacuoles, migration using flagella, increasing surface area/volume ratio to form resistance, and metabolic processes [11]. Freshwater phytoplankton populations are seasonally variable [13] and are regulated by both chemical and physical factors. Nutrient concentrations, nutrient ratios (stoichiometry), and light are essential growth indicators.

Zooplankton are floating and drifting animals with limited power of locomotion. Majority of them are microscopic, unicellular or multicellular forms with size ranging from a few microns to a millimeter or more. In addition to size variations, there are differences in morphological features and taxonomic position. By sheer abundance of both types and their presence at varying depths, the zooplankton is used to assess energy transfer at secondary trophic level, feeding on phytoplankton and enabling the conversion of plant material into animal tissue. The zooplankton constitutes the basic food for higher animals including fishes, particularly their larvae [6].

Benthic invertebrates are commonly applied for the integrated quality assessment of rivers based on the requirements of the WFD. They may offer numerous advantages in biomonitoring, which explains their reputation as the most commonly used group in assessing water quality. Among the reasons, according to Rosenberg and Resh, are included: it is generally well known group, which enables reliable identification and predicting of response to stress; organisms belonging to this group are generally characterized by low mobility. Thus, use of macro invertebrates in monitoring enable effective spatial analyses of the effect of stressors; there is an array of widely distributed species among the group, which allow extensive use of same indicators; it is diverse component of the aquatic environment, which offers a spectrum of responses to the stress; sampling can be done easily, using simple inexpensive equipment [29].

The Danube has a great importance in Romania: covering 237,391 km^2 and with nearly 22 million inhabitants, the country is almost entirely within the Danube Basin being the end carrier of all wastewater discharges from all countries into the Black Sea. The intensive economic development in the communist period between 1960 and 1989 resulted in a significant worsening of the water quality of the Danube and inland rivers. Navigation poses a significant pressure on the Danube: of 455 incidents involving shipping reported from 1983 till 2003, 30 resulted in serious water pollution. Considerable areas of the Danube Delta have been embanked and drained, and the total length of the channel network in the delta doubled between 1920 and 1980 (at present 3,500 km). Despite an overall improvement in water quality over the past few decades, the Danube and its tributaries remain exposed to multiple points and non-points pollution sources [25]. Also, intensive agriculture, fish farming, and most recently recreation and tourist industry had led to a decrease of the river's self-cleaning capacity and to an increase of polluted effluents resulting in a continuous deterioration of river quality [36, 39–41]. Hazardous substances like heavy metals, persistent organic pollutants (pentachlorophenols, PCPs; polycyclic aromatic hydrocarbons, PAHs and organochlorine pesticides), hormone active substances and micro-pollutants are becoming an increasing issue in the Danube and Danube Delta. Regarding organic pollutants, some of them and readily bind to particle fraction in surface waters. Via sedimentation processes, these chemicals are deposited to the bottom remaining for a long time in sediment due to their long half-life times [27]. These organic pollutants retained in sediment can be taken up and retained in benthic chains to higher trophic levels [30], and then can obviously result in the decrease of biodiversity, in the simplification of macro invertebrate structures, in the distortion of functional feeding group composition. Examination of persistent organic pollutant levels in sediment may give basic information on the contamination status, sources and ecological risks of these chemicals in the aquatic environment [22]. Some recent data about contamination with organochlorine pesticides, PCBs and polybrominated biphenyl ethers of sediment and biota (intervertebrates, 11 fish species and cormorant tissues) reported on the presence of DDTs in all samples collected from Danube Delta in 2001 [4].

The study aims to understand how pollutants are transmitted from the water to the biotic components due to interdependent relationships in order to take measure and reduce the negative effects of pollution.

21.2 Materials and Methods

21.2.1 Study Area

During 2003–2009 period there were collected samples from two control sections situated in Danube Delta on St. Gheorghe Branch, Uzlina and Murighiol. The main objective was to highlight the dynamics of biotic community in terms of spatial and temporal relationship based on the identification and quantification of the most important inorganic and organic pollutants, the evaluation of their compliance with standards, and finally the long-term trends of the ecosystem's status.

The St. Gheorghe Branch is the most southern branch carrying 23 % of the Danube water discharge and 21 % of the Danube sediment discharge. This highly sinuous branch was cut off between 1984 and 1988 by an artificial canal opened to shipping which caused significant changes of the river flow velocity in the natural channel.

In *Uzlina (45°4′N-29°14′E)* situated on the natural channel, samples were collected from the Cormoran Complex, a place with intensive naval activity. Cormoran Complex has its own domestic wastewater treatment station, and the treated water is dumped into the river. However, many houses in this area are discharging water directly into the river.

In *Murghiol (45°2′N-29°10′E)*, samples were collected from the artificial canal near Murghiol Lake. These two locations situated on two different channels, in an area of natural fish reproduction and a pelican colony, are tourist villages in continuous expansion with ports and fuel stations for boats (see the map of the Danube Delta from Fig. 21.1).

21.2.2 Sample Collection

All biological methods of water quality assessment are based on the fact that all existing species, populations and communities of plants and aquatic animals do not react the same way to a particular type of pollution. In practice, biological evaluation involves different activities such as: sampling, processing of samples (sorting, identification and counting), processing data and interpretation.

The samples were collected monthly from April to October (2003–2009). In April 2005 and 2006 was not possible to collect samples, because of the strong floods period. The sampling and preservation step was done according to the recommendations of specific international guides [7, 14–17].

The samples for phytoplankton were collected away from shore to eliminate contamination by periphytic species of algae. For qualitative samples was used a phytoplankton net which gave us information about the presence or absence of phytoplankton species. For quantitative samples, was collected a constant volume of water (1 L) in a plastic bottle.

Fig. 21.1 Danube Delta map – Uzlina and Murighiol control sections – St. Gheorghe Branch (https://maps.google.ro/maps?hl=en)

The zooplankton collection involved primarily the filtration of 50 L water by net and then collecting the water in a 0.5 L bottle. This method was suitable both for qualitative and quantitative analysis.

The benthic invertebrates samples were collected directly from the bottom sediment using a Van Veen Sampler (Duncan & Associates, United Kingdom).

Phytoplankton, zooplankton and benthic invertebrates‘ samples were preserved in 4 % formaldehyde solution.

For physical and chemical determinations, 10 L of water samples were collected from each sampling location and stored in glass bottles. The sediment samples were taken from 2 to 3 m depth within the Danube using a Van Veen Bottom Sampler. All samples were kept in cooling boxes at 4 °C during transportation and the analyses were performed immediately after receiving the samples in the laboratory.

21.2.3 Reference Comparison Values

The Romanian Order MEWM no. 161/2006 [26], transposed from Directive 2000/60/EC introduced a classification of the quality factors that describe the ecological condition of surface water. This classification consists in five conditions for surface water: *very good* (I), *good* (II), *moderate* (III), *poor* (IV) and *bad* (V). The parameters imposed for chemical and physic-chemical characterization of water quality are: temperature, oxygen, nutrients, pollution with dangerous substances, mineralization and pH. The Norm provides two categories of chemical conditions for all aquatic, sediment and biota systems: good chemical conditions (all quality parameters for the indicators are situated in the limits imposed by the quality standards) and bad chemical conditions (one or more parameters exceed the limits imposed by the quality standards).

21.2.4 Methods and Equipment

The analysis of the biotic communities in both sampling sites were focused on quantitative (numerical density, biomass, abundance after numerical density and biomass) and qualitative components (dominant species, indicator species). Identifications and counts for quantitative and qualitative estimates were performed on preserved subsamples.

For *phytoplankton* determination the most common counting method used to quantify phytoplankton samples was the Utermöhl [34] technique as modified by Nauwerck [24]. This method involved settling a known volume of sample (1 mL) into a counting Kolwitz chamber using an optical microscope equipped with phase/contrast illumination, 10X, 40X, and 100X objectives. Cells were identified and counted using the 10X objective on transects that covered 100 % of the chamber surface. Cell fragments were not counted. Viable cells that are partially in a counting field on the right hand side were counted, but those on the left were omitted. For colonies, a small portion of the colony was counted, and the number of cells is then estimated. Filaments are counted individually. Cell counts were converted to wet-weight biomass by approximating cell volume. Estimates of cell volume for each species were obtained by measurements of the cells of an individual species and application of the geometric formula best fitted to the shape of the cell [31, 38]. The qualitative species were determinate using Süβwasserflora von Mitteleuropa key [8, 9, 18, 19, 23].

Information on the fauna composition and the relative abundance of different *zooplankton* taxa and their species were obtained by counting the plankters present in the samples. The counting was done under the Motic optical microscope and when the specimen of a particular group was seen, a tally mark was made on the sheet. The total numbers of specimens were later calculated for the whole sample depending on the percentage of subsamples examined. For sorting and identification of species was used freshwater illustrated key.

In order to quantify *benthic invertebrates*, the preserved samples were washed to remove preservative under running water through a sieve of 0.50 μm, then sorted by groups of indicators, counted and analysed using Motic stereomicroscope with digital camera (Motic, Germany).

Polyaromatic hydrocarbons, PAH's (Fluoranthene, Benzo(b)fluoranthene, Benzo (k)fluoranthene, Benzo(a)pyrene, Benz(a)anthracene, Benzo(g,h,i)perylene, Indeno (1,2,3-cd)pyrene, Naphthalene, Phenanthrene, Anthracene, Chrysene, Pyrene) were determined with Agilent 1100, HPLC equipment with fluorescence detection. The quantitative analysis of total Mineral oil content was performed with an FT-IR System type Perkin Elmer Spectrum BX II. The quantitative determination of pesticides was performed on a GC System (Agilent Technologies 6890 N) with ECD detector for organochlorine pesticides (α-HCH, β-HCH, γ-HCH, δ-HCH), with NPD detector for organophosphorus pesticides (Malathion, Parathion, Dichlorvos) and Triazine pesticides (Atrazine, Simazine, Propazine). For the quantification Phenol Index specific equipment for pretreatment of water samples and a Perkin Elmer Lambda 25 UV/VIS Spectrometer were used. Polychlorobiphenyls (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, and PCB 180) were analyzed on a GC System Agilent Technologies type 6890 N, with ECD detector.

21.2.5 Quality Assurance (QA) and Quality Control (QC) Data

The samples were analyzed in the Department of Pollution Control, which is accredited by RENAR (Romanian Accreditation Association) and follows the requirements of ISO/IEC 17025/2005 standard. Also, NRDI ECOIND operates in integrated Quality Environment Management System, according to ISO 9001:2008 and ISO 14001:2005. For all the analyzed parameters, the laboratories use standard methods (ISO, SR ISO, EN, SR EN, SR, STAS, EPA), reference materials and certified reference materials. All these standard methods were verified and the main performance parameters (limit of detection, limit of quantification, linearity, accuracy, precision, selectivity, the uncertainty of measurement) were established with the existing equipments in the laboratories.

21.3 Results and Discussion

21.3.1 Surface Water

To understand the dynamics of the phytoplankton-zooplankton systems, one could use the analogy of self-sustaining plants and the animals that graze on them [2, 35]. Because phytoplankton is so integral to the overall health of its environment, it is important to study the changes in their population caused by different ecological conditions such as variation resources and the introduction of other plankton species. The research of the biotic communities in both control sections – Uzlina and Murighiol – was achieved in the systemic conception point of view and based on the assumption that these associations are considered subsystems of aquatic

Table 21.1 Dynamics of dominant phytoplankton species during 2003–2009 in Danube Delta control sections

	Dominant species[a]	
	2003	2009
Uzlina		
Phytoplankton	(B) *Diatoma elongatum* (o-β) (B) *Asterionella formosa* (o-β) (CL) *Pediastrum duplex* (β) (CL) *Ulothrix tennerima* (β) (CL) *Pediastrum boryanum* (β)	(B) *Diatoma elongatum* (o-β) (B) *Navicula gracilis* (o-β) (B) *Synedra acus* (β) (CL) *Pediastrum boryanum* (β)
Murighiol		
Phytoplankton	(B) *Diatoma elongatum* (o-β) (B) *Navicula gracilis* (o-β) (E) *Euglena acus* (β-α) (B) *Gyrosigma acuminatum* (β)	(B) *Diatoma elongatum* (o-β) (B) *Navicula gracilis* (o-β)

[a] *B* Bacillariophyceae, *CL* Chlorophyta, *E* Euglenophyceae

ecosystem biocenosis. The analysis of the phytoplanktonic samples from surface water focused on the quantitative and qualitative components. The Bacillariophyta, Euglenophyta, Chlorophyta associations were constant and dominant components in aquatic ecosystems structure for numerical density and biomass – oligo-betamesosaprobic diatoms, betamesosaprobic chlorophyte (Table 21.1).

Early spring phytoplankton development in Uzlina control section in 2003 started with the dominance of Bacillariophyta 96 % of total functional groups. The well-developed populations in April 2003 were representatives *Diatoma elongatum (o-β)* and *Asterionella formosa (o-β).* Due to strong floods recorded in April 2005 and 2006 the samples have not been collected. Diatoms species retained dominance in phytoplankton under conditions of extreme flooding in 2005 when water depth reached its maximal value. The maximum value of remanent biomass was recorded in June 2004 (0.58 mg/dmc).

The obtained results indicated that flood water had altered the physical and chemical environment of Danube Delta systems, and also the phytoplankton structure and composition. The increased algal biomass leads to an important uptake of dissolved oxygen causing degradation of organic matters with impact on benthic invertebrates. The highest numerical density value of phytoplankton was recorded in Uzlina control section in April 2003 (378.000 ex./dmc) (Fig. 21.2), decreasing it in the other years sampling campaigns, respectively May–October 2004–2008 (Figs. 21.3 and 21.4).

In Murighiol control section, for numerical density and remanent biomass the phytoplankton is very well represented than in Uzlina location. Concerning numerical abundance, dominated the oligo-betamesosaprobic or betamesosaprobic diatoms in all sampling months. In terms of abundance expressed as a percentage of biomass in all sampling campaigns were dominated by oligo-betamesosaprobic diatom species – *Navicula gracilis* or betamesosaprobic chlorophyte – *Pediastrum boryanum* (Fig. 21.5).

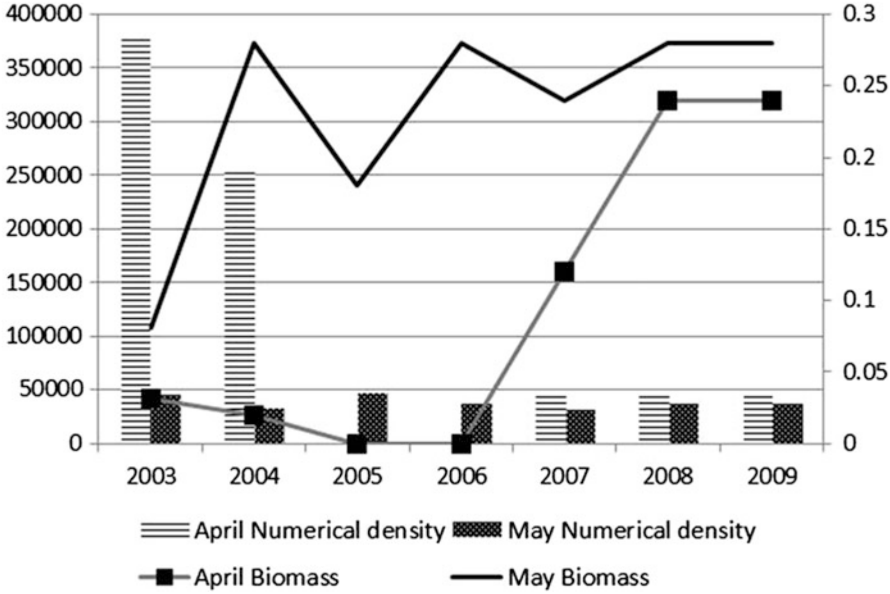

Fig. 21.2 Variation of numerical density and biomass of phytoplankton in Uzlina (spring 2003–2009)

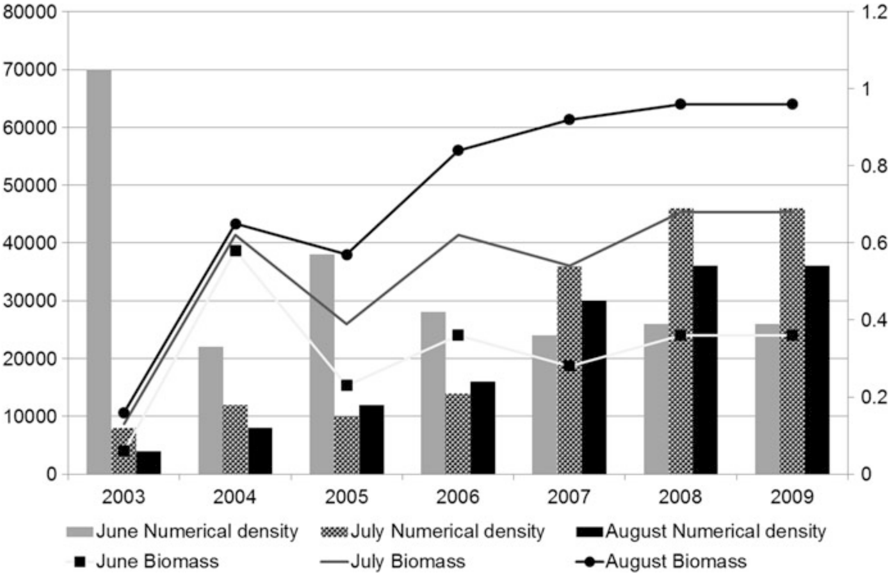

Fig. 21.3 Variation of numerical density and biomass of phytoplankton in Uzlina (summer 2003–2009)

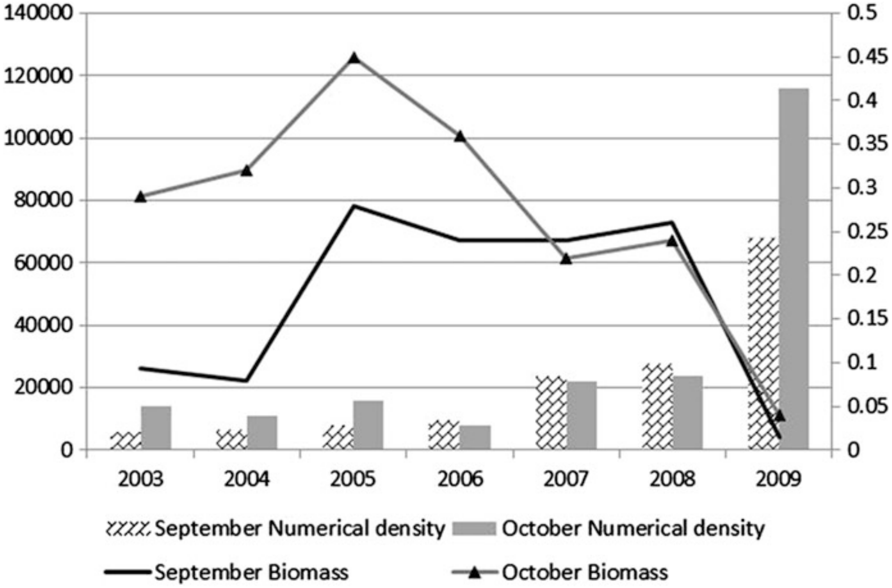

Fig. 21.4 Variation of numerical density and biomass of phytoplankton in Uzlina (autumn 2003–2009)

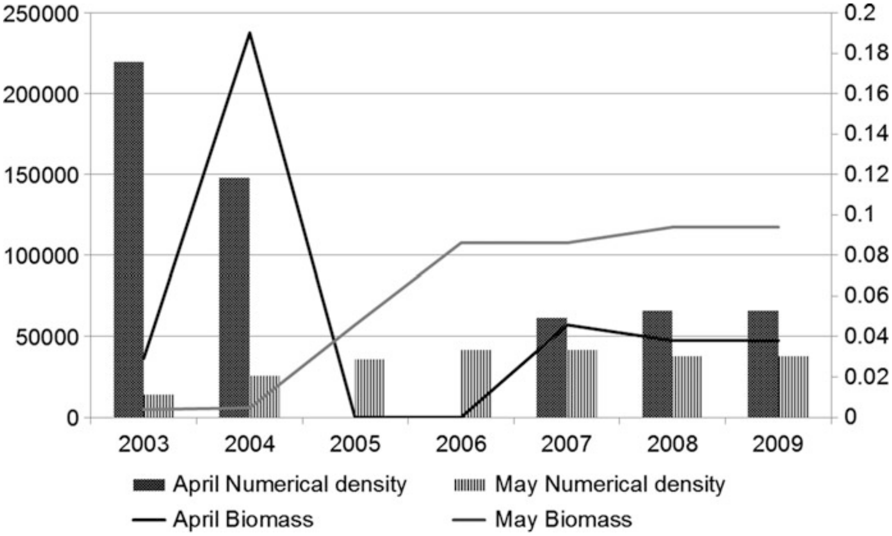

Fig. 21.5 Variation of numerical density and biomass of phytoplankton in Murighiol (spring 2003–2009)

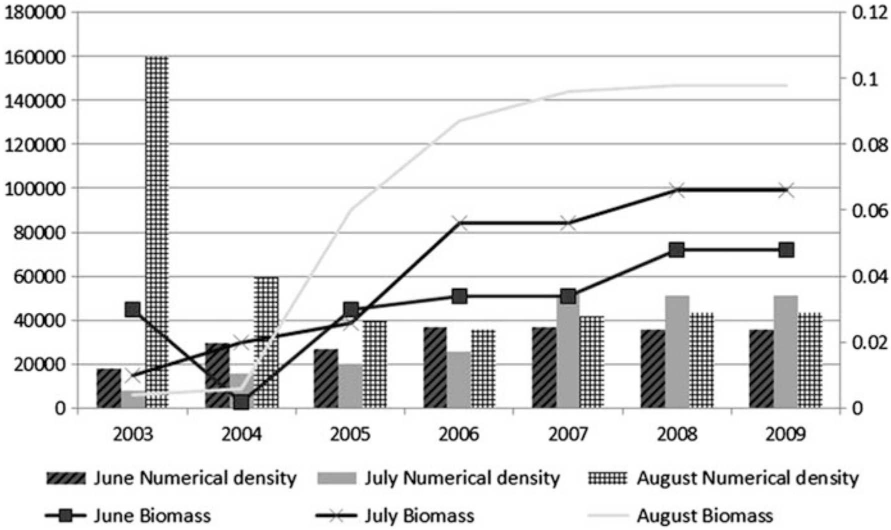

Fig. 21.6 Variation of numerical density and biomass of phytoplankton in Murighiol (summer 2003–2009)

The phytoplankton biomass had an increasing trend observed in the summer of 2007 and 2008 in Uzlina and Murighiol due to immobilization of nutrients (Figs. 21.4 and 21.6), but the highest value of remanent biomass was recorded in April 2004 (0.19 mg/dmc) (Fig. 21.5). Also, numerical density had an increasing trend in September 2009 in Murighiol (Fig. 21.7) and in October 2009 in Uzlina.

The analysis of the zooplanktonic community in Uzlina and Murighiol also focused on the quantitative (numerical density, biomass, abundance after numerical density and biomass) and qualitative component (taxonomic composition, dominant species).

Numerical density, biomass and remanence values recorded for the entire period almost the same order of magnitude. From point of view, numerical abundance and biomass throughout the sampling period predominates in both control sections betamesosaprobic rotifers species: *Keratella quadrata frenzeli* better represented in Uzlina and Murighiol betamesosaprobic copepods, betamesosaprobic rotifers and oligo-betamesosaprobic cladocers species: *Bosmina longirostris* (Table 21.2). The highest numerical density value of zooplankton was recorded in Murighiol in June 2003 (3,320 ex./dmc), also in September (2,840 ex./dmc), in Uzlina in April 2004 (880 ex./dmc) and was observed the absence of the zooplankton species in the two control section in April, May 2005–2006 and September 2007–2008. The maximum value of remanent biomass was recorded in October 2009 (0.14 mg/dmc) in Uzlina and in April 2003 in Murighiol location. The spatial distribution of zooplankton in Uzlina and Murighiol control sections was not equal; Uzlina was characterized with lower densities and lower number of taxa then in Murighiol during all investigated period.

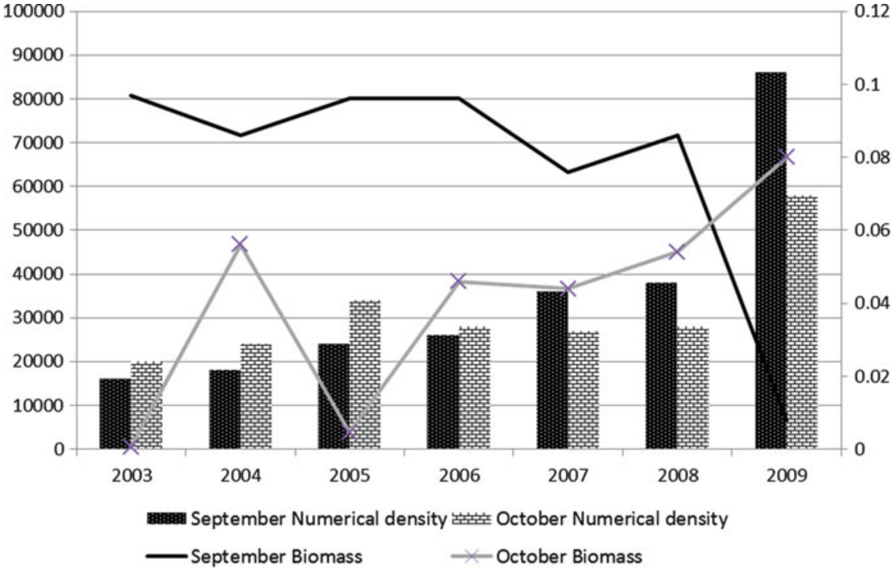

Fig. 21.7 Variation of numerical density and biomass of phytoplankton in Murighiol (autumn 2003–2009)

Table 21.2 Dynamics of dominant zooplankton species during 2003–2009 in Danube Delta control sections

	Dominant species[b]	
	2003	2009
Uzlina		
ZPK[a]	(Rot) *Keratella quadrata frenzeli* (β)	(Cop) *Cyclops strennus metanauplis* (β-α)
	(Cop) *Eucyclops serrulatus* (o-β)	(Rot) *Keratella quadrata frenzeli* (β)
	(Rot) *Trichocera stylata* (o-β)	(Rot) *Flatyias polyacanthus* (β)
	(Cop) *Acanthocyclops bicuspidatus*(o-β)	
	(Cld) *Bosmina longirostris* (o-β)	
Murighiol		
ZPK[a]	(Rot) *Keratella quadrata frenzeli* (β)	(Cop) *Cyclops strennus metanauplis* (β-α)
	(Cld) *Bosmina longirostris* (o-β)	(Rot) *Keratella quadrata frenzeli* (β)
	(Cop) *Cyclops strennus metanauplis* (β-α)	
	(Rhiz) *Difflugia corona* (β)	
	(Rot) *Brachionus angularis angularis* (β)	

[a] ZPK – Zooplancton

[b] *Rot* Rotifers, *Cop* Copepoda, *Cld* Cladocera, *Rhiz* Rhizopoda

21.3.1.1 Organic Pollutants

Generally, the concentrations of organic pollutants found in the tested surface water were not high. In 2005, in almost all months and both locations were founded phenols, the highest value was recorded in Uzlina in September (0.105 mg/L).

Relatively higher levels of mineral oil occurred in the spring of 2007 in Murighiol (0.23–0.27 mg/L) as well as in Uzlina (0.26–0.32 mg/L), decreasing drastically in the next period to below the limit of detection (LOD) of the analysis method.

Considering the fact that the mineral oil and phenol index occurred in both locations at the same time and in similar concentrations, it can be concluded that the contamination took place through accidental discharges elsewhere before river bifurcation. Organochlorine pesticides, such as heptachlor and Lindane (γ-HCH) were found in water only in spring months. Thus, heptachlor was detected in spring 2007 and 2008 at both location in concentration of 0.01 μg/L (50 times higher than the limit), and Lindane occurred in spring 2008 in a mean concentrations of 0.034 μg/L. The occurrence of organochlorines in surface water only during spring could be caused by their illegal use as pesticides in agriculture and vector control. Other persistent organic pollutants detected during spring 2008 in the surface water of the two channels were PCBs (concentrations 0.025–0.48 μg/L). Of the 12 PAHs investigated, benzo[a]anthracene, phenanthrene, fluoranthene, benzo[b]fluoranthen, benzo[k]fluoranthene, benzo[a]pyrene were founded in different periods and years of the monitoring programme. In spring period of the year 2004 were founded the highest density of PAH compounds in both locations. In 2006 were detected also PAHs in surface water (benz(a)anthracene and fenanthrene); their concentrations were higher than the admissible values in three different months. Benzo[b] fluoranthene was occurred in the artificial canal during April and May 2008 in high concentrations (0.028–0.031 μg/L) exceeding the limit sets for a good water quality status [39–41].

21.3.2 Sediments

The macro invertebrates analysis in the two sampling sites have aimed the quantitative component and qualitative determination.

In fact, were dominant gasteropodes and lamellibranchiates species, and the oligocheta association together chironomidae organisms that are links important in aquatic ecosystems [33]. The maximum values of numerical density were recorded in June 2005 and May 2009 in Uzlina (4,340 ex./sqm), in June 2009 in Murighiol location (7,300 ex./sqm). Spatial and temporal distribution of the numerical density and biomass for macro invertebrates in each of the sampling locations for all period study is represented in Figs. 21.8, 21.9, 21.10, and 21.11.

For the entire study period, benthic macro invertebrates community was better represented by gasteropods in Uzlina control section throughout the spatial and temporal variation of numerical density and remanent biomass than in Murighiol. These decomposition processes have replaced sensitive species with tolerant, often foreign species (*Corbicula fulminea*). The highest value of remanent biomass were observed in May 2008 in both sampling sites, in Uzlina was 1,086 g/sqm and in Murighiol 1,692 g/sqm.

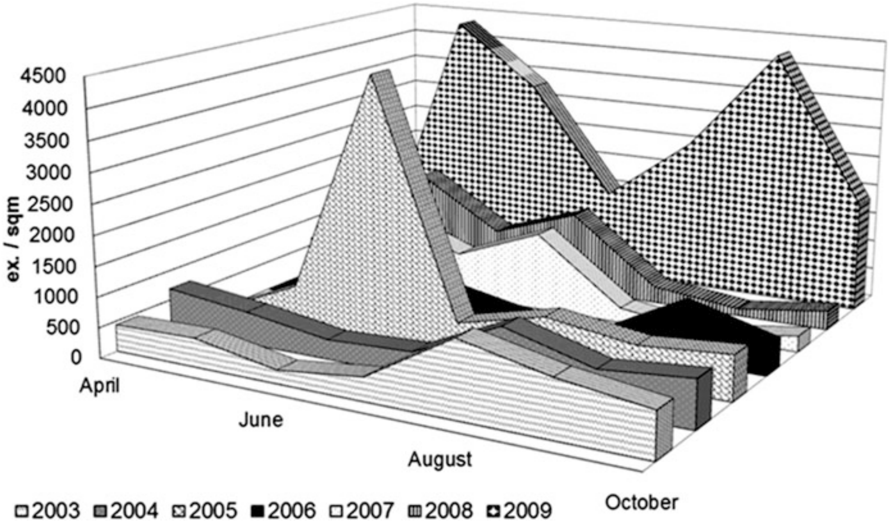

Fig. 21.8 Numerical density of benthic macroinvertebrates in Uzlina location during 2003–2009

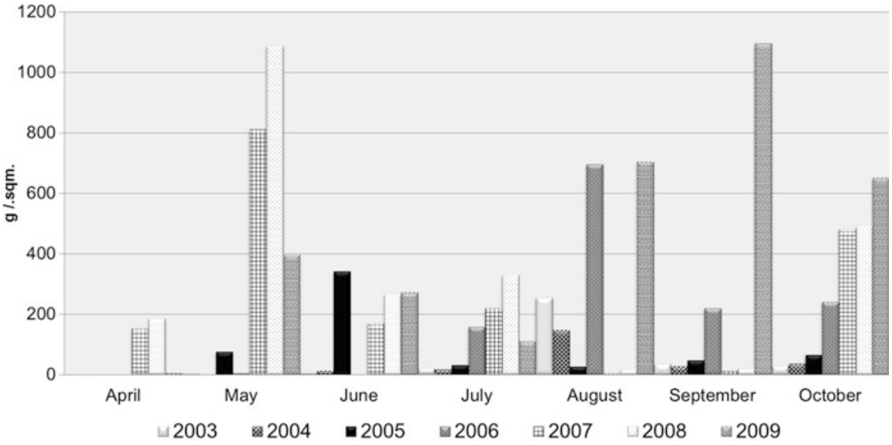

Fig. 21.9 Benthic macro invertebrates biomass in Uzlina location during 2003–2009

Comparative analysis of macroinvertebrates characteristics in the Uzlina and Murighiol for 2003–2007 were dominant gasteropodes and lamellibranchiates species and in 2004–2006 the associations of oligochaeta with chironomidae organisms are important links in the aquatic ecosystems. In Murighiol dominated oligochets associations as an important bioindicators in the integrated system monitoring. Increased phytoplankton production resulted the decreasing oxygen concentration at the sediment. For benthic invertebrates, this meant more food but reduced oxygen availability.

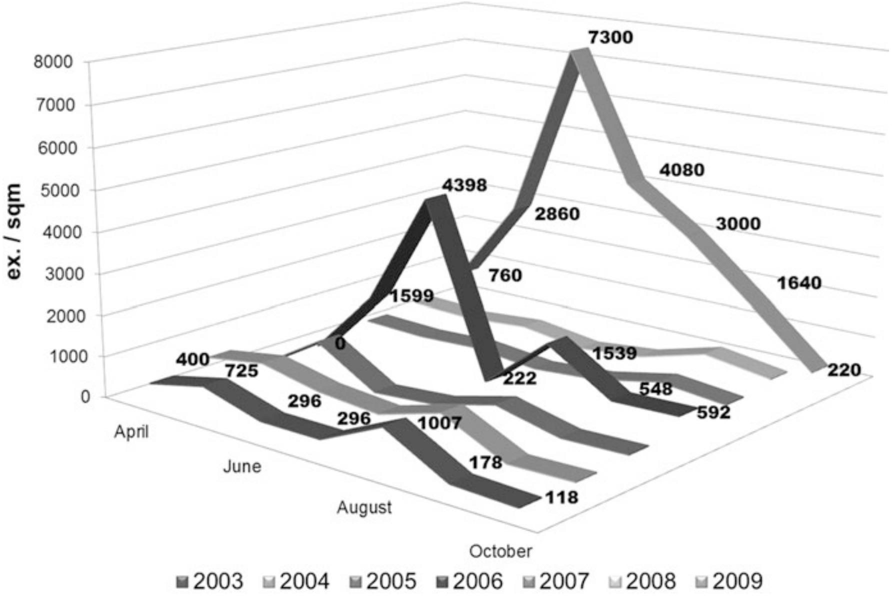

Fig. 21.10 Numerical density of benthic macroinvertebrates in Murighiol location during 2003–2009

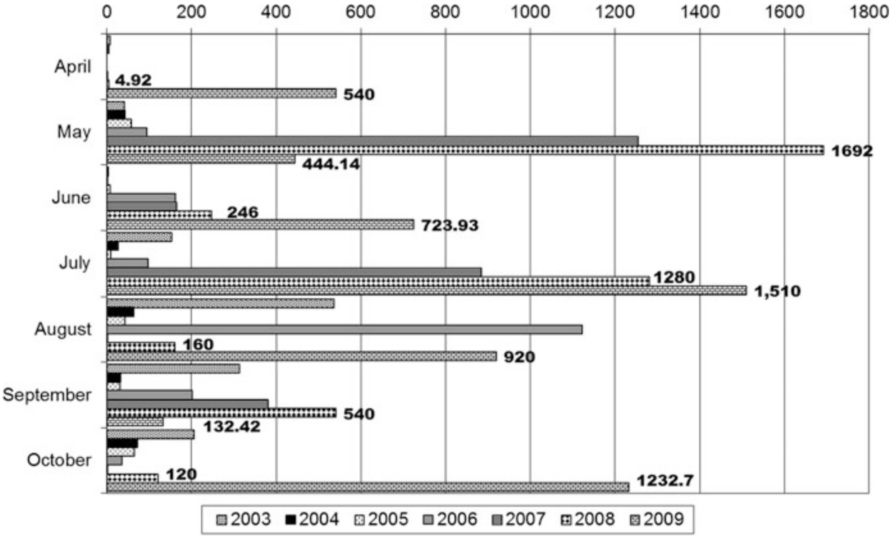

Fig. 21.11 Benthic macro invertebrates biomass in Murighiol control section during 2003–2009

As a result, characteristic species indicator of eutrophic conditions gradually colonise (in this case, Oligochaeta and Diptera). Oligochaetes were more affected by enrichment than chironomids as they are less mobile and they depend more on the inner sediment for their food and reproduction [20]. In terms of biomass, Oligochaeta, Diptera and crustaceans species identified Danube Delta systems although most abundant groups, they have a minor role in total biomass. Due to their size, Gasteropoda and Bivalvia species made up 90 % of the whole biomass. The monitoring of the specific component of the benthonic biocenosis played an important role in establishing the water quality for the determination of the pollution risk, because the benthic invertebrates species respond in the most realistic way to the environmental changes. The zoobenthos density and diversity are dependent on the bottom sediment quality, the condition of running, the presence and/or absence of pollution sources [3].

21.3.2.1 Total Content of Metals

From all metals studied, Cd, Cu, Hg, Ni and Zn represent a particular concern because of their high total concentration levels found in the sediments. Small quantity of mobile Cu and Ni, less than 1 %, was found in both locations, these elements have been bound in the fractions with high stability. The large amounts of Fe, Mn, Zn and Pb found in mobile fractions of the sediment samples may have significant adverse impact on chemical and ecological status of the river water, especially during the low-flow seasons and also, if occur some changes in the system stability such as: discharge of reductants or oxidants in the surface water, mechanical mixing of the sediment and water in natural flood or anthropogenic disaster. Overall, the relatively high proportion of traces associated with the extractable fractions is indicative of significant heavy metal input of anthropogenic origin and of high potential mobility.

21.3.2.2 Organic Pollutants

In Uzlina Location, mineral oil was found in sediment samples in concentration levels ranging between 28.0 and 375.4 mg/kg, higher peaks being observed mainly in spring months of years 2007, 2008 and in the period May-August of 2009. Concentrations of mineral oil adsorbed in sediments were gradually reduced to below the LOD of the method of analysis, probably because of microbial degradation and/or being transported by the water streams into the Black Sea. One of the most contaminated sediment sites with petroleum hydrocarbons in the Black Sea is considered to be situated near the Danube Delta (220 mg/kg) and is associated with river inputs [28]. Our earlier studies [39–41] have shown that mineral oil occurrence in all the river compartments has an anthropogenic origin, probably by an accidental spillage of this pollutant into the river. A major ecological problem was the detection in 2006 of high PAH levels (4.5–13.5 mg/kg dry matter in Murighiol, and 0.35–7.7 mg/kg dry matter in Uzlina) in sediments. PAHs are persistent substances and, therefore, pollution may be older than from investigation period of 2006. In Uzlina Location PAHs concentrations are generally increasing in 2008 (1.12–2.78 mg/kg) as compared to previous year values (1.01–1.27 mg/kg), but their concentrations were not so high than in Murighiol location. Here, their concentrations ranged from 1.24 to 13.5 mg/kg (1 mg/kg represents the admissible value). We mention also that benzo(b)fluoranthene found in water body was detected in sediments in 2007 in April (6.44 mg/kg) and May (5.38 mg/kg) as well as in 2008 in April (0.09 mg/kg), May (0.43 mg/kg) and June (0.21 mg/kg). In 2008, the PAH concentrations in Murighiol (1.24–10.26 mg/kg) exceeded the value of qualitative standard set as 1 mg/kg in both years. Their concentrations in Uzlina location are generally increasing in 2008 (1.12–2.78 mg/kg) compared to the previous year values (1.01–1.27 mg/kg), but their concentrations were not so high than in Murighiol location. Anthropogenic PAHs enter the riverine environment through a variety of routes including domestic and industrial outfalls, atmospheric deposition or direct spillage of petroleum products. An approach commonly used in the literature to distinguish between petrogenic and pyrolytic PAH origin is based on PAHs isomeric ratios, such as anthracene/anthracene + fenanthrene (Ant/178), fluoranthene/fluoranthene + pyrene (Flu/202) [5, 37, 43, 44]. PAHs in sediment with Ant/178 <0.1 are from petroleum contamination while Ant/178 >0.1 are of combustion source. Anthracene and fenanthrene were detected at both locations only from April till May in each year, and the ratios Ant/178 were in the range of 0.23–0.90 indicating a combustion source in sediments. In 2008, when PAH contamination was observed, the ratios Flu/202 were less than 0.4 from April till October with a mean of 0.27 in Uzlina and 0.34 in Murighiol, indicating a petroleum contamination source. These characteristics indicate that the associated PAHs could be a mixture of pyrolytic and petrogenic origin. The total PAH concentrations in sediments from the studied area are similar to the levels found in Inner Clyde Estuary (0.63–23.711 mg/kg) [37] and Pearl River Delta (1.291–9.871 mg/kg) [21]. In the Black Sea, highest PAH concentrations were observed at the sediment sites influenced by the Danube (0.638 mg/kg) [12]. The results for organochlorine pesticide levels (OCPs), found occasionally in sediment samples collected from Uzlina and Murighiol sampling points. Heptachlor identified in water-body in June 2007 was also detected in sediment from Uzlina in April (2.30 μg/kg) and Murighiol in May (4.68 μg/kg). Lindane was found in sediments in concentrations ranging from 1.30 to 8.00 μg/kg in Murighiol and from 2.29 to 6.00 μg/kg in Uzlina. B-HCH isomer has also been detected during spring in concentrations ranging between 1.60 and 7.50 μg/kg in Uzlina, but only in April in Murighiol (2.61 μg/kg). DDT compounds occurred in sediments only in 2008 from April till June in concentrations ranging from 2.70 to 70 μg/kg, the highest value being recorded in Murighiol. Other OCPs detected in 2008 were α-HCH (1.30–1.75 μg/kg) in Murighiol, aldrin (0.018–0.27 μg/kg) and endrin (12–24 μg/kg) at both sampling points, whereas δ-HCH, dieldrin, triazine pesticides and phenylurea herbicides were not detected. In addition, organochlorine pesticides were found in sediment in April and June 2009. Only limited amount data on the organochlorine residues in sediments from Danube Delta exist. Sediment samples collected in 2001 from three lakes of the Danube Delta have shown high organochlorine values. HCHs ranged from 0.9 to 9.0 μg/kg with a higher contribution of lindane (range 31–76 %). DDTs were found in higher concentrations than HCHs, and ranged from 0.7 to 33 μg/kg [4]. Elevated concentrations of lindane (up to 40 μg/kg) and DDTs (up to 72 μg/kg) have been reported in the Black Sea sites influenced by the Danube Delta [10]. Comparable concentration levels of lindane (5.00 μg/kg wet wt.) were also recorded in the samples collected from Kizilirmak river, as well as DDTs in the Mert stream sediments (71 μg/kg wet wt), both discharging into Black Sea coast of Turkey [1]. This study has shown that some organochlorine compounds (lindane, heptachlor, endrin, DDTs) were present mainly during spring in sediments samples collected from both sampling points indicating substantial usage of these compounds as pesticides in the river area.

21.4 Conclusions

The structural changes of biological compartments in Danube Delta systems in terms of pollution and natural phenomena (floods, droughts) was evaluated. In phytoplankton community, oligo-betamesosaprobic diatoms, betamesosaprobic chlorophyte were constant and dominant components in the investigated aquatic systems. The spatial distribution of zooplankton in Uzlina and Murighiol control sections was not equal; betamesosaprobic rotifers species were better represented in Uzlina and betamesosaprobic copepods and oligo-betamesosaprobic cladocers species in Murighiol. As result of increased phytoplankton production, the oxygen concentration decreased in the sediment. For benthic invertebrates, this meant more food but reduced oxygen availability. As a result, Oligochaeta and Diptera indicator species of eutrophic conditions gradually colonise. Also, the changes in flow regimes and large amounts of Cu and Pb which were found in mobile fractions of the sediment samples have led to significant impact of benthic invertebrates diversity. It was observed the loss of sensitive taxa which have been replaced by tolerant ones, often foreign species. Several organic toxic compounds (lindane, heptachlor, endrin, DDTs) were detected in water and sediment samples during the monitoring period. For all metals studied, Cd, Cu, Hg, Zn and Ni appear to be a particular concern because of their high concentration levels found in both control sections sediment. In terms of ecological status, during 2003–2009 the Danube Delta systems are an eutrophic system equilibrated for a good ecological status [26].

The main actions to be taken to counteract potential adverse effects on the structure of biotic communities are: strong reduction in nonpoint inputs of pollutants, measures to decrease nutrient input from agriculture, building of wastewater treatment plants with nitrogen and phosphorus removal stage and transboundary cooperation for reducing oil pollution from shipping.

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Chapter 22
Quantitative Fire Risk Assessment Procedure at Pesticide Storage Facilities in Romania

Lucrina Ştefănescu, Török Zoltan, Francisc Senzaconi, and Alexandru Ozunu

Abstract Accidents involving fires occurring at facilities used for the storage of chemicals have determined the elaboration of specific legislation such as the SEVESO Directives. Pesticides represent major-threat chemical substances which, due to improper handling and storage, can induce fire and explosion risks, endangering the life and safety of population and causing major environmental pollution. The paper focuses mainly on these risks and their analysis methods, although health and environmental risks associated to the manufacturing, usage and transport of pesticides are of major concern, too. The procedure described in the paper uses a calculation method where the possibility to develop a fire is quantified using a set of fire scenarios, each with a certain occurrence probability, a certain fire surface and a certain time duration of the fire.

Keywords Pesticides • Quantitative risk assessment • Fire risk • Pesticides storage facilities

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22.1 Introduction

Environmental and human risks induced by pesticides are generally acknowledged worldwide. Health and environmental hazards associated to pesticides occur in each phase of pesticides handling: first during fabrication, by generation of toxic emissions and polluting liquid and solid wastes, then during transport through possible leakage and accidents causing the loss of containment, undoubtedly during their usage on cereal crops, vegetables or fruits, and last but not least during their storage and disposal.

The storage of pesticides involves several hazards such as: pollution of soil by seepage due to inadequate lining of the storage facility, pollution of underground and surface waters, pollution of air and other environmental media, as well threat of human and animal life by explosions and fires due to human errors or to improper storage conditions.

Incidents involving fires at pesticides storage facilities and plants were reported in: Basel, Switzerland (Warehouse 956 at the Muttenz works owned by Sandoz), in 1986 [6]; Canning, Nova Scotia in 1986; Birmingham Alabama, USA in 1997 [8]; Helena, Arkansas, USA in 1998; Antananarivo, Madagascar in 1997 [1]; Grandview, Washington in 2005; Dundas Ontario in 2007, to mention only a few. Most of these resulted in major fish kills in local rivers, clouds of pollution affecting the inhabitants and ecosystems, pollution of air, waters and soil, even death of firemen, injuries of people and long-term breathing problems. The largest disaster caused by the release of toxic pesticide components at a pesticide manufacturing plant was that occurring at Union Carbide pesticide plant, Bhopal, India, in 1984. The consequences were catastrophic: 2,500–5,000 fatalities and 200,000 people suffering from acute health effects [2].

A report of the Institute of Terrestrial Ecology stated that chemical accidents in general (such as Seveso, Bhopal, Mexico City – LPG disaster, Alaska – Exxon Valdez) causing major devastation tend to occur at low frequency, diverting attention from much smaller but much more frequent accidents [11].

In Romania, pesticides have extensively been used in agriculture since 1948, including products based on DDT and other products based on chlordane, dieldrin, endrin, aldrin, heptachlor and toxaphene. Most of these products were imported, except those based on DDT and heptachlor which were produced locally [3]. Some of these pesticides have been banned, and most of them have been stored in large amounts in storage facilities, and later destroyed. The 2002 and 2003 inventory of obsolete pesticides storage facilities listed 709 storage facilities of which 237 were not appropriately equipped [3]. Most of these obsolete pesticides have been destroyed as a compulsory condition of the European Union accession.

However, there are also active pesticides storage facilities in Romania, where pesticides are stored for marketing and later on agricultural purposes. Some of these storage facilities are subjected to the Seveso Directives and represent the object of risk assessment procedures required by the safety report.

Table 22.1 The list of some current pesticide storage facilities in Romania (Source: General Inspectorate for Emergency Situations, Ministry of Administration and Interior, 2012)

Location	Field of use	Substances	The current amount in storage/storage capacity (t/t)	Seveso status
Malu Mare, Preajba village	Phytosanitary products storage facility	Various substances such as DECIS Mega 50 EW, DEMITAN 200 SC, FASTAC 10 EC, PHOSFOROL, etc.	Depending on the season/106	Minor risk
Diosig	Retail commerce of chemical products	Herbicides, insecticides, fungicides	0.569/30.5	Minor risk
Cauaceu	Retail commerce of chemical products	Pesticides	24.7/24.7	Minor risk
Arad	Insecticides	Insecticides	0/1.5	Non Seveso
Arad		Toxin gas	0/1,000 l	Non Seveso
Arad	Merchandising of fertilizers	Insecticides, fungicides	0/70	Non Seveso
Valea lui Mihai	Storage and merchandising of seeds, chemical fertilizers, pesticides	Ammonium nitrate fertilizers	50/100	Non Seveso
Săcuieni			100/200	Non Seveso
Salonta			100/200	Non Seveso
Marghita			50/100	Non Seveso

Table 22.1 presents the list of some current pesticide storage facilities in Romania. However, this list is not comprehensive and should not be considered as the complete list of existing pesticide storage facilities in Romania.

22.2 Quantitative Fire Risk Assessment Methodology

The quantitative analysis of the major accident risk is a gradual process which includes three general steps. The first step is the selection of possible and credible major accident scenarios. The second step, that of major accidents occurrence probability and frequency analysis consists in assessing the probable frequency of each identified event and the accident scenarios frequencies. The last step is the consequence assessment of accident scenarios and it is composed of the definition of the terms resulting from each event, the assessment of the consequence magnitude of each scenario and the presentation of individual and social risk.

The analysis of fire risk is based on two Dutch methodologies acknowledged at European level: Risk analysis method for CPR-15 warehouses – document of the Ministry of Housing, Spatial Planning and the Environment [14] and Reference Manual Bevi Risk Assessments of the National Institute of Public Health and the Environment [10].

In these cases, when large quantities of pesticides are stored, a detailed risk analysis is necessary to contain the assessment of the individual and social risk, accounted for by the possibly catastrophic consequences on population and environment. These consequences are mainly caused by the atmospheric dispersion of highly toxic compounds produced during the fire and carried away to large distance [4, 9].

For the possibility of a fire the fire compartment has to contain combustible materials. The estimation of the quantities can be difficult, because the cargo is changing. Therefore, the estimation of toxic combustion products and quantities can be difficult, too. Generally, toxic combustion products are formed during the fire if the stored chemicals contain heterogeneous atoms like N, S, Cl, F, Br, Mn, Zn and so on.

In case there are no detailed statistics about the stored quantities of pesticides in the storage facility, the default average structural formula can be used, as the chemical structure representative to the mixture of the materials stored in the storage facility.

The default average structural formula is the following:

$$\mathbf{C3.6H5.3O0.4N0.9S1.3P0.01Cl0.8Mn0.08Zn0.01Sn0.01}$$

This approach, as generally accepted practice is recommended in the WHFIRE manual [15].

Regarding the unburned toxic materials during a storage facility fire, airborne powders or evaporated liquid may escape to the atmosphere, without being burned due to inefficiency of the combustion process. Only highly toxic chemicals with LD_{50} for rats of less than 250 mg/kg are considered relevant [15].

A fire scenario describes a possible way of the development of a fire and can be defined by the combination of the factors, which determine the burn rate.

Factors influencing the size of a fire in each scenario are the fire area (i.e. floor area, number of compartments involved etc.), the ventilation rate of the compartment per hour and the fire duration of maximum 30 min, equal to the exposure time [10]. The probability of a fire scenario occurring is determined by the size of the fire compartment and by the fire fighting system operating in the fire compartment.

The selection of the fire probability in a storage facility is presented below. The specific literature indicates the frequency of the release scenario of toxic combustion products to be 8.8E-4 year^{-1} if the fire protection level is 1 or 2, and 1.8E-4 year^{-1} if the fire protection level is 3 [10]. These frequencies are applied to each fire compartment.

The probability of a given size fire depends also on the ventilation rate and on the probability of not closing of the doors between the fire compartments.

Upon the formation of toxic combustion products, only the formation of NO_2, SO_2, HCl, HF and HBr and their dispersion are considered in the risk assessment [10, 14].

The final result of the risk analysis is the quantitative determination of individual and societal risks. Individual risk represented by the risk contours describes the frequency of death of an individual exposed to an accident at the facility.

Societal risk is represented by the so-called F-N (Frequency vs. Number of individuals) curve which describes the cumulative frequency of lethal events causing at least a certain number of deaths.

22.3 Results and Discussion

The results of this paper present a case study illustrating a simplified example of Individual Risk estimation in case of a storage facility fire in a single compartment of 400 m^2 surface area, with the purpose of presenting the risk assessment procedure. The storage facility is considered to be in the vicinity of a residential area. The calculation of individual risk considered only the dispersion of HCl gas during the fire, with an average release rate of 10 kg/s for 30 min. The individual risk curve is presented only for down-wind distance from the source along the axis of the toxic cloud.

The social risk calculation represents the subject of further analysis based on population density data, which was not included in the case study.

Figure 22.1 presents the variation of concentration of HCl versus the downwind distance from the source. The LC_{50} concentration (inhalation, rat = 7,004 $mg/m^3/30$ min) [5] was found at 296 m and the IDLH concentration of 50 ppm [7] was found at 5,094 m distance from the source.

Figure 22.2 presents the probability of death versus distance due to 30 min exposure to the HCl cloud. The probability of death (P_d) was estimated using Eq. 22.1 [13]:

$$P_d = 0.5^* \left[1 + erf\left(\frac{\mathrm{Pr} - 5}{\sqrt{2}} \right) \right] \tag{22.1}$$

where: *Pr* is the Probit function (Eq. 22.2) of HCl exposure to concentration *C* (mg/m^3) for an exposure time *t* (minutes):

$$\mathrm{Pr} = -37.3 + 3.69^* \ln(C^* t) \tag{22.2}$$

One can notice from Fig. 22.2, that in this case study the consequences can be severe at distances shorter than 600 m from the source.

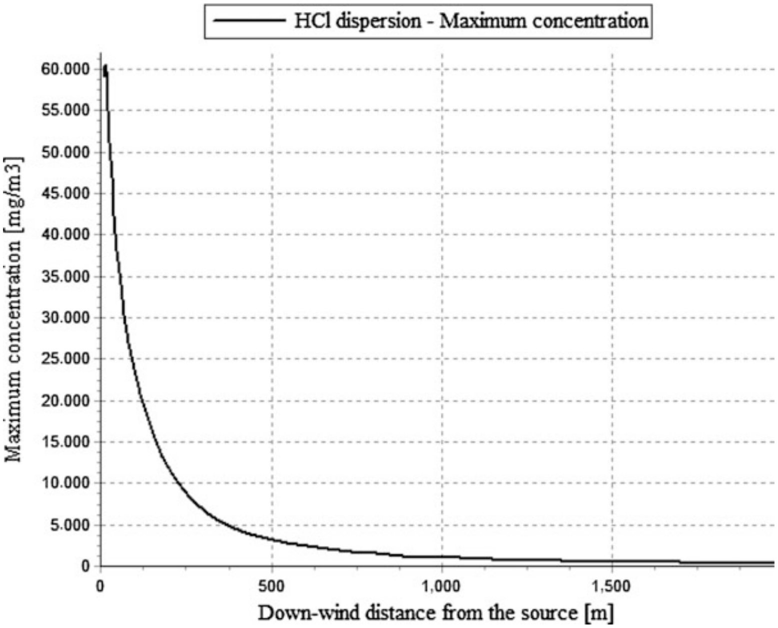

Fig. 22.1 Maximum concentration of HCl vs. down-wind distance from the source

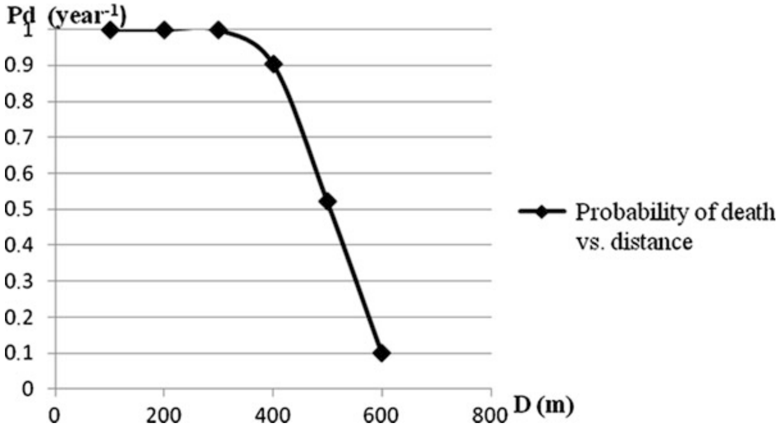

Fig. 22.2 Probability of death versus distance

Considering the frequency of 8.8E-4 fire events/year and the probability of death, the individual risk can be estimated using Eq. 22.3 [13]:

$$\Delta IR = f_s{}^* P_d{}^* P_m{}^* P_\varphi \qquad (22.3)$$

where: f_s – the frequency of fire event (year^{-1});

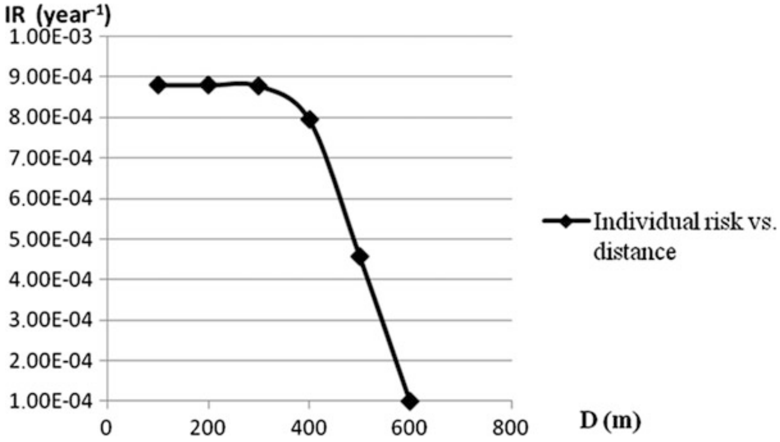

Fig. 22.3 Individual risk versus distance

P_d – probability of death;

P_m – probability of meteo stability class – it is considered to be 1 in this case study;

P_φ – probability of wind direction – it is considered to be 1 in this case study;

As it can be concluded from Fig. 22.3, the individual risk exceeds the acceptable limits for residential areas (between 10^{-5} and 10^{-6}/year) [12] in the vicinity of the pesticide storage facility. Therefore, the construction of residential buildings and economic use facilities is forbidden in the immediate vicinity of the pesticide storage facility.

22.4 Conclusions

A large number of pesticide storage facilities in Romania are built close to residential areas and several of these are subjected to the Seveso Directive, therefore a safety report is required to demonstrate the safety level. The Romanian legislation does not specify the need of individual and social risk estimation in the risk assessment procedure. Therefore, the authors of this paper recommend that in the case of Seveso-type pesticide storage facilities, the individual and social risks should be estimated, for a more efficient land-use planning procedure. Land-use planning is a very important element to be considered in fire risk assessment at pesticide storage facilities.

The proposed risk assessment methodology is based on two Dutch methodologies acknowledged at European level and it considers both the probability analysis and the consequence analysis, including the calculations of individual and social risk. The results obtained from the risk assessment provide a relevant overview of the exposure level of the population to risks induced by the pesticide storage facilities.

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Chapter 23
Distribution and Quantification of Pesticides in Processed Wheat Grain Fractions

Simona Dobrinas, Elisabeta Chirila, and Alina Serban (Olgos)

Abstract Food safety is an area of growing worldwide concern on account of its direct bearing on human health. The presence of harmful pesticide residues in cereals has caused a great concern among the consumers. For investigating the carryover of pyretroids, organochlorine and organophosphorus pesticide residues in the cereal food chain from grain to consumer, a study was set up on seven varieties of Romanian wheat. Over each variety of grain it was added a mix of pesticides. The residue levels of pesticides were determined in processed wheat grain fractions: bran, semolina and flour. The goal was to assess the absorption quantity of pesticide mixtures and their distribution in wheat fractions. The pesticides concentrations were below the maximum residue limits (MRLs) for wheat. The highest amounts of pesticides were present in bran and the least in flour.

Keywords Bran • Semolina • Flour • Pesticides • GC-ECD • GC-NPD

23.1 Introduction

Cereal grains are raw materials for the main foods at the basis of all of the regional diets in the world. Wheat processing is represented by milling of wheat into bran, semolina and flour manufacturing.

Studies on grain following the effect of storage, milling and cookie processing on the residues of pesticides were investigated by many researchers [3, 5, 6, 8, 11]. Despite the considerable amount of research that has been carried out on the application of pesticides to wheat, there are limited investigations on pyretroids.

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The objective of the present study was to determine the distribution of pesticides residues in processed wheat grain fractions. In this purpose the residue content in the seven varieties of wheat grain (before milling) was quantitatively determined by gas chromatography with nitrogen phosphorus detection (GC-NPD), respectively electron capture detection (GC-ECD). The seven varieties of Romanian wheat analyzed were: Dropia, Delabrad 2, Izvor, Faur F, Gruia, Boema and Glosa. Over each variety of grain it was added a mix of pesticides (organophosphorus pesticides, organochlorine pesticides and pyretroides). Then it was analyzed the contamination level of each fraction resulting after grinders: bran, semolina and flour in order to assess the absorption quantity of pesticide mixtures and their distribution in wheat fractions.

23.2 Experimental

23.2.1 Sample Collection

In this study, seven different types of wheat samples have been investigated: Dropia, Delabrad 2, Izvor, Faur F, Gruia, Boema and Glosa. These samples were collected from the state reserve existing in custody of certain companies to ensure quality and quantity of existing stocks. All samples have been obtained in 2012, transported to the laboratory and stored at 4 °C until being analyzed.

23.2.2 Application of the Pesticides

In a small-scale model of a commercial storage vessel with the inner surfaces of stores covered with thin metal sheet, were introduced 100 g wheat samples. Then mixes of organochlorine pesticides, pyretroids respectively organo phosphorus pesticides were prepared. The 50 g mix of each pesticide was sprayed directly onto the samples. These mixes were used to obtain better accuracy in the determination of very low residue contents that were expected in some milling streams with very low extraction rates. Immediately after the treatment, the batches of treated grain per variety were thoroughly mixed to provide a homogenous distribution of pesticide on grain. For each variety, the batch was delivered to the milling plant to be processed 24 h after treatment.

23.2.3 Extraction

The wheat samples processed in the milling plant (8 ± 0.001 g) were homogenized with 34 mL acetone/methylene chloride (50:50 v/v) and left overnight for the static extraction. The extract was filtered and centrifuged at 4,000 rev/min for 10 min.

Then 10 mL of extract was decanted and evaporate almost to dry using a rotary evaporator. The obtained residue was dissolved in 3 mL mixture isooctane/toluene (9:1 v/v) containing internal standard and then centrifuged 2 min at 4,000 rev/min. The samples were applied onto GC-ECD, GC-NPD without clean up [10].

23.2.4 Gas Chromatography

Gas chromatography was performed using a Varian 520 gas chromatograph equipped with a nitrogen phosphorus detector, electron capture detector and a capillary column (HP–5 fused–silica – 29.6 m × 0.32 mm × 0.25 μm) using helium carrier gas at a flow rate of 1.5 mL/min. The oven temperature programmed was: initial temperature isothermal, at 170 °C, for 5 min, then from 170 to 200 °C at 10 °C/min, then from 200 to 210 °C at 1 °C/min, for 3 min, finally held for 3 min at 260 °C. Injector and detector temperatures were 250 °C, respectively 300 °C. The injection was carried out splitless and the injection volume was 1 μl. Quantification of the pesticides was performed by comparing the peak areas to that of a calibration curve of standards. Correlation coefficients were found to be above 0.98 in all cases, indicating a good linearity.

23.3 Results and Discussion

Determined levels of pesticides in each fraction of wheat samples resulting after grinders: bran, semolina and flour are presented in Figs. 23.1, 23.2, 23.3, 23.4, 23.5, 23.6, and 23.7.

From the obtained data it was observed that the pesticides concentrations were below the MRL [1, 2, 7] and furthermore, most of them are below the detection limit.

Only cypermethrin (0.2376 ppm) was detected in wheat variety Delabrad 2 and two organochlorine pesticides: folpet at a concentration of 0.1016 ppm and metoxychlor at 0.0247 ppm. After contamination with pyretroids mix and organochlorine pesticide mix was observed that: deltamethrin, fenvalerate, iprodione, endosulfan-sulphate, bifenthrin, β endosulfan, op DDT, captan, op DDE, lindan and α endosulfan have not been absorbed into the grain. These pesticides were not found into any of the three fractions (bran, semolina or flour). α HCH and cypermethrin were found only in semolina and heptachlor only in flour with a concentration of 0.0122 ppm. Folpet and DDT pesticides were detected only in bran. Aldrin, chlorothalonil, β HCH, endrin and metoxiclor were found in all three partitions in concentrations ranging from 0.0155 to 0.284 ppm. In Delabrad 2 only methyl-parathion was found (0.0026 ppm) between organophosporus pesticides. After the application of organophosphorus pesticides mix was observed that sulfotep was not found in any of the three partitions and in bran were distributed

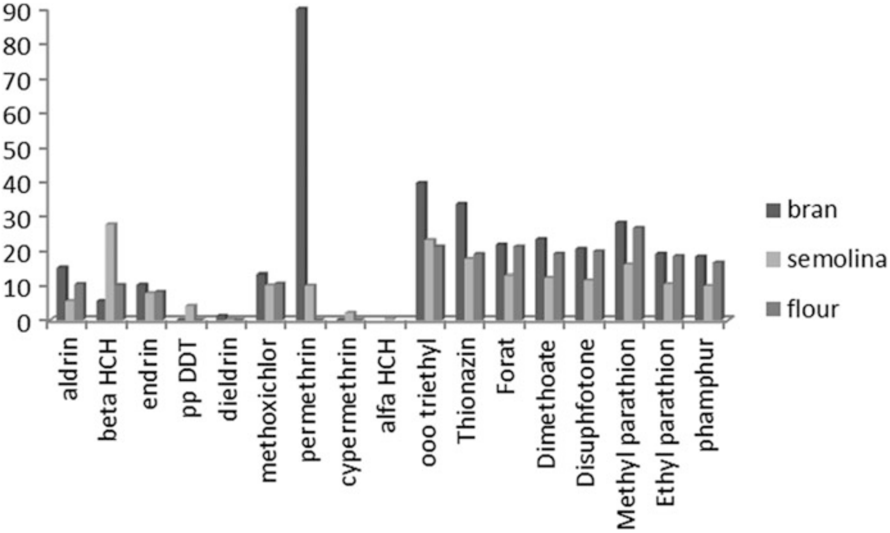

Fig. 23.1 Distribution of pesticides residues in the three processed Delabrad 2 wheat fractions

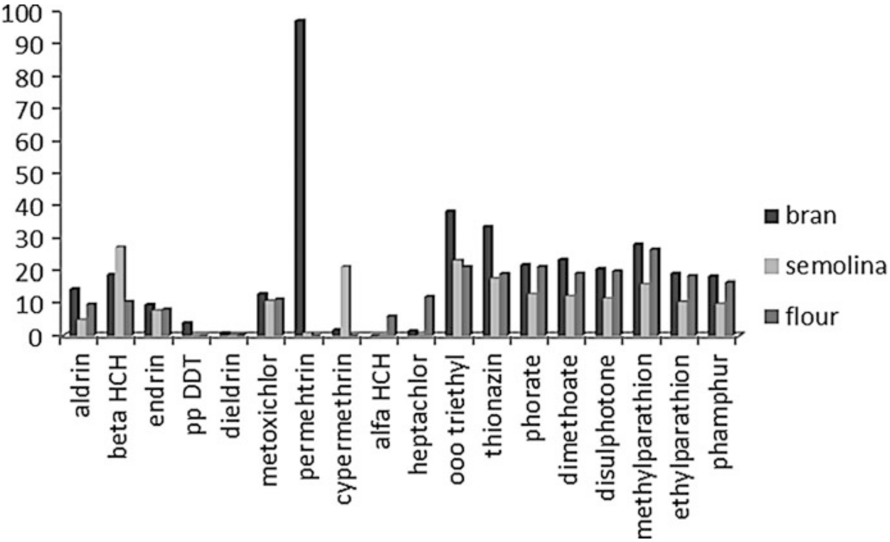

Fig. 23.2 Distribution of pesticides residues in the three processed Dropia wheat fractions

in the highest amount all organophosphorus pesticides: famfur (0.1966 ppm), ethyl-parathion (0.1223 ppm), methyl-parathion (0.3557 ppm), disulfoton (0.1179 ppm), dimethoate (0.148 ppm), thionazin (0.3426 ppm), ooo triethyl (0.3743 ppm) and forat (0.104 ppm).

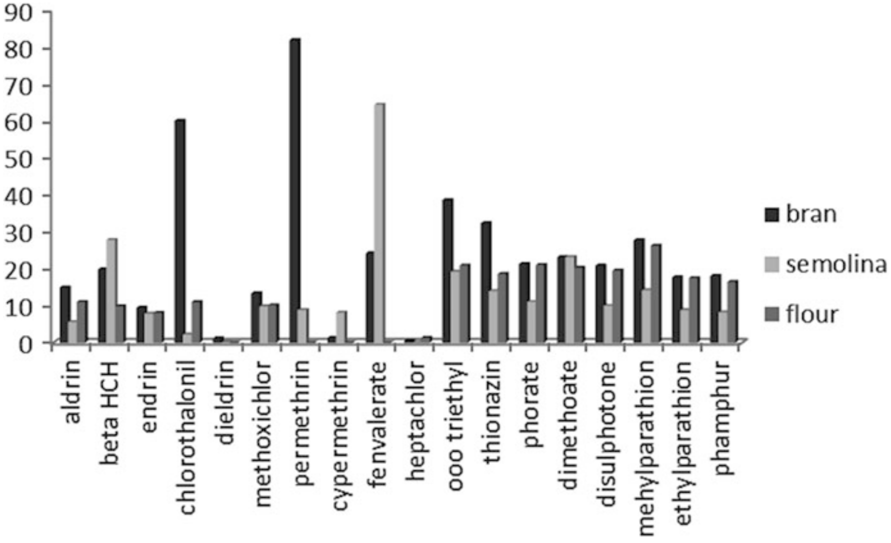

Fig. 23.3 Distribution of pesticides residues in the three processed Glosa wheat fractions

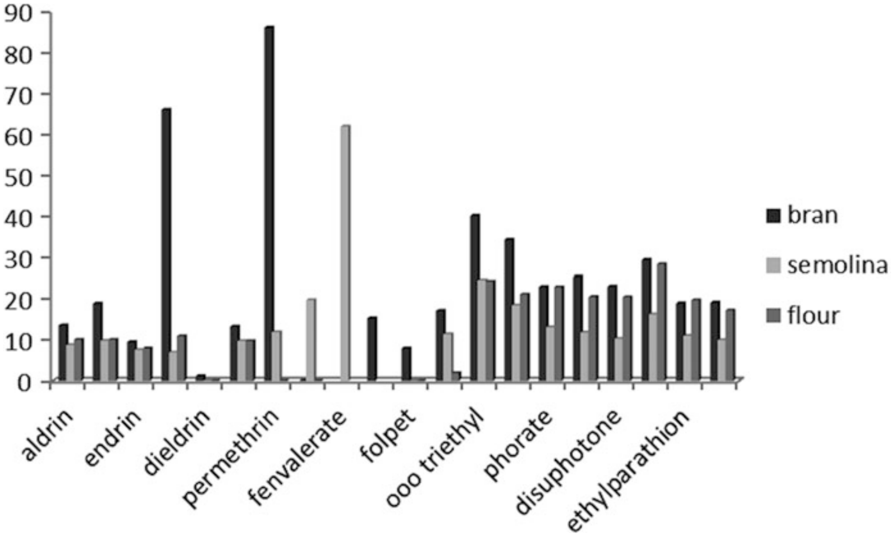

Fig. 23.4 Distribution of pesticides residues in the three processed Faur F wheat fractions

After analyzing the varieties Dropia and Glosa it was found that no organophosphorus pesticide was detected and only two pyretroids and organochlorine pesticides were detected: cypermethrin and metoxychlor. After contamination is observed a much better absorption of pesticide mix, only unabsorbed pesticide has been sulfotep (it was not found into any of the three fractions).

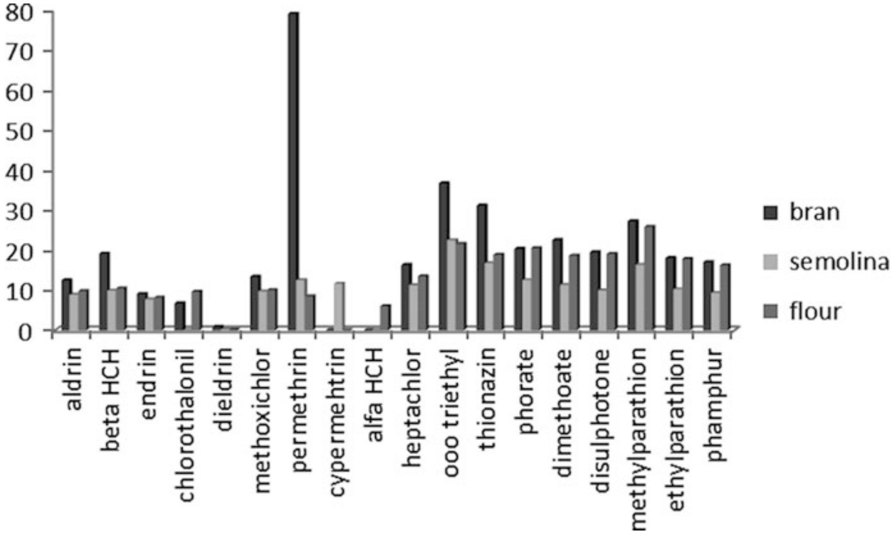

Fig. 23.5 Distribution of pesticides residues in the three processed Gruia wheat fractions

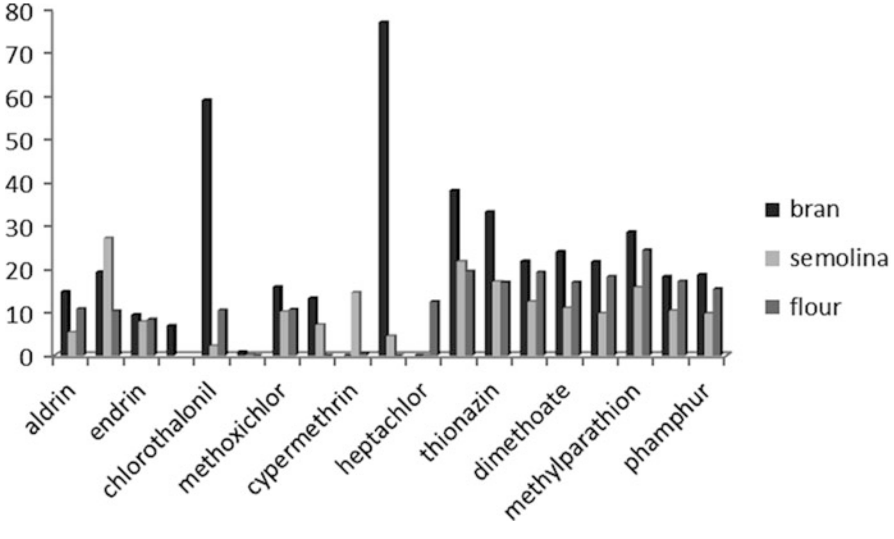

Fig. 23.6 Distribution of pesticides residues in the three processed Boema wheat fractions

Thus in bran it was assigned the highest amount of all other eight pesticides in varying concentrations from 0.106 to 0.3654 ppm. In semolina were encountered pesticide amounts ranging between 0.0529 ppm (forat) and 0.2163 ppm (ooo triethyl). α HCH and cypermethrin were found in Dropia semolina and α HCH and fenvalerat were found in Dropia flour. In Dropia bran were detected in very low concentrations folpet (0.094 ppm) and pp DDT (0.021 ppm). In Glosa variety were

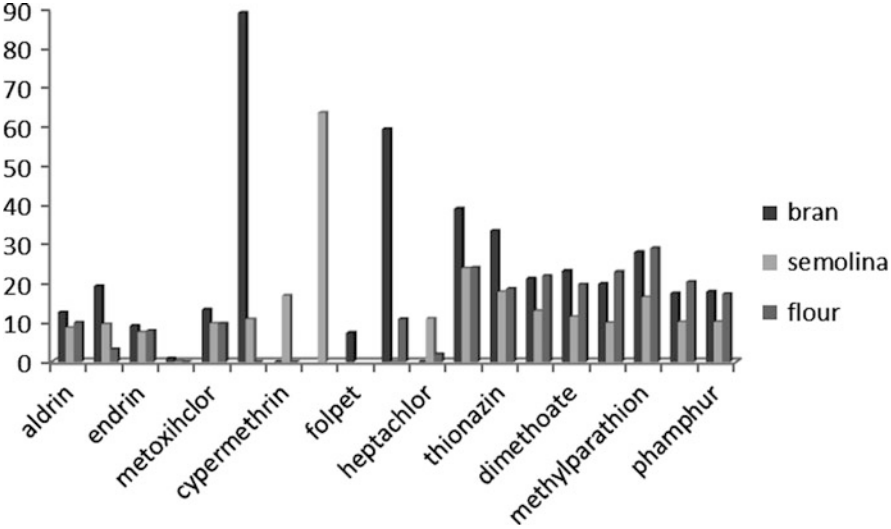

Fig. 23.7 Distribution of pesticides residues in the three processed Izvor wheat fractions

detected in all three fractions the organochlorine pesticides: aldrin, endrin, metoxiclor, β HCH and chlorothalonil.

Sulfotep was not detected in the other wheat samples Gruia, Boema, Izvor and Faur F as in previous varieties.

In Gruia type of grain was detected methylparathion, folpet, metoxychlor and cypermethrin before the mix treatment and in Izvor type was detected thionazin, ooo triethyl and methylparathion. So in Gruia bran was distributed in higher concentration famfur, methylparathion, disulfoton, dimethoate, thionazin, ooo triethyl and folpet. Pesticide forat is the only exception (has a higher concentration in semolina). The same distribution was registered for Izvor wheat sample, with the exception of disulfoton pesticide, which was encountered in higher concentration in flour.

In Boema variety it was not detected any organophosphorus pesticide, but in Faur F variety was detected tionazin and methylparathion (the lowest detected concentrations). In bran were distributed all other pesticides in concentrations ranging from 0.1048 to 0.3808 ppm. As in Gruia variety pesticide forat is the only exception (has a higher concentration in semolina).

The results show that Izvor, Boema and Faur F wheat samples considered in the present study contained detectable residues of various pyretroids and oranochlorine pesticides (folpet, metoxychlor, fenvalerate, cypermethrin, β HCH, permetrhrin, chlorotalonil) before the mix treatment. Similar to the results of this study, it was reported that methoxychlor has been determined in all wheat samples [4].

In case of Gruia, Boema, Izvor and Faur F varieties the organochlorine pesticides were distributed in flour in lower concentrations and pesticide folpet was found only in bran. The results showed that the pesticides were distributed in the bran in highest amount and in a smaller amount in semolina. The distribution in flour was the smallest one. In application of pesticides, considerable amounts of them remain on the surface of wheat and some penetrate to internal parts of wheat. Consequently, residue levels in bran were found to be higher than in wheat [9].

23.4 Conclusions

The pesticides concentrations were below the maximum residue limits (MRLs) for wheat. The highest amounts of pesticides were present in bran and the least in semolina. All fractions absorbed in large amounts endrin, methoxychlor, triethyl and thionazin. These pesticides were distributed differently in the studied fractions of wheat. Deltamethrin and sulfotep were not detected in any samples. The lowest absorption capacity had wheat variety – Boema and the largest absorption capacity had wheat variety – Dropia.

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Chapter 24
Organochlorine and Organophosphate Pesticides Determination in Plants Used for Cosmetic Purposes

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Abstract Recent information about the presence of pesticides in plants used for cosmetic purposes has been reported in the media and on the internet. Pesticides are toxic substances applied on plants that are going to be food or other products like cosmetics. Therefore it is not difficult to understand why people are concerned about these substances. Our goal was to determine the organochlorine and organophosphate pesticides occurrence in five plant materials used in the composition of value-added organic cosmetic products manufactured in Constanta county, Romania. The studied plant materials were sea buckthorn fruits, snowdrops and violets flowers, poplar buds and sea buckthorn male buds. The trace pesticides concentrations were determined using gas chromatographic technique, after the extraction and cleanup steps. There have been found 15 types of organochlorine pesticides and 8 types of organophosphate pesticides. The total organochlorine pesticides concentrations varied between 0.0173 and 0.4604 mg/kg dry plant and the total organophosphate pesticides concentrations between 0.0028 and 2.5900 mg/kg dry plant. All individual concentrations of determined pesticides ranged below the limits established by EC regulations for plants used as animal feed.

Keywords Pesticides • Organic cosmetics • GC • Plant materials

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24.1 Introduction

Pesticides are being used to increase production and quality in crops over the past few decades. Excessive usage of pesticides contaminates the crop itself and the environment, eventually causing disease to human beings [8]. Real and perceived concerns about the impact of pesticides on human health and the environment have led to strict regulation of both their application and residue levels in food and water supplies. Maximum residue limits of pesticides in food commodities and drinking water are commonly set by national regulatory authorities and international bodies, such as the Codex Alimentarius.

Cosmetic products are widely used and are directly applied to human skin. While the skin provides a protective barrier, certain ingredients may penetrate the skin and become systemically available. There is a growing interest in the pharmaceutical and cosmetic industry to use plant extracts for various fields of applications [3, 11, 12].

Since 1992 in Constanta, Romania, there is a value-added brand of natural cosmetics which contain a combination between 47 plant essences obtained from fresh flowers, buds and roots grown on the company's own lands in Dobrogea County.

Recent reports in the media and on the internet show an increasing concern about the presence of toxic chemicals in cosmetics. These toxic substances may result from the intentional addition of some preservatives [1] or from the contact of raw materials (mainly plants) with organic pollutants like pesticides from air, soil or water. They persist in various media and some can be transported over long distances to regions where they have never been used.

Suitable (reliable and cost-effective) analytical methods are needed mostly in order to enforce pesticide residue regulations, to provide toxicological risk assessment data, and to study pesticide fate in the food chains and the environment. Most analytical methods in the literature involve extraction of pesticide residues from food by organic solvents, surfactants, supercritical fluids or solid-phase extraction followed by GC, HPLC, GC-MS or ICP determination [2, 4–7, 10].

The aim of this study was to determinate the levels of organochlorine pesticides (OCL) and organophosphate pesticides (OP) from five types of plant materials used in cosmetics collected from rural sites belonging to Dobrogea county, Romania, using gas chromatography with ECD respectively termoionic detection.

24.2 Experimental

24.2.1 Sampling

Five types of plant materials have been investigated: snowdrop (*Galantus nivalis* sp.) and violet (*Viola odorata* sp.) flowers, poplar (*Populus nigra* sp.) buds and sea buckthorn (*Hippopae rhamnoides* sp.) male buds and sea buckthorn fruits. All plants have been collected from Topraisar village, Dobrogea county. The flowers and the buds were collected in March 2012 and the sea buckthorn fruits in October 2011.

The fruits have been stored in a freezer and before analysis have been kept to room temperature for 24 h. Other plant materials (flowers and buds) were dried in air, on special wood grid for 10 days.

24.2.2 Sample Preparation

In order to obtain the extracts containing OCL and OP pesticides, 8.33 g of grinded sample have been mixed with 34 mL acetone: isooctane 1:1 (v:v) and left 24 h at room temperature. The mixture was then centrifuged for 10 min at 4,000 rpm.

For OCL analysis, 10 mL of extract were evaporated to dryness and after redissolved with 3 mL isooctane; to 1 mL of isooctane extract were added 50 μL OCL internal standard (mirex) solution; 1 μL of the resulted solution was automatically introduced in GC.

For OP analysis, 10 mL of extract were evaporated to dryness and after redissolved with 3 mL isooctane; to 1 mL of isooctane extract were added 84 μL OP internal standard (ethion) solution; 1 μL of the resulted solution was automatically introduced in GC.

24.2.3 Reagents and Equipments

All used reagents were supplied by Merck Darmstadt, Germany: standards of pesticides – HCH, Lindane, p,p′- DDT, p,p′- DDE, p,p′- DDD, aldrin, endrin, heptachlor, endosulfan, metoxychlor, mirex, oootriethyl phosphorothioate, thionazine, sulfotep, phorate, dimethoate, disulfoton, methyl parathion, ethyl parathion, famphur, ethion and solvents: acetone, isooctane.

A Varian 520 gas chromatograph (GC) equipped with HP–5 fused–silica capillary column (29.6 m × 0.32 mm × 0.25 μm) and electron capture detector (ECD) for OCL pesticides analysis/thermoionic detector for OP pesticides analysis has been used for determinations.

Helium was used as the carrier gas with flow rate 1.5 mL/min and nitrogen makes-up gas at 30 psi. The injector temperature was 250 °C. The initial temperature was 170 °C; the temperature increases with 10 °C/min until 200 °C, held 3 min; increases 1 °C/min. until 210 °C; increases 30 °C/min until 260 °C and then held for 3 min. Other chromatographic conditions were: purge time ON – 2.5 min; purge time OFF – 7 min; split/splitless inlet vent – 17.14 mL/min; septum purge vent – 4.3 mL/min.

24.3 Results and Discussions

Our goal was to determine the organochlorine (OCL) and organophosphate (OP) pesticides in five plant essences obtained from different plant materials: sea buckthorn fruits, snowdrops and violets flowers, poplar buds and sea buckthorn male buds, using gas chromatographic technique. OCL were widely used in agriculture in the past, and most of them are resistant to photochemical, biological and chemical degradation for a long period of time [12]. The levels of OCL residues found in the investigated samples are given in Table 24.1.

From the 16 analysed OCL pesticides, most were found in poplar buds (12), followed by snowdrop flowers (10), sea buckthorn fruits (4), sea buckthorn male buds and violet flowers (3). The highest total OCL concentration occurred in poplar buds and the smallest in sea buckthorn male buds. This could be explained by the influence of air pollution, poplar being taller than sea buckthorn tree.

OP are more toxic than OCL, but they degrade quickly by hydrolysis on exposure to sunlight, air, and soil. This is why they represent an attractive alternative to the persistent OCL. Table 24.2 presents the levels of OP residues in the investigated plant materials. From the nine analysed OP individual pesticides, most in were found in snowdrop flowers (5) followed by sea buckthorn fruits and poplar

Table 24.1 OCL pesticides content in plants used in cosmetics (mg/kg dry weight)

		Plant materials				
		Flowers		Buds		Fruits
Pesticides	Retention time (min)	*Galantus nivalis*	*Viola odorata*	*Populus nigra*	*Hippopae rhamnoides*	*Hippopae rhamnoides*
α HCH	9.137	0.0223	<QL	0.0239	0.0003	<QL
γ HCH	9.332	<QL	<QL	<QL	<QL	<QL
β HCH	9.747	0.0460	<QL	0.2866	<QL	<QL
Heptaclor	10.074	0.0063	<QL	0.0043	<QL	0.0003
λ HCH	10.585	<QL	<QL	0.0024	<QL	<QL
Aldrin	10.921	0.0236	<QL	0.0676	<QL	<QL
Heptaclor e	11.443	<QL	<QL	<QL	<QL	0.0040
Endosulfan 1	11.925	0.0120	<QL	0.0099	<QL	<QL
pp′ DDE	12.559	<QL	<QL	<QL	<QL	<QL
Endrin	13.388	0.0113	<QL	0.0079	<QL	<QL
pp′ DDD	13.777	0.0157	0.0001	0.0111	0.0028	0.0059
Endosulfan 2	15.081	0.0014	0.0324	0.0072	<QL	<QL
pp′ DDT	15.622	<QL	<QL	<QL	0.0012	<QL
Endrin ald.	17.104	<QL	<QL	0.0002	<QL	<QL
Endosulfan s	17.475	0.0176	0.0076	0.0161	0.0130	0.0087
Metoxychlor	18.308	0.0248	<QL	0.0232	<QL	<QL
Total		**0.1810**	**0.0401**	**0.4604**	**0.0173**	**0.0189**

QL quantification limit

Table 24.2 OP pesticides content in plants used in cosmetics (mg/kg dry weight)

		Plant materials				
		Flowers		Buds		Fruits
Pesticides	Retention time (min)	*Galantus nivalis*	*Viola odorata*	*Populus nigra*	*Hippopae rhamnoides*	*Hippopae rhamnoides*
ootriethyl phosphorothioate	6.674	0.0118	0.0004	0.0131	0.0056	0.0171
Thionazin	9.461	0.0007	0.0008	0.0027	0.0008	0.0180
Sulfotep	9.878	<QL	<QL	<QL	<QL	<QL
Phorate	10.747	0.0011	<QL	<QL	<QL	<QL
Dimethoate	10.871	<QL	<QL	<QL	0.1133	<QL
Disulfoton	11.071	0.0013	0.0016	<QL	<QL	<QL
Methyl parathion	11.446	<QL	<QL	0.0324	<QL	0.0039
Ethyl parathion	11.614	0.0015	<QL	0.0006	<QL	2.5510
Famphur	11.765	<QL	<QL	<QL	<QL	<QL
Total		**0.0164**	**0.0028**	**0.0488**	**0.1197**	**2.5900**

QL quantification limit

buds (4), sea buckthorn male buds and violet flowers (3). The highest total OP concentration occurred in sea buckthorn fruits and the smallest in violet flowers.

In the international regulations for pesticides levels in different samples there are no limits regarding pesticides in raw plant materials used for cosmetic and medical purposes. The only existing limits are in plants used for animal feed, and there are between 0.01 and 0.1 mg/kg dry weight [9].

24.4 Conclusions

The paper presents original studies concerning the organochlorine and organophosphate pesticides residues determination in plant materials used in cosmetics from Constanta, Romania in order to quantify the environment pollution influence on plant.

The total organochlorine pesticides content varies between 0.0173 and 0.4604 mg/kg dry weight and the total organophosphate pesticides content varies from 0.0028 to 2.59 mg/kg dry weight.

There are no imposed limits in the international regulations for pesticides levels in raw plant materials used for cosmetic and medical purposes. Compared with the EC regulations for pesticides residues in plants for animal feed, the individual investigated pesticides concentrations do not exceed the existing limits.

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Chapter 25
Green Chemistry Protocols: Specific Ionic Liquids as Recyclable Reagents, Catalysts, Solvents and Extractors

Fliur Z. Macaev

Abstract Low-temperature melting salts or ionic liquids (ILs) were determined to be useful as alternative solvents, catalytic media and simply catalysts in various processes of organic synthesis. In this paper reviewed the available literature and our own data on the synthesis, physical and chemical properties, and future uses of imidazolic ionic liquids in different kind of reactions. Ionic liquids form a two-phase system, which greatly facilitates the separation of products from the catalyst. In addition, they can be used repeatedly without any activity loss and without a need of regeneration. Another important advantage is represented by the strength (for some systems – superacidic) of metal-chlorinated ionic liquids, a property that allows for the transformation process to occur at low temperatures. These properties permit ionic liquids to be full participants in "alternative" chemical processes, even if we don't take into account the productivity and selectivity gains presented in this review.

Keywords Ionic liquids • Reagents • Catalysts • Solvents • Persistent toxic substances • Liquid/liquid extraction

25.1 Introduction

One of promising research areas today is the chemistry of melted salts, which are usually called "ionic liquids" (ILs) [10, 17, 20]. Ionic liquids in most cases are nitrogen-containing organic substances, except the derivatives of phosphorus, which account for not more than 10 % of the total number of specific liquids described in the literature [17, 63]. Ionic liquids derived from 1*H*-imidazole **1** are

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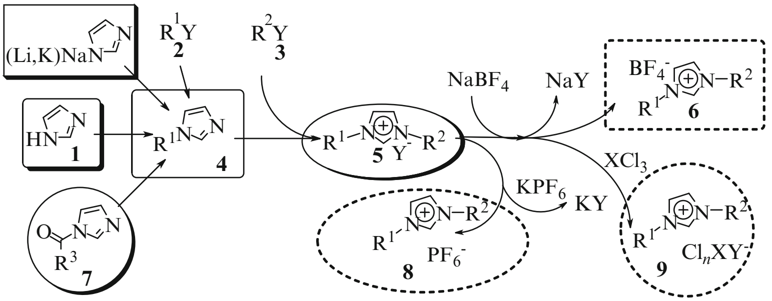


$R^1 = R^2 =$ alkil, Bn, $SiMe_3$, etc.; R^3 =OH, alkil, Ar, Im, etc; X=Na,K,Li,Al,Fe; Y=Cl,Br,I

Scheme 25.1 Synthesis of ionic liquids derived from 1H-imidazole

one of the most representative and interesting groups due to their properties [54, 58]. We shall begin the study of the chemical transformations of **1** with the analysis of the data of the synthesis of 1,3-dialkyl imidazolium salts. The second part of the paper will focus on functionalized ILs. Finally, data will be given on the use of ILs as recyclable reagents, catalysts and solvents or extractors of PTS, without any special sub-section dedicated to chiralic representatives [4, 13]. The known methods of N-alkylation of imidazole **1** include: the reaction with halo alkanes **2**; the reaction of imidazole alkaline metals salts with halo alkanes **2**; the reaction with trialkylphosphates; thermal decarboxylation of N-alkoxycarbonylimidazoles **7**; the reaction with alcohols, in the presence of acidic catalysts; quaternization of N-acylimidazoles **7** with subsequent deacylation and others (Scheme 25.1).

The synthesis of 3-bis-((S), 2-methyl-butyl)-1*H*-imidazole-1-ium bromide (yield 21 %) can be carried out by alkylation of trimethylsilylimidazole **4** ($R^1 = SiMe_3$) with (S)-1-bromo-2-methylbutane [28]. Syntheses of mono N-substituted imidazoles **4** are performed in most cases in absolute conditions, or with the use of at least double equivalents of the initial 1*H*-imidazole **1**. Imidazoles **4** may react with the halo alkanes to give the salts **5** (Y = halogen). This group of substances is involved in the reaction with $AlCl_3$ or $FeCl_3$, leading to ionic liquids of structure **9** [5, 6, 24, 48, 55]. Borates **6**, phosphates **8** and aluminates **9** can be synthesized by exchange reactions involving salts of the corresponding acids on N,N′- dialkyl imidazoles **5**. Also described are the syntheses of ionic liquids "*in situ*" directly in the reactor. In this case, the N-alkyl imidazoles **4** and the halo metals are mixed together in the reactor, and the ionic liquid is formed just before the beginning of the chemical process or the catalytic reaction [32].

For the purification of ionic liquids from chloride-ions electrochemical methods or ion exchange on ionites are used [2, 44, 53]. As to other approaches, the synthesis of ionic liquids with methyl and ethyl sulphate anions should be mentioned [25]. When synthesizing ionic liquids in the absence of a solvent (quaternization stage) a significant amount of heat is eliminated. The solution to this problem lies in performing the reaction under microwave or ultrasound radiation [1, 34, 51, 62].

Eventually, the reaction product yield increases, the reaction time diminishes, the content of impurities in the product is minimized, and in some cases, the stage of the purification of the final product could be avoided.

25.2 Task-Specific Functionalized ILs

25.2.1 Preparation of the Specific 1,3-Disubstituted ILs

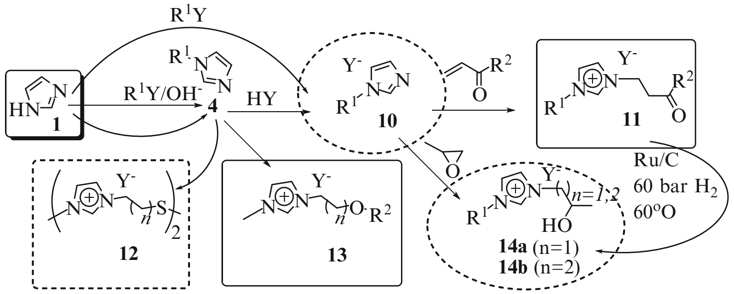
Specific derivatives of **11–14** with functional groups in side chain are also known (Scheme 25.2).

Imidazolium salts **10** are added to α, β-unsaturated carbonyl compounds to give salt **11**. This is called the Michael reaction and it involves conjugate addition [65]. Variants of functional group such as ether or thio- may be present in the final substrates **12** or **13** [7, 11, 23, 30, 53].

Hydroxy functionalized ionic liquids **14a** can be prepared by the reduction of ketone **11** (R^2 = Me) or the treatment of 2-methyloxirane with salts **10** [8, 26]. In addition to the above-mentioned ionic liquids primary alcohols **13** may be synthesized directly by quaternization **4** [26]. The synthesis of imidazolium salts with amino-, carboxy-, sulfo- and nitrile groups in the side chain should also be mentioned [19, 22, 56, 67].

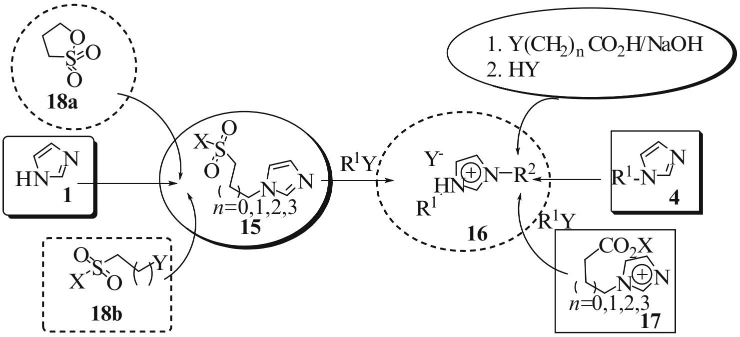
Imidazole **1** easily transforms into salts **16**. There are many reagents (like **18a,b**) that are added to imidazoles **4,15** according to (Scheme 25.3). The same result can be achieved in one step with carboxy-functionalized imidazoles **17** [36–42, 57, 66].

Specialized literature also describes the precursors of carboxy-functionalized imidazolium salts (Scheme 25.4) [16, 27, 36–38, 49, 50]. There are two possible ways. The initial product is always mono-substituted imidazoles [16]. In this case symmetrically dinitrile imidazole chlorides are obtained. The anionic exchange of chlorides with HPF_6, $NaBF_4$ and $LiTf_2N$ gives the corresponding imidazolium



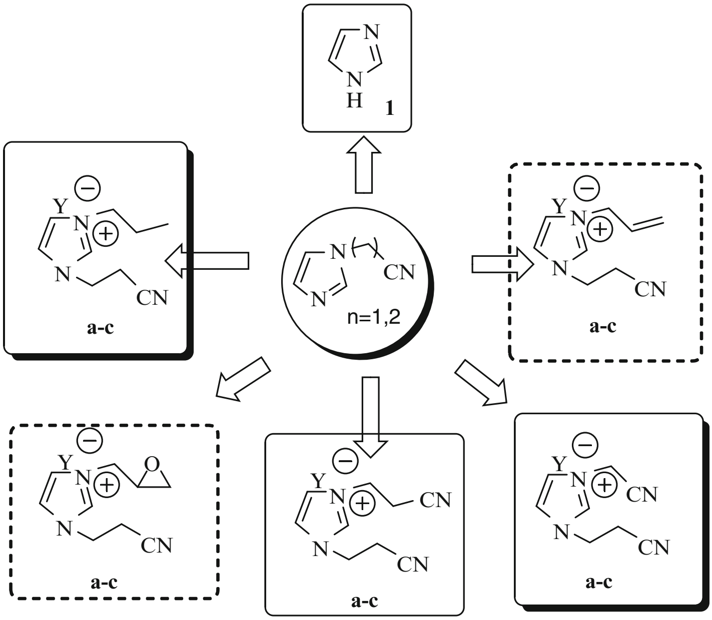
R^1=alkil, Bn, etc.; R^2=H, OH, alkil, O-alkyl, etc; Y=Cl,Br,I,BF_4,PF_6, $MeSO_3$, $(CF_3SO_2)_2N$

Scheme 25.2 Synthesis of the specific 1,3-disubstituted ionic liquids



R^1=alkil, Bn,(CH$_2$)$_n$CO$_2$H, etc.; X=Cl,H, OH;Y=Cl,Br,I,BF$_4$, PF$_6$, HSO$_4$

Scheme 25.3 Synthesis of the carboxy-functionalized ionic liquids



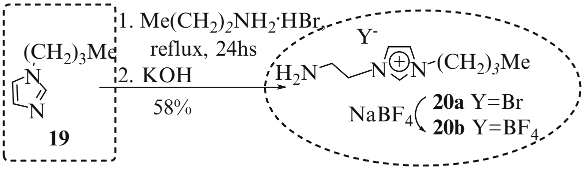
Y : a=Br or Cl; **b**=PF$_6$; **c**=BF$_4$

Scheme 25.4 Synthesis of the nitrile-functionalized ionic liquids

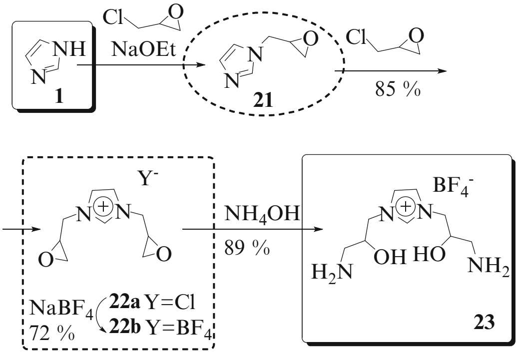
salts. The synthesis of the functionalized ionic liquids with the primary amino group in the side chain is presented in (Scheme 25.5) [3].

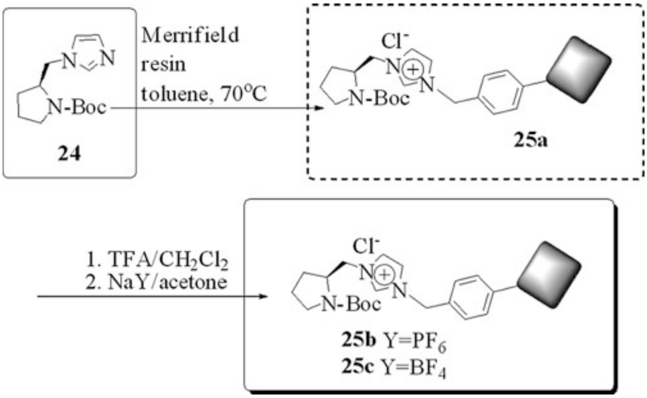
The reaction of imidazole **19** with 3-brompropylamine is easily carried out at room temperature. NaBF$_4$ are added to aqueous ethanol solution of bromide **20a** to give ionic liquid **20b**.

Scheme 25.5 Synthesis of the primary amino-funktionalized ionic liquids



Scheme 25.6 Synthesis of the amino-alcohol functionalized ionic liquids



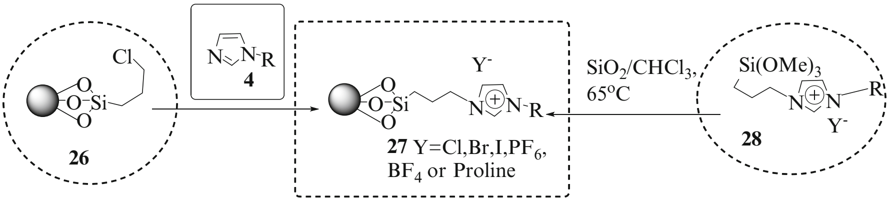


Scheme 25.7 Synthesis of the proline-functionalized ionic liquids supported on organic polymers

Oxirane group can be introduced into the 1 and 4 positions of imidazole **1** by the treatment with 2-chloromethyloxirane following the quaternization of the product **21** until the chloride **22a** (Scheme 25.6) [12].

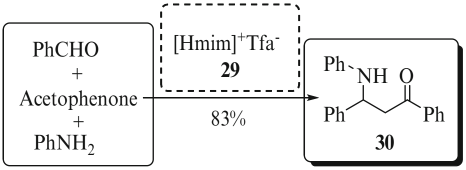
The opening of the epoxides **22b** to amino-alcohols **23** has been accomplished at 2 atm.

Lately, the number of the investigations dedicated to linking or immobilizing the ionic liquids on Al_2O_3, SiO_2, microporous polymers, clays and media based on coals and mesoporous materials has increased. Polymers immobilizing ionic liquids can be prepared in a number of ways. In one method, chiral imidazolium salts **25a-c** have been obtained starting with imidazole **24** according to (Scheme 25.7) [35].



Scheme 25.8 Synthesis of the funktionalized ionic liquids supported by S_iO_2

Scheme 25.9 Mannich reaction catalyzed by Bronsted acidic ionic liquids



If the activated silica gel contains a functional group such as OH, corresponding ionic liquids **27** may be the products from chloride **26** or salts **28** (Scheme 25.8).

This group of substances demonstrates the advantages over homogeneous ion liquids in simplifying the separation after the reaction, facilitating the regeneration which follows reusing [29, 47, 59, 60].

25.2.2 *Specific ILs as Recyclable Reagents, Catalysts, Solvents and Extractors*

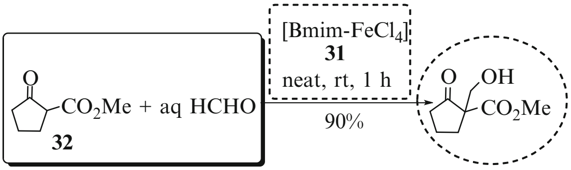
It is known that up to 90 % of chemical processes on earth occur under catalytic conditions. High specificity and/or efficiency are the main requirements of catalysts.

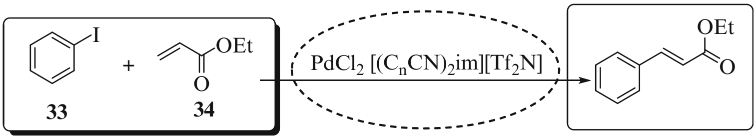
In the last decade we witnessed a significant increase of the interest in the use of 1,3-disubstitutedimidazole salts in organic synthesis. This is due to the fact that they are positively different from traditional solvents in their resistance towards oxidative actions, their non-volatility, and, in some cases, their catalytic activity.

Bronsted acidic ILs were used as solvents and catalysts of tree-component Mannich reactions of aldehydes, amines, and ketones at room temperature [68]. Higher yield of **30** were obtained in the presence of $[\text{Hmim}]^{+}\text{Tfa}^{-}$ **29** (Scheme 25.9) in comparison with other Bronsted acidic ILs.

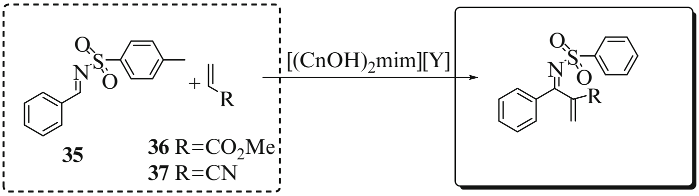
Isomerization of 2-methyl-3-buthenenitrile in 3-pentenonitrile was performed in a two-phase system (ionic liquid/n-heptane/ $Ni(cod)_2$, $[Ph_2P\textit{m}\text{-}PhSO_3Na]$) with 96 % conversion and 93 % selectivity [45, 64]. It has been found that this system does not lead to the isomerization or the degradation of the n-heptane itself. The latter phenomenon has been observed with $Al_2Cl_7^-$ anion containing ionic liquids [52, 61].

Scheme 25.10 Ionic liquids as catalyst for the hydroxymethylation of beta-ketoester





Scheme 25.11 Heck coupling of iodobenzene with ethyl acrylate in ionic liquids



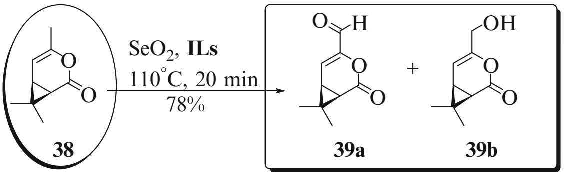
Scheme 25.12 Baylis-Hillman reactions in ionic liquids

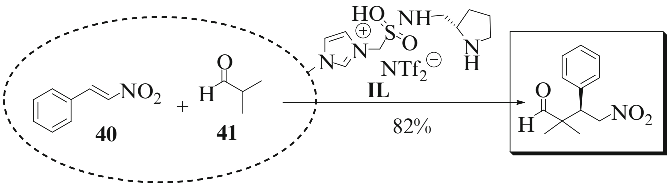
Aldehydes are obtained by the hydroformylation of olefins on Rh, Pt, Co, or Ru-catalysts, which are quite expensive. However, acetylacetonic and carbonylic metal complexes dissolved in imidazole with the hexafluorophosphate as the anion showed high activity and selectivity in obtaining products of hydroformylation. The iron-containing ILs [Bmim-$FeCl_4$] **31** proved to be an efficient and recyclable catalyst for the hydroxymethylation of β-ketoester **32** using aqueous formaldehyde and a low catalyst loading of up to 0.1 mol-% without co-solvents or additions surfactants (Scheme 25.10) [5].

Nitrile-functionalised ILs have been prepared and the reactivity of these salts with $PdCl_2$ studied by Fei et al. at [16]. The catalytic activity of the different palladium salts in Heck coupling of iodobenzene **33** with ethyl acrylate **34** (Scheme 25.11) has been evaluated in some of the nitrile-functionalised ILs and compared with that of nonfunctionalized ILs, providing insights into the nature of actual catalyst. Moreover, palladium nanoparticles were identified, but the nature of the catalyst strongly depends on the ILs employed.

Baylis-Hillman reactions involving the poor Michael acceptors, cyclic enones, were usually quite sluggish under normal conditions. Many attempts have been made to explore efficient catalysts to accelerate this kind of reaction. Hydroxyl functionalized IL has been explored as a catalyst for reaction of N-arylmethylidene-4-methylbenzenesulfonamides **35** with methyl acrylate **36** and acrylonitrile **37** [46] (Scheme 25.12).

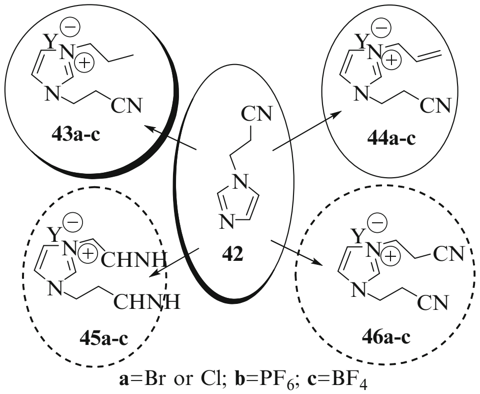
Scheme 25.13 The allylic oxydation reactions in ionic liquids





Scheme 25.14 Assymetric Michael coupling of trans-beta-nitrostyrene and isobutyraldehyde catalyzed by ionic liquids

Scheme 25.15 Nitrile-functionalized ionic liquids from 3–(1H–imidazol–1–yl) propanenitrile



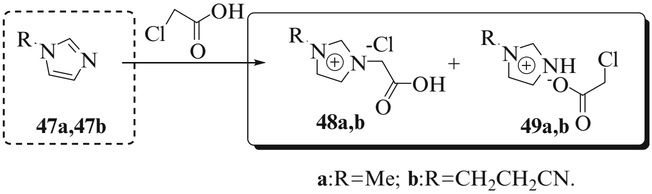
The allylic oxidation of the lactone **38** (Scheme 25.13) has also been investigated [14, 31].

It was shown that increasing the amount of SeO$_2$ up to a 3 eq. and carrying out the reaction in the ionic liquid leads to a single product **39**a. When compound **38** is heated with SeO$_2$ in ionic liquids at 50 °C, the product is the mixture **39**a,**b** (total yield 60 %).

The pyrrolidine-functionalized chiral ILs shown to be effective and reusable catalysts for asymmetric Michael addition of tarns-β-nitrostyrene **40** and isobutyraldehyde **41** (Scheme 25.14) [70].

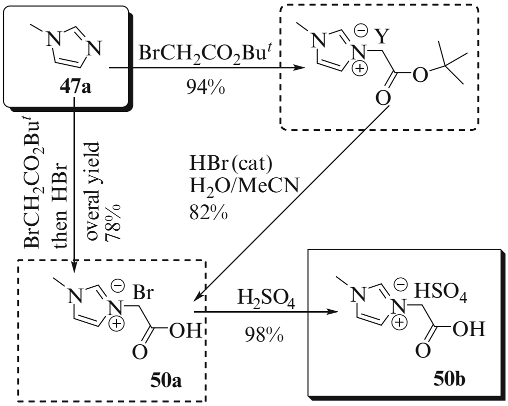
A convenient method of preparing hydroxy derivative **41** consists in the treatment of compound **40** with system -aqueous formaldehyde/0.1 mol-% of [bmim][FeCl$_4$]. The utilization of titanium tetrachloride decreased the yield of the product **41** (Scheme 25.14).

We also began by designing solvents/catalysts which would be prepared from imidazole **42** (Scheme 25.15).



Scheme 25.16 Ionic liquids from monochloroacetic acid

Scheme 25.17 Ionic liquids from tert-butyl 2-bromoacetate



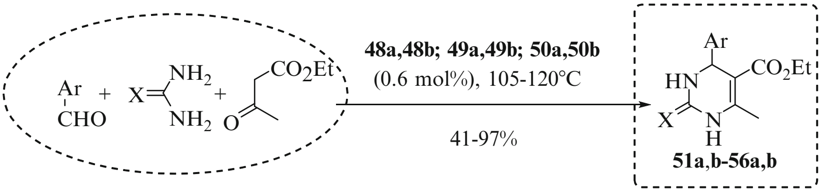
The imidazolium salts **43a, 44a, 45a, 46a** (Y = Br or Cl), which can be prepared by the treatment of imidazole **42** with alkyl halides, can be converted to ionic liquids 4**3b, 44b, 45b, 46b** as well as **43c, 44c, 45c, 46c** by the treatment with inorganic salt ($NaBF_4$ or KPF_6) [36–42, 57].

Carboxymethyl group can be introduced by the treatment of N-methyl imidazole **47a** (R = Me) with monochloroacetic acid in acetonitrile (Scheme 25.16).

The mixture **48a, 49a** is presented as oil-like liquid. Imidazolium salts **50a,b**, important reagents with a number of potential synthetic uses can be prepared in other ways (Scheme 25.17). When imidazole **47a** is treated with tert-butyl 2-bromoacetate, quaternization that is analogous to scheme 25.17 occurs. Deprotection gives a free carboxy-group of substance **50a**. A particularly mild method for the conversion of salt **50a** to imidazolium salt **50b** uses H_2SO_4 (Scheme 25.17).

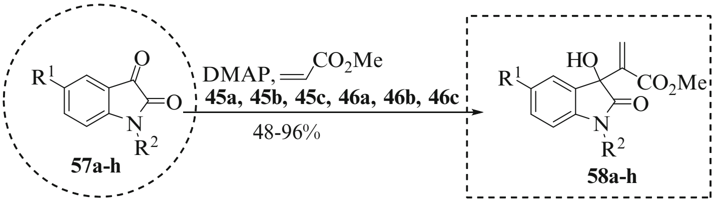
It was established that using ionic liquids as organocatalysts of Biginelli reaction leads to the decrease of the reaction time and the increase of the product yields, as compared to the activity of other ionic liquids (Scheme 25.18). It should be noted that in most cases, the yields of the 3,4-dihydropyrimidine-2(1*H*)-thiones are higher than those of their oxygen-containing analogues.

Considerable attention has recently been focused on the conversion of the simple starting materials into highly functionalized products such as α-hydroxy- or α-amino-alkyl activated olefins. The 2-(3-hydroxy-2-oxo-2,3-dihydro-1*H*-indolyl)



51 : Ar=Ph, X=O (a), S (b);
52 : Ar=3,4-$(MeO)_2C_6H_3$, X=O (a), S (b);
53 : Ar=2,4-$(MeO)_2C_6H_3$, X=O (a), S (b);
54 : Ar=2,4-$Cl_2C_6H_3$, X=O (a), S (b);
55 : Ar=4-$MeOC_6H_4$, X=O (a), S (b);
56 : Ar=4-$(Me_2N)C_6H_4$, X=O (a), S (b);

Scheme 25.18 Ionic liquids as organocatalysts of Biginelli reaction



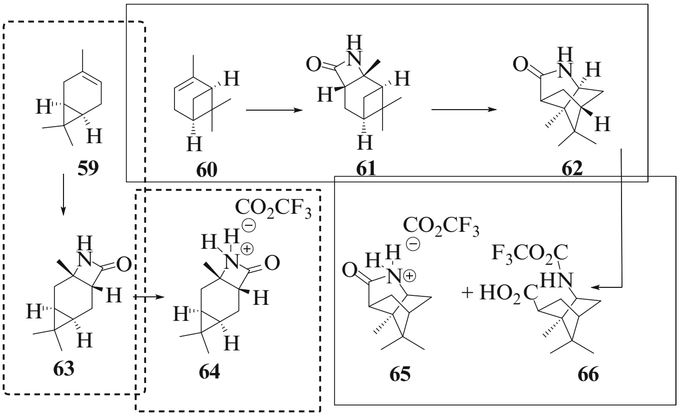
a) R^1=H, R^2=-Et, **b**) R^1=H, R^2=-C_3H_7; **c**) R^1=H, R^2=-CH_2CH=CCl-Me;
d) R^1=H, R^2=-CH_2-CH=CH_2; **e**) R^1=-Me, R^2=-CH_2-CH=CH_2;
f) R^1=H, R^2=-CH_2-CCH; **g**) R^1=H, R^2=-CH_2-C_6H_5; **h**) R^1=H, R^2=-C_4H_9

Scheme 25.19 Coupling of isatines and methyl promoted by ionic liquids

acrylates sequence plays a very important part in organic as well as medicinal chemistry. We established, that the two-component system consisting of ionic liquid and dimethylaminopyridine (1:8) is an efficient catalyst for obtaining 2-(3-hydroxy-2-oxo-2,3-dihydro-1*H*-3-indolyl) acrylates **58a-h** (Scheme 25.19) [36–42, 57, 58].

The reaction was performed under stirring during 1 h at room temperature of the mixture of isatines **57a-h**: methyl acrylate : dimethylaminopyridine : ionic liquid at a molar ratio of 1 : 1.56 : 0.5 : 0.06.

In the current age of high dependency of chemical industries on the resources coming from oil and gas which are getting increasingly scarce, the focus is shifting towards renewable feedstock. In this prospective, natural monoterpenes produced by a wide variety of plants represent a group of inexpensive and abundant starting materials for fine chemical synthesis. It is noteworthy that used specific ionic liquids are synthetic chemicals arising from petroleum. As this resource continues to be consumed at a prodigious pace, and given the rather turbulent conditions present in some of the major oil-producing parts of the market, alternative materials, possible based on biorenewable monoterpenoids, are of



Scheme 25.20 Optically active lactam-functionalized ionic liquids from biorenewable monotterpenoids

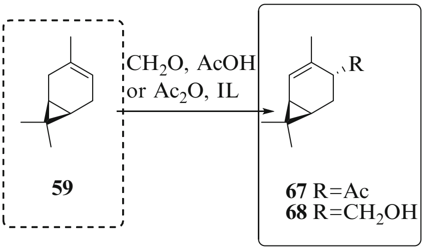
considerable interest and great practical benefit. Shortly our interest in area of green solvents-catalysts from renewable resources prompted us to focus our attention on the optically active lactams from (+)-3-carene **59** and α-pinenes **60** [43] (Scheme 25.20).

The stirring of lactams **62** or **63** in trifluoroacetic acid at room temperature gave the mixture of trifluoroacetates **65/66** or **64** in quantitative yields. We were proposed use of the lactam-functionalized ionic liquids **64** and **65/66** as catalysts to a synthesis of ethyl 1,2,6-triphenyl-4-(phenylamino)-1,2,5,6-tetrahydropyridine-3-carboxylate *via* a solvent free one-pot multicomponent approach. In all tested cases the product was obtained with good yields. Slightly higher selectivity has been observed in the reaction between benzaldehyde, ethyl acetoacetate and aniline using mixture **65/66** as a catalyst in comparison with catalysts **64**. It should be to note that all catalysts can be recovered during isolation of ethyl 1,2,6-triphenyl-4-(phenylamino)-1,2,5,6-tetrahydropyridine-3-carboxylate.

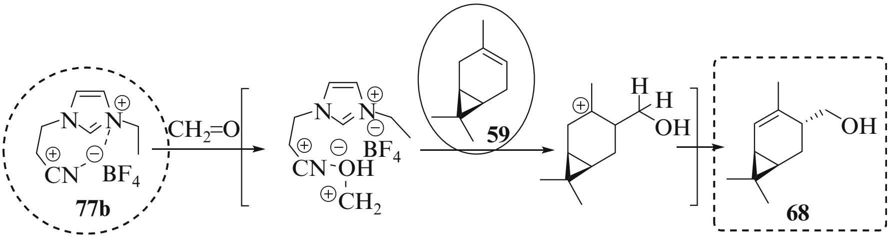
Increased requests to ecologically pure selective chemical processes need to develop a new type of compounds, posses several useful properties (combination of solvent and catalyst for the same compound), and possibility to use of it without any additional regeneration and purification.

Ionic liquids (IL) posses all above mentioned properties, but the list of it is very short. Furthermore, the use of ILs may enhance the regio- and stereo-selectivity of reactions. Well known, one of the tasks of the synthesis of a bioactive compound is preparation of required enantiomer in optically pure form. Bicyclic monoterpene (+)-3-carene **59** is widely used for resolving this type of problems. A structural future of compound **59** is the presence of the reactive $C = C$ double bond and bicyclic bridging system. This fact opens perspectives for new synthesis with retention of the bicyclic framework of monoterpene **59**.

(+)-4a-Acetyl-2-carene **67**, and (+)-4a-hydroxymethyl-2-carene **68**, are widely used to synthesize precursors of commercially important insecticides. Known methods for preparing 4-substiututed 2-carenes **67, 68** include heating **59** with



Scheme 25.21 Kondakov's and Prins's reactions in ionic liquids



Scheme 25.22 Proposed catalysis based on ionic liquids

$ZnCl_2$ in Ac_2O solution or with paraformaldehyde in AcOH. The new type of ionogenic solvents/catalysts for Kondakov's and Prins's reactions was proposed us (Scheme 25.21) [39–41].

It was observed that heating of (+)-3-carene **59**, acetic anhydride, or with paraformaldehyde in AcOH and 6 mol% **77a**, **77b** or **77c** at +60 °C afforded the (+)-4a-acetyl-2-carene **67**and (+)-4a-hydroxymethyl-2-carene **68**. The catalytic activity of the synthesized imidazolinium salts is probably due to initial formation of an acylium ion from Ac_2O that involves the nitrile associated with the anion.

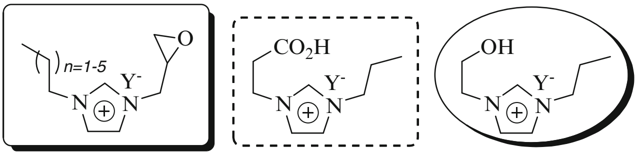
The carbonium ion formed by addition of the acyl-cation to the double bond of (+)-3-carene **59** is stabilized by elimination of a proton to regenerate double bond of (+)-4a-acetyl-2-carene **67**.

On the other side the α-hydroxy carbonium ion generated *via* adding of a proton to formaldehyde reacts with (+)-3-carene **59** to give the hydroxyl-carbonium ion that is stabilized via elimination of a proton (Scheme 25.22).

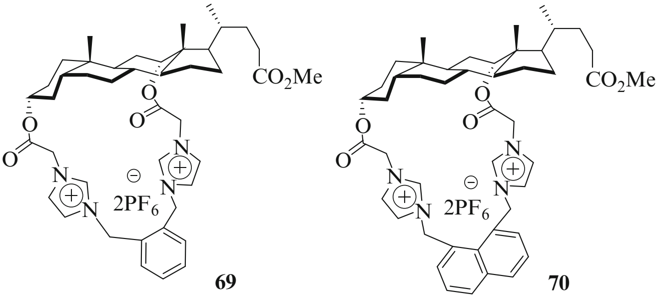
Finally the generations double bond of (+)-4a-hydroxymethyl-2-carene **68** was realizing.

During the last few years, extraction of kind of metal ions by using ionic liquids have been developed and reviewed [33, 69] as well. Mercury compounds extraction from aqueous solutions was carried out us [42] analogously published method [18].

Liquid/liquid extraction was carried out by contacting equal volumes of ionic liquid and an aqueous solution of $HgCl_2$ under stirring at room temperature. Ionic liquids (Scheme 25.23) showed a remarkable ability to extract Hg(II) ions from the aqueous phase and different trends depending different ionic liquids/Hg(II) molar rations. For example, use nitrile containing ionic liquid produced a corresponding increase of Hg(II) ion percentage partitioned in the organic phase then use 5:1 M ratio. On the contrary, in the case of carboxyl group containing ionic liquids,



Scheme 25.23 Ionic liquids for extraction from aqueous solutions



69

70

Scheme 25.24 Deoxycholic acid-based cyclic ionic liquids

a 2:1 M ratio is high enough to achieve the complete transfer of Hg(II) in the organic phase. Moreover, comparable results were gained with allyl side chain group with combination of 2,3-epoxypropyl chains, pointing out that further modifications in the hydrocarburic moiety of imidazolium salt did not significantly affect the efficiency of Hg(II) ion extraction. It should be mentioned that our ionic liquids shown practically the same “extractors” properties as reported by [18].

New deoxycholic acid-based cyclic receptors bearing imidazolium moieties bridged with o-xylene group **69** and 1,8-dimethylenenaphthalene group **70** have been synthesized (Scheme 25.24) [9]. Anion binding studies revealed that receptors having naphtalenic group as spacer exhibit very high selectivity for fluoride ion over other anions while receptors with o-xylene group shown a preference for the chloride ion.

25.3 Conclusions

In the last 10 years significant progress has been made in the field of ionic liquids. In the future, more complex target compounds, new methodology will be required for rapid construction of low-temperature melting salts bearing unusual substitution patterns. Thus, works are continued in an effort to elucidate innovative new processes using task-specific ionic liquids to obtain products with target properties.

Acknowledgments I thank to all members of my team: researchers, PhDs, technicians and students for their suggestions have made a much better our research. Their names are listed in the references. Financial support of NATO is acknowledged.

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Chapter 26
Relative Liver Weight in Rats Subacutely Exposed to Polychlorinated Biphenyls

Aleksandra Buha, Vesna Milovanović, Biljana Antonijević, Zorica Bulat, and Vesna Matović

Abstract Polychlorinated biphenyls (PCBs) are pollutants abundantly present in the environment. Before they were banned, commercial mixtures of PCBs had been produced in many countries and were used for a number of technical purposes. This is considered to be the main source of the current levels of PCBs found in the environment due to their lipophilic properties and high persistence. The aim of this study was to evaluate dose-response relationship for the effects of the commercial PCBs mixture on relative liver weight by Benchmark dose (BMD) approach, and to derive the BMD10 for the effect. In this study, commercial mixture of PCBs, Aroclor 1254 was used in a 28-day toxicity study in young adult Wistar rats. The rats were provided with food and water ad libitum and maintained in a controlled environment. Commercial mixture of PCBs dissolved in corn oil or corn oil (control) was administered by oral gavage at 1 μL/g. Rats were given doses of 0.5, 1, 2, 4, 8, 16 mg PCBs/kg b.w./day. After 28 days rats were sacrificed and their livers were weighted. Relative liver weight was calculated as ratio of liver weight and body weight. Using PROAST software a dose-response relationship has been confirmed for the influence of PCBs on relative liver weight and associated CED10 was 0.154 mg/kg bw/day, and its lower confidence limit (CEDL) was 0.1127 mg/kg bw/day proving CED10/CEDL ratio under 10. Our results showed that relatively low doses of PCBs may produce effect on liver weight causing liver hypertrophy in a dose response manner.

Keywords Polychlorinated biphenyls • Benchmark dose • Relative liver weight • Rats

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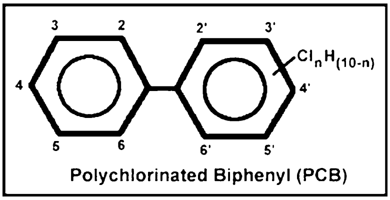

Fig. 26.1 Chemical structure of polychlorinated biphenyls

26.1 Introduction

Polychlorinared biphenyls (PCBs) are ubiquitous environmental contaminants which are considered to be persistent organic pollutants that bioaccumulate in individuals and biomagnify in food chain. Being highly persistent in living organisms, together with other organochlorides, PCBs comprise the bulk of organochlorine residues in human tissues [15].

The structure of all PCBs consists of biphenyl molecule with minimum of 1, and no more than ten chlorine atoms attached (Fig. 26.1). Theoretically, 209 different PCB congeners are possible, depending on the place of Cl, although only 130 congeners are found in commercial PCB mixtures. These synthetic organic chemicals have no known natural sources in the environment; they were commercially manufactured as industrial mixtures known for their stability under broad range of chemical, thermal and electrical conditions. They have been used as heat transfer agents in electrical transformers, flame retardants, adhesives, carbonless copy paper, as well as pesticide extenders. After the public's outcry concerning the discovery of PCBs detrimental health effect and apparent link between PCBs and widespread environmental problems, PCBs were the first industrial compounds to experience a world-wide ban on production. The production of PCBs in USA known under commercial name Aroclor ceased in 1979 and international ban on production was enacted at the Stockholm Convention on Persistent Organic Pollutants in 2001. However, exposure to PCBs due to their resistance to biodegradation and their lipophilicity which allows them to biomagnify in the food chain is still significant [1]. General population may be exposed to PCBs by contaminated food and by contaminated air. Food consumption, especially consumption of fish, meat and poultry remains to be the major source of PCBs intake in humans. The concentration of these chemicals in blood, fat tissue and mothers' milk have decreased over the past 30 years, but are still detectable in blood of the general population all over the globe [13].

Exposure to PCBs has been associated with neurobehavioral, immune, developmental, reproductive and hepatic abnormalities in both humans and experimental animals [4, 19, 23]. International Agency for Research on Cancer (IARC) has determined that PCBs are probably carcinogenic to humans and assigned them to group 2A [11]. The environmental Protection Agency (EPA) has determined that PCBs are probable human carcinogens and assigned them the cancer weight-of-evidence classification B2 [12].

Hepatotoxicity of PCBs is well-documented in animals exposed to acute, intermediate (subacute and subchronic), or chronic poisonings with commercial PCBs mixtures or single congener by different relevant routes of exposure [2, 6, 7, 17, 22]. PCB-induced liver effects in animals include microsomal enzyme induction, liver enlargement, increased serum levels of liver-related enzymes and lipids, altered porphyrin and vitamin A metabolism, and histopathological changes. Hepatotoxic effects are considered one of the most sensitive endpoints for PCBs mixtures [3], relative liver weight being one of the relevant parameters for determination of no observable adverse effect levels (NOAEL) and lowest observable adverse effect level (LOAEL).

Risk assessments for health effects that may result from exposure to environmental agents such as PCBs require an analysis of the dose-response relationship that can be defined by NOAEL/LOAEL levels. However, this approach has several disadvantages which can be overcome by benchmark dose (BMD) approach.

The aims of this study were to evaluate dose-response relationship for the effects of the commercial PCBs mixture, Aroclor 1254 on relative liver weight by using BMD approach, to derive critical dose effect (CED_{10}) for this effect and to compare it with NOAEL values obtained for liver in previous studies.

26.2 Materials and Methods

Male albino Wistar rats weighting 200–250 g free of typical rodent pathogens were used through the study. The animals were housed in cages with a 12-h day and night cycle at the temperature of 20–24 °C and relative humidity between 40 and 60 %. The rats were provided with food and tap water ad libitum and were treated in compliance with Guidelines for Animal studies no. 9667-1/2011. Rats had been accommodated to the experimental conditions for 1 week before they were randomly assigned into control and treated groups, seven animals each. Six treated groups were receiving Aroclor 1254 dissolved in corn oil in the doses of 0.5, 1, 2, 4, 8 and 16 mg PCBs/kg body weight/day by oral gavage in a volume of 1 μl/g body weight for 28 days. Control animals were receiving only corn oil also by orogastric tube during 28 days. Doses were selected on the basis of literature data [3, 16, 21]. On the day following the final dose, rats were sacrificed. The liver was removed, dissected out and weighted. Relative liver weight was calculated as ratio of liver and body weight at the time of scarification.

In order to establish significantly important differences between the observed groups, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) as post hoc test. The level of significance for all tests was set at $p < 0.05$. For this statistical analysis SPSS software (version 11.5) was used.

Collected data on relative liver weight were then analyzed by the Benchmark dose approach using PROAST software. PROAST is a software package that has been developed by the Dutch National Institute for Public Health and the Environment for the statistical analysis of dose-response data and deriving a BMD in risk

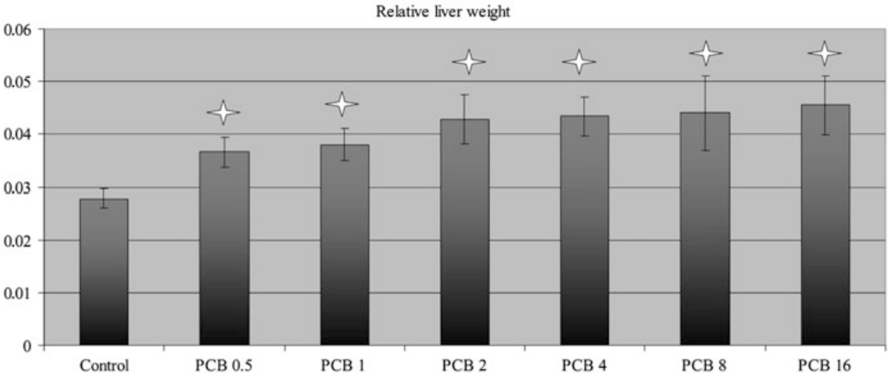

Fig. 26.2 Relative liver weights in rats exposed to different doses of PCBs. The values are expressed as mean values ± SD and statistical evaluation was performed by ANOVA followed by LSD test. *Stars* represent significant difference between the treated group and control group ($p < 0.01$)

assessment. The dose-response data are statistically evaluated by fitting a dose-response model to the data followed by established low but measurable change in response-Benchmark response (BMR) and the associated dose level-BMD. The BMDs lower confidence level (BMDL) is generally used as the reference point. The terms critical effect size (CES) and critical effect dose (CED) can be used instead of BMR and BMD when obtained data are continuous [18]. In this study, CED_{10} was determined since critical effect size at 10 % level appears to minimize the adversity and adaptivity, as well as animal variations [20].

26.3 Results

Relative liver weight was significantly higher in all treated group when compared to control group with a maximum increase of 63.84 % observed in the group receiving the highest dose of 16 mg PCBs/kg bw/day. As it can be seen in Fig. 26.2, the increase in doses of PCBs was followed by elevated relative liver weight.

Therefore, the next step was to characterize a dose-response relationship for the effect of PCBs on relative liver weight (Fig. 26.3). Lower confidence leveled of CED at CES of 10 % was calculated from the best fitted curve. PROAST software has proved dose-response relationship and CED_{10} was shown to be 0.154 mg PCBs/kg with the lower confidence level of 0.1127 mg PCBs/kg. The obtained CED/CEDL ratio, which is used as measure of statistical uncertainty, was under 10 proving that data on relative liver weight can be considered relevant.

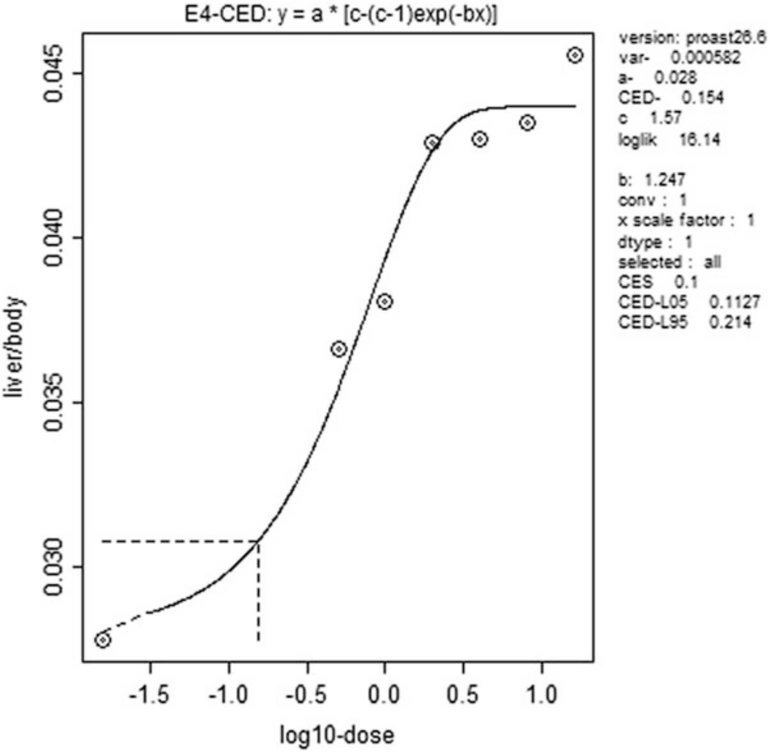

Fig. 26.3 Dose-response curves of the increased relative weight of liver against PCB doses. Exposure dose (x-axis) is on a logarithmic scale. CES for this endpoint was set at 10 % (*horizontal dotted line*), while a corresponding CED is represented by the *vertical dotted line*; L05 and L95 represent the lower and upper bound of 90 % confidence level

26.4 Discussion

Common way of analyzing dose-response data collected from animal studies is to statistically test each dose group against control groups for significant difference and to determine NOAEL. Due to serious objections against this approach, Crump [8] introduced the BMD concept as an alternative to the NOAEL approach. This approach is currently gaining more and more attention and acceptance in the process of risk assessment of various chemicals. The scientific committee of European Food Safety Authority (EFSA) recommended this approach for deriving reference points for risk assessment claiming that BMD approach is scientifically more advanced approach than NOAEL/LOAEL approach [10]. Moreover, BMD approach is supported by the REACH legislation guidance document [9]. It is scientifically accepted that BMD approach is applicable to all toxicological effects estimating the shape of the overall dose-response relationship for a particular endpoint.

In this study, Aroclor 1254 (known to produce the most prominent effects on liver) was applied to adult rats in 28 days gavage study. Benchmark dose approach was appropriate since all doses in a study were associated with a significant adverse response and therefore no NOAEL could be estimated.

Relative liver weight was significantly higher in all treated animals when compared to controls. Literature data confirm increased absolute and relative liver weights in rats treated either with PCBs or with single congener. Thus, in rats fed with estimated dose of 1 mg/kg/day Aroclor 1254 for 4 days relative liver weight and serum total cholesterol were increased while no changes were observed in rats fed with estimated dose of 0.5 mg/kg [6]. The lowest reported hepatic effect level (microsomal enzyme induction) was 0.03 mg/kg in orally treated rats with Aroclor 1242, 1248, 1254, or 1260 through food for 4 weeks, while increased relative liver weight was determined at the dose of 2.5 mg/kg [14]. Andrews [2] found increased liver-to-body weight ratio in rats intragastrically treated with 10 or 25 mg PCBs/kg during 10 weeks. Dietary ingestion of 0.25 mg/kg Aroclor 1254 for 35 days induced hepatic microsomal enzymes in rats, while increased liver weight occurred only at a higher dose of 1.25 mg/kg [5]. No changes in liver weight were found in rats exposed to dietary dose of 0.033 mg/kg Aroclor 1242 for 30 days [7]. However, recent investigations carried out on bank voles treated orally with 10 and 50 mg/kg Aroclor 1254 for 12 weeks showed significant increase in liver weight [22]. Treatment with single congener also affects liver weight. Dose dependent increase in liver weight was observed in male rats receiving doses higher than 300 mg PCB 180/kg by gavage for 28 days [17]. However, the results of this study indicate that even the lowest applied dose of 0.5 mg PCBs/kg can induce changes in relative liver weight. Furthermore, hepatic NOAEL values obtained in previously conducted studies, i.e. NOAEL of 1 mg/kg during 5 weeks in study on rats [2] and NOAEL of 0.25 mg/kg during 35 days in study on rats [5] were higher than CED_{10} value obtained in this study. The results of BMD analysis showed that the dose of 0.1127 mg PCBs/kg Aroclor 1254 produce the response in relative liver weight that is likely (95 %-confidence) to be smaller than 10 %.

26.5 Conclusion

Based on performed BMD analysis it can be concluded that relative liver weight is a sensitive parameter for PCBs exposure, since relatively low doses produced effect on liver weight causing liver hypertrophy in a dose-response manner. The CED_{10} value obtained for this effect suggests that BMD approach can be used for determining critical levels of change in the effect pattern that may later serve as a basis for risk assessment. Further investigations that would determine possible histopathological changes in liver caused by these doses of PCBs are needed.

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Chapter 27
Analysis of Organochlorine Pesticides in Black Sea Sediments

Simona Dobrinas and Adriana Culea

Abstract A study was conducted to investigate the pesticides residues in sediments collected from different Constanta harbour berths Black Sea water. Determination of these pollutants is carried out in order to evaluate the pollution potentials and hazard in Black Sea sediments. An Agilent gas chromatograph equipped with an electron capture detector (GC-μECD) was used for organochlorine pesticides (OCP's) analysis from sediments samples, after extraction and clean-up step. The OCP's total concentration (sum of 11 compounds) is generally quite low (in order of μg/kg).

Keywords OCPs • Sediments • GC-μECD • Black Sea

27.1 Introduction

According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most dangerous and persistent organic chemicals are pesticides [13].

Owing to their high capacities for bioaccumulation and toxicities in organisms, these compounds pose threats to ecosystems and human health [6]. Pesticides have great affinity for fatty molecules what makes it easier to be accumulated in the fat tissue of organisms; as a consequence they can be biomagnify along the food chain, where its degradation products are more toxic or permanent than the original compound [7]. In fish, the tissue accumulation grade in males is proportional to the age [12] and its trophic level [9].

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Their intensive use throughout the agricultural world for crop protection in the past has led to their ubiquity in the environment [5].

Residues of OCPs continue to detect in many areas. OCPs can be introduced into the coastal environment by a variety of processes, such as discharge of domestic sewage and industrial wastewater, runoff from non-point sources, agricultural inputs, and atmospheric deposition. The marine sediment is one of the most important reservoirs of these contaminants. Because of their low water solubility, OCPs have a strong affinity for suspended particulates and subsequently settle down to sediments. Marine sediments are thought to be one of their major sinks [14]. Therefore, the investigation of distribution of OCPs in sediments can provide a valuable record of contamination in the aquatic environments [3].

The Black Sea is the world's largest land-locked sea and has an area of $4.2 \cdot 105\ \mathrm{km}^2$ with average depth of 1,240 m, respectively. Ninety percent of its water mass is anoxic, thus it contains the world's largest anoxic water mass [8].

The aim of this study is to determine the concentration of organochlorine pesticides in sediments collected from different berths of Black Sea port of Constanta. Determination of these pollutants is carried out in order to evaluate the pollution potentials and hazard in Black Sea sediments.

27.2 Experimental

27.2.1 Sampling

Different sediments samples were purchased from the berths of Black Sea port of Constanta.

Constanţa harbour is both a maritime and a river port. Daily, more than 200 river vessels are in the port for cargo loading or unloading or waiting to be operated. It has a handling capacity of 100,000,000 tons per year and 156 berths, of which 140 berths are operational. Is importing crude oil, diesel, gas and exporting refined products and chemical products, iron ore, bauxite, coal and coke.

Surface sediment samples were taken with a grab sampler in November of 2009. The top 1-cm layers were carefully removed with a stainless steel spoon for subsequent analysis. All the samples were packed in aluminum boxes and immediately stored at 20 °C until required.

27.2.2 Reagents and Solutions

Standards of pesticides: 2,4,5,6 tertrachlor-m xilen, α-HCB, ß-HCB, Heptachlor, Endosulfan I, Dieldrin, Endrin, 4,4-DDD, 4,4-DDT, Methoxychlor, 2,10-Chlorphenyl were supplied by International Atomic Energy Agency, Monaco laboratory.

Florisil was assayed for preconcentration step as sorbent material of variable polarities. It (60–100 mesh) was obtained from Fluka (packed in Switzerland) and was activated overnight (12 h) at 130 °C before use. Anhydrous sodium sulphate (granulated for residue analysis – Merck) was activated at 200 °C for 2 h before use. As eluents two organic solvents there were used: n-hexane, supplied by Merck, Darmstadt, Germany and dichloromethane supplied by J.T. Baker.

27.2.3 Extraction and Clean-Up

Each sediment sample was placed into a homogenizer jar and mixed with anhydrous sodium sulphate in an amount three times greater than the weight sample. The homogenized subsamples were freeze-dried and OCPs were extracted with Soxhlet apparatus. Approximately, 8 g sediment was placed into a thimble filter. The Soxhlet extraction used takes 8 ± 0.5 h with hexane (250 ± 10 mL) as solvent.

The extracts were then preconcentrated to a 15 ± 2 mL volumes on a vacuum using a rotary evaporator. The extract was further treated with activated copper powder to remove sulfur compounds. A Florisil column chromatography was used to clean-up the extracts. A home-made glass column containing a piece of glass wool on a glass frit was filled with 5 g of activated Florisil and about 1 g of anhydrous sodium sulfate was added on the top. The organochlorine pesticides were eluted with hexane: dichlormethane (3:1) mixture.

The elutes were concentrated to about 1–2 mL on a rotary evaporator and then transferred to 10 mL glass tubes with small amounts of hexane. The solvent in the glass tube was entirely evaporated under a gentle stream of nitrogen and the precipitates were redissolved in 1 mL hexane. The extracts were analyzed with an Agilent gas chromatograph (model 6890 HP) equipped with an electron capture detector (GC-µECD). The internal standard used was 2,4,5, trichlorobiphenile (10 ng/µL).

27.2.4 Instrumental Analysis

Gas chromatography was performed using a Agilent 6890 gas chromatograph equipped with a electron capture detector and a capillary column (HP-5 30 × 0.32 mm × 0.25 µm) using helium carrier gas at a flow rate of 1.5 mL/min. The oven temperature programmed was: initial temperature isothermal, at 60 °C, for 1 min, then from 60 to 300 °C at 20 °C/min, for 10 min. Injector and detector temperatures were 250 °C, respectively 300 °C. The injection was carried out splitless and the injection volume was 1 µL. Quantification of the pesticides was performed by comparing the peak areas to that of a calibration curve of standards.

27.3 Results and Discussion

The residual concentrations of the organochlorine pesticides in surface sediments are summarized in Tables 27.1 and 27.2. OCP's total concentration (sum of 11 compounds) in sediments collected in the Black Sea is generally quite low (in order of µg/kg).

As shown in Tables 27.1 and 27.2, the total concentration of organochlorine pesticides in surface sediments revealed a wide range of fluctuation, from 0.0212 to 2.7200 µg/kg dry weight with a mean concentration of 0.5254 µg/kg.

The highest concentration was found in sediments from berth 112 (1.9 µg/kg – 2,10 chlorophenyl) while the lowest concentration (0.0002 µg/kg) was found in sediments collected from berth RR3 and berth 122 for dieldrin, respectively for α-HCB. The detected OCPs in sediments of the Black Sea are within the limits permitted by the National Environment Agency (0.01 mg/kg) [10]. DDD which

Table 27.1 Concentrations of organochlorine pesticides in surface sediment (µg/kg) of Constanta harbour

	Berth	Berth	Berth	Berth	Berth
Analyte	61	68	111	112	113
Tetrachloro-*m*-xylene	0.060	0.070	0.006	0.005	0.060
α-HCB	0.100	0.003	0.0003	0.002	0.020
ß-HCB	0.010	0.007	0.002	0.003	0.030
Heptachlor	0.050	0.009	0.004	0.002	0.060
Endosulfan	–	0.002	0.006	0.0004	–
Dieldrin	–	0.004	0.002	0.0002	0.050
Endrin	0.050	0.002	0.001	0.001	0.100
p,p′-DDD	0.180	0.007	–	0.005	0.200
p,p′-DDT	0.070	0.004	0.002	0.001	0.300
Methoxychlor	–	0.013	0.010	0.020	–
2,10-clorophenyl	1.460	0.023	0.006	0.003	1.900

Table 27.2 Concentrations of organochlorine pesticides in surface sediment (µg/kg) of Constanta harbour

	Berth	Berth	Berth	Berth	Berth
Analyte	114	122	RR3	RR4	RR5
Tetrachloro-*m*-xylene	0.030	0.050	0.003	0.0006	0.004
α-HCB	0.006	0.005	0.0009	0.0002	0.0008
ß-HCB	0.001	0.008	0.002	0.0004	0.001
Heptachlor	0.010	0.020	0.001	0.0007	0.002
Endosulfan	0.0008	0.0004	–	–	0.001
Dieldrin	0.010	0.008	0.0008	0.0006	0.001
Endrin	0.004	0.003	0.0006	0.001	0.002
p,p′-DDD	0.002	0.0007	0.0009	0.002	0.002
p,p′-DDT	0.0007	0.002	0.001	0.004	0.020
Methoxychlor	0.020	0.003	0.009	–	0.040
2,10-clorophenyl	0.007	0.004	0.002	0.020	0.009

ranged from 0.0007 to 0.200 μg/kg dw was detected in 90 % of samples. Concentrations of DDD and DDT in Black Sea sediments were comparable to those of the Baltic Sea sediments [11], Black Sea sediments from Rusia [4] and lower than those encountered in Alexandria harbor, Egypt [2]. The DDT compounds in sediments may be mainly derived from DDT-treated aged and weathered agricultural sources [1].

27.4 Conclusions

Recorded values of OCPs were not especially high in comparison to levels reported from sediments of other coastal environments, but their presence indicates a significant degree of pollution and permits the identification of principal contamination sources. Based on our experimental findings, it should be concluded that the environmental actuality of Black Sea is still contaminated by some pollutants. It is also necessary to monitor their distribution in the atmosphere, sediment and marine mammals, and to evaluate their long-term harmful effects to marine organisms in the Black Sea.

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Chapter 28
Pesticides and Residues in Different Media with Especial Regard to Waters in Hungary

Zsuzsanna Dávidovits, Ágnes Sebestyén, and Gyula Dura

Abstract Agriculture practices using chemicals endanger soils and drinking water supplies worldwide. But, without chemicals, most agricultural systems cannot meet the requirements of our modern world. In soils, pesticide residues accumulate due to soil water retaining capacity. But after a time, pesticides eventually appear in surface waters and groundwater (Solymosi, Definition of concentration of pesticide agents with different SPE methods out of groundwater and surface water, PhD thesis, Veszprém, 2006). Regular water quality monitoring and the reduction of pesticide use are recommended in river catchment areas, and especially in the catchment of those surface and subsurface waters, which are used as source water for drinking water supplies. Low level contamination of drinking water sources may occur, especially in intensive agricultural areas, even though there is regular water quality monitoring, and occasionally it can contaminate the finished drinking water as well (Vágvölgyi, Introduction to HU-SRB calls and support system: an example of the BIOXEN project, Hungary – Serbia IPA Cross – border Co – operation programme: Bioxen, Development of xenobiotic – degrading bioaugmentation products, http://www2.sci.u-szeged.hu/microbiology/BIOXEN/indexhu.html, 2011). Our aim was to give a comprehensive picture on the average yearly occurrence of pesticides and pesticide degradation products in Hungarian drinking water supplies. Data of 3 years were analyzed, using the Hungarian Drinking Water Quality Database of National Institute of Environmental Health (NIEH). In 2008–2010, up to 2 % of all drinking water samples analyzed for pesticides per year were above the limit values. The main limitation of the monitoring and data interpretation is the low number of pesticides measurements, a large proportion (about 89 %) of the water supply zones were not analyzed for pesticides in this period.

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Keywords Drinking water • Pesticides • Database

28.1 Introduction

Pesticides are substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest [2]. ‘Pesticides’ include: organic insecticides, organic herbicides, organic fungicides, organic nematocides, organic acaricides, organic algicides, organic rodenticides, organic slimicides, related products (*inter alia*, growth regulators) [5].

Agriculture practices using chemicals endanger soils and drinking water supplies worldwide. But most agricultural systems cannot meet the current requirements on the volume and stability of yield without the use of chemicals. So there are two important aspects of agriculture, which need to be balanced: the amount of the chemicals used should meet the amount what is necessary to raise the crop and maintain its health, but should not exceed it. To calculate the trade-off, regional and nationwide water quality measurements, mass transport studies, simulation models and applied mathematical methods are needed [7].

The currently applied pest control methods do not restrict the effect of the pesticides to the place of application, or the desire action range. Even the most carefully designed treatments have some side effects, i.e. environmental pollution of soil, surface water and groundwater.

In soils, pesticide residues accumulate for a time due to the soil’s water retaining capacity. But pesticides are eventually transported through various transportation pathways and make their appearance in surface water and groundwater (Fig. 28.1).

Pesticide pollutions can originate from various sources. First, there are the negative effects of intentional pesticide usage (e.g. spraying against aquatic insects or weeds). Agricultural pesticides can contaminate waters through leaching or runoff. Furthermore, there are the direct or indirect pollution of industrial sewage or discarded liquid agents. Havaria incident during pesticide production, packaging, storage or transport can also contribute to environmental pollution. Surface water and groundwater pollution should be prevented to the greatest possible extent, because its implications on human health and aquatic ecosystems, both directly (via water toxicity) and indirectly (via the food chain). Some pesticides (and/or the pesticide degradation products) generate taste and odour changes in the water, even at very low concentration. These changes can make the polluted water unacceptable for drinking. Furthermore, some pesticides (and/or the pesticides’ degradation products) are toxic to non-target organisms, like phytoplankton and other aquatic invertebrates [8].

Potential carcinogenic and mutagenic effects, and the impacts on fetal development and on the immune and hormonal system of certain pesticides are becoming more precisely understood. Pesticides are known to reduce biodiversity, thus degrading the quality of soils. Nitrogen fixation and the decomposition of organic materials are getting slower as a consequence. Through the food chain (consumption of polluted produce or drinking water) – directly or indirectly –humans are also affected. Since the human population is exposed to pesticide residues through the

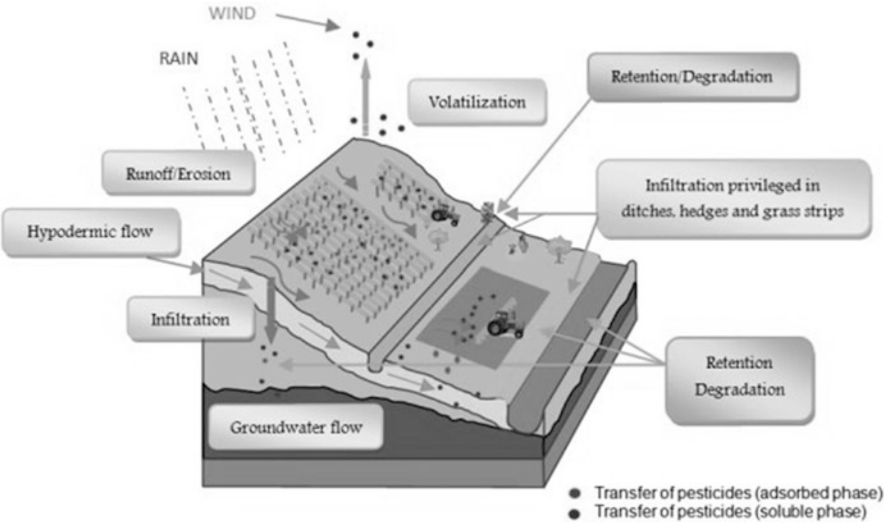

Fig. 28.1 Main pesticides transfers at the catchment area scale (http://coursenligne.u-strasbg.fr/pages.jsp?idTheme=6424&idsite=630&idRub=1275&rubSel=1275)

above pathways, to protect human health, optimal microbial degradation of pesticides in the soil should be ensured. With the help of pesticides degrading microorganisms in the soil ecosystem, soils can in time be cleared from pesticide residues and even ecological (or organic) cultivation can be possible [8].

To prevent the above detailed negative health or environmental outcomes, regular water quality monitoring and the reduction of pesticide usage are recommended in river catchment areas, especially in the catchment of those surface and subsurface waters, which are used as a source of drinking water supply. Low level contamination of drinking water sources may occur in heavily used agricultural areas despite the regular water quality monitoring [9]. Occasionally pollution can affect the finished drinking water as well. Aim of the present study was to assess the prevalence of pesticide pollution in the drinking water supplies of Hungary by data mining of the National Drinking Water Quality Database.

28.2 Health Effects of Some in Hungary Typical Occurring Pesticide Agents

Table 28.1 shows the hazard information and the main properties of the most frequently measured agents.

Aldrin and dieldrin are chlorinated pesticides that were used against soil-dwelling pests and for wood protection. Dieldrin was used against insects of public health importance. The use of dieldrin is restricted or banned in many countries

Table 28.1 The main hazard information about the most frequently measured pesticides

Agent	Hazard information[a]	Properties
Atrazine	Unclear	Triazin herbicide. Prohibited in most of EU members is prohibited, carcinogen. In Hungary there is "essential consent for use" for this agent[b]
Propachlor	Slightly hazardous	It obstructs the protein synthesis, the growth of root will be damaged
Metolachlor	Slightly hazardous	It obstructs the protein synthesis, the growth of root will be damaged
Trichlorfon	Slightly hazardous	Organic phosphoric acid ester. It is used for exterminate house flies, tick. It dissolves rapidly in water
Carbaryl	Moderately hazardous	It is used for worm extermination and plant thinning

[a] Classification of pesticides based on their hazard (*LD50* (mg/kg) *of rats*)

I. a. Extremely hazardous	III. Slightly hazardous
I. b. Highly hazardous	IV. Unlikely to present acute hazard hazardous

[b] If there is not alternative, less harmful agent against a pest, the carcinogen, hormone-disrupting agent can be restricted allowed in a restricted way

since the early 1970s. Aldrin transforms rapidly to dieldrin in the environment and in the body. Dieldrin is a persistent and has low mobility in soil. Aldrin and dieldrin are listed as Persistent Organic Pollutants. They have multiple toxic mechanisms, both are toxic to experimental animals, there is evidence on human healths effects from case studies. The target organs are the nervous system and the liver. Aldrin and dieldrin have been classified to Group 3 by IARC. Guideline value in drinking water is 0.03 μg/l [10].

Atrazine is used as a selective systemic herbicide to control annual weeds. According to current classification, it is unlikely that atrazine has genotoxic or carcinogenic risk to humans. It has reproductive toxicity in experimental animals. Atrazine was observed to attenuate the luteinizing hormone surge and disrupt the estrous cycle; teratogenic effect was not detected Its chloro-*s*- triazine metabolites have the same toxicity profile. The main effect of hydroxyatrazine was kidney toxicity; there was not evidence for carcinogenicity, neuroendocrine-disruption, or genotoxicity of these metabolites. The WHO guide values in drinking water are 100 μg/l for atrazine and its chloro-s-triazine metaboiltes, 200 μg/l for hydroxyatrazine metabolites [10].

Carbaryl is a broad-spectrum carbamate insecticide. Its primary mode of toxicity is the inhibition of brain cholinesterase. In experimental animals (male mice) it caused vascular tumors. The health-based guide value is 50 μg/l [10].

Metolachlor is a selective pre-emergence herbicide. It is fairly mobile in soil, so under certain conditions it can contaminate groundwater. In animal experiments, the effects were decreasing kidney weight, body weight and survival in female mice, additionally increasing of liver tumors in female mice and a few nasal tumors in male mice. There is no evidence for carcinogenicity and genotoxicity. The WHO guideline value is 10 μg/l [10].

28.3 Regulation of Pesticides in Water in Hungary

1. Directive 2000/60/EC (Water Framework Directive): extends to all of human activity that have negatively affected the status of waters, obstruct to approach or save them. Thirty three chemicals including 13 agents of pesticides to be monitored are listed in this directive that are particular hazard to the status of the waters [3].
2. Government Decree No. 220/2004. (VII.21.) on protection of surface water quality standards: this decree regulates the quality of surface water, the list of indicative water pollutants and the hazard substances for the surface water quality, including a few pesticide agents and their decomposition products. In Annex 1 part C are required the agents their emission is illicit: hexachloro cyclohexane (HCH), aldrin, dieldrin, endrin, isodrin, DDT, pentachlorophenol [4].
3. Ministry Decree No. 21/2002. (IV. 25.) on operation of water utilities: this decree requires the list of measured parameters in water wells. The measured pesticide agents: acetochlor, propachlor, 2,4-D, atrazine, lindane, malation, MCPA, metil-paration, simazine, 2,4,5-T, DDT/DDD/DDE, dezetil-atrazin, diazinon, hexaklórbenzol, dezipropil atrazine*, endrin*, phorat*, hexazinone*, chlorpiriphos*, metribuzin*, prometrin*, propazin*, terbutilazin*, terbutrin* (* = based on local pesticide using and the list of Public Health Office) [6].
4. 98/83/EC of 3 November 1998 (Drinking Water Directive) on the quality of water intended for human consumption: this directive regulates the limit values, the monitoring and reporting systems of the drinking water quality for the members of European Union [1].
5. Government Decree No. 201/2001. (X.25.) on the quality standards and monitoring of drinking waters. This Decree implements the Drinking Water Directive in Hungarian legislation. The Decree regulates the limit values of pesticides in drinking water. The limit value applied to each individual pesticide concentration is 0.1 μg/l, and to total pesticides concentration is 0.5 μg/l. More strict limit value applies to aldrin, dieldrin, heptachlor and heptachlor epoxide (0.03 μg/l) [5].

28.4 Methods

Data mining in the Hungarian Drinking Water Quality Database was used to analyze the presence of pesticides in Hungarian drinking water. The Database compiles the results of drinking water compliance monitoring (including physical, microbiological, biological, organic and inorganic chemical parameters) in Hungary based on reports of local public health services and waterworks. Microsoft Office Excel 2003 and Microsoft Office Access 2003 programs were used during the analysis.

28.5 Results and Discussion

According to the Hungarian Drinking Water Quality Database, in 2008 there were 531 pesticide measurement in drinking water, seven samples were above the limit value, so the non-compliance was 1.32 %. In 2009 were 396 samples were analyzed for pesticides, none of them was above the limit value. Nine of the 414 pesticide measurements in drinking water in 2010 exceeded the limit value, which is 2.17 % non-compliance (Table 28.2). All samples were taken at point of use.

The total number of measurements was geographically very variable in the different Hungarian counties and the capital. Number of measurements was above average in Pest and Szabolcs-Szatmár-Bereg counties, and very low in Bács-Kiskun, Győr-Moson-Sopron and Vas counties in the analyzed 3 years (Fig. 28.2). There is not sampling and measurement of drinking water for pesticides content in the most of water supply zones (87 % in 2008, 90 % in 2009 and 92 % in 2010, 79 % none of these years).

The scope of pesticide measurements in drinking water samples depends on the local pesticides usage. The list of examined pesticides is defined annually by the

Table 28.2 Total pesticides content in tap water samples and percentage of non-compliance with requirements (2008–2010) based on database of NIEH

	2008	2009	2010
Total amount of samples	531	396	414
Amount of non-compliance	7 (1.32 %)	0	9 (2.17 %)
The most frequently detected of pesticides in drinking water	Atrazine Propachlor Metolachlor Endosulfan		Metolachlor Propachlor Carbaryl Propanil Trichlorfon

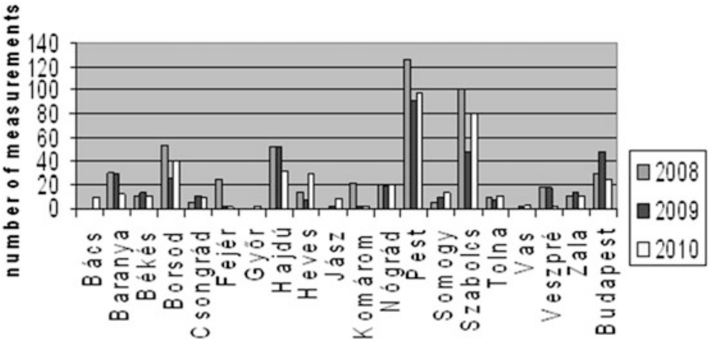

Fig. 28.2 Geographical distribution of pesticide measurements in drinking water by counties and the capital (2008–2010)

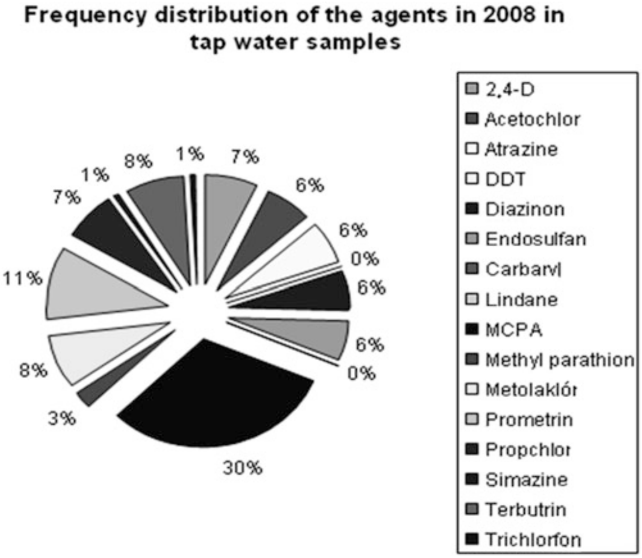

Fig. 28.3 Frequency of pesticide measurements in drinking water in 2008

Public Health Office, based on local information from environmental inspectorates, public health services and plant protection stations [5] (X.25.).

Figures 28.3, 28.4, and 28.5 present the frequency of measurements for the various pesticides in drinking water in 2008, 2009 and 2010.

28.6 Discussion

The presence of pesticides Hungarian drinking water supplies was observed in the 2008–2010 period, though the frequency was low. The non-compliance was 1.32 and 2.17 % in 2008 and 2010, respectively; there was no non-compliance in 2009. Overall non-compliance for 2008–2010 is 1.16 %. The total number of results is low, does not meet the required number of samples, and there is not sampling and measurement of drinking water for pesticides content in the most of water supply zones (about 89 %). The maximum concentration of the most frequently detected pesticide in drinking water in the 3 year period was 10–16 times higher than the limit value (Table 28.3). Only carbaryl exceeded the health-based limit value of WHO [10], but it can still pose a health risk to the consumers.

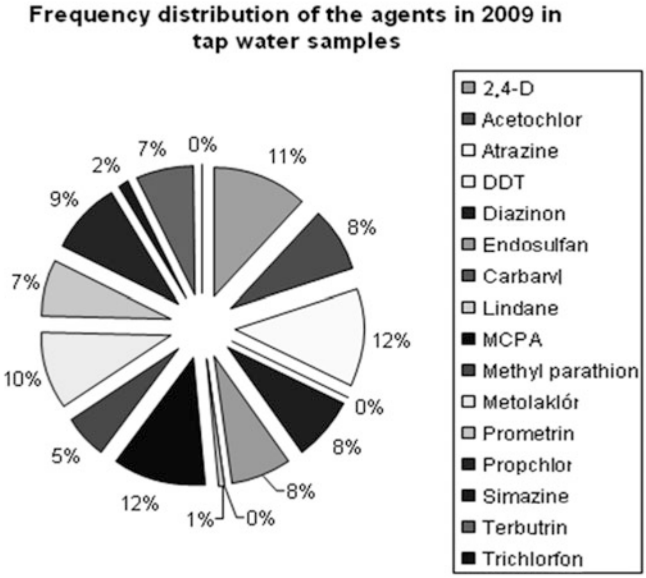

Fig. 28.4 Frequency of pesticide measurements in drinking water in 2009

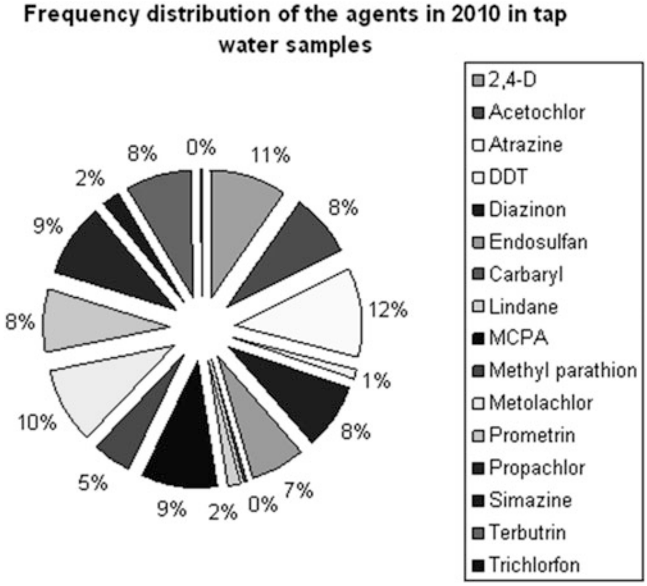

Fig. 28.5 Frequency of pesticide measurements in drinking water in 2010

Table 28.3 The maximum values of the most frequently measured pesticides' agents in drinking water period in 2008–2010

Agent	Limit value (μg/l)	Maximum value (μg/l)
Atrazine	0.10	1.3
Propachlor	0.10	1.4
Metolachlor	0.10	1.6
Trichlorfon	0.10	1.1
Carbaryl	0.10	0.18

28.7 Conclusion

The inappropriate use of pesticides can cause both environmental and human health risks. According to current monitoring results, pesticides are detected in drinking water samples in Hungary. In 2008–2010, up to 2 % of all drinking water samples analyzed for pesticides per year were above the limit values based on Hungarian Drinking Water Quality Database. There is not detected not-allowed agent examined the occurring agents. There is statistical data on the amount of purchased pesticides, but there is no compiled data on their application (dosing, application area etc.). Pesticides are often used in unreasonably high quantity, or not in the appropriate period. To conclude, more attention needs to be paid to the health effect of these chemicals and to the updating of unpermitted agents. Also, targeted detection methods need to be applied to pesticides and their degradation products. The monitoring system of pesticides content in drinking water need to be developed, minimum annual measurement is necessary in all of water supply zones.

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Chapter 29
Possible Use of Certain Mineral Raw Materials of Moldova for DDTs and HCHs Adsorption from Aqueous Solutions

Oleg Cadocinicov and Oleg Petuhov

Abstract Estimation of the possibility of use mineral raw materials for adsorption of some chlorinated pesticides (DDT and metabolites, HCH-isomers) from "spiked" water solutions was the aim of this study. Certain mineral raw materials, occurring at the area of Moldova (tripolites, bentonites, diatomites etc.), were used as accessible adsorbents during laboratory experiments. For that scope, the "spiked" water solution containing DDTs and HCHs were prepared. "Spiking" of water solution was fulfilled taking into account water solubility of the analytes of interest, their maximum allowed concentration in water, instrumental limit of detection and limit of quantification. During lab experiments the "spiked" solution has been mixed with the adsorbents. Then after separation the concentration of the analytes in adsorbents and water solution was analyzed. Sample preparation was carried out by means of liquid-liquid extraction, solid-phase extraction, microwave assisted extraction, while analytical determination – by gas chromatography with mass-detection. For determination of sorption parameters of mineral sorbents gas adsorption analyses has also been performed. As a result, the experimental data have been collected, proving that Moldavian mineral raw materials demonstrate adsorption efficiency regarding DDT and metabolites. Finally, tripolites showed the best adsorption efficiency amongst all studied mineral raw materials.

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Keywords Obsolete pesticides • HCH • DDT and metabolites • Water quality • Mineral adsorbents • Adsorption • Gas chromatography • Gas adsorption analysis

29.1 Introduction

Notwithstanding that usage of such insecticides, as dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) is banned in the most of parts of the world (in some parts extremely limited), these pollutants are still dangerous from environmental point of view due to their persistence and ability of bioaccumulation. In Stockholm Convention on Persistent Organic Pollutants [1] the mentioned pesticides are also present. Since Stockholm Convention different regulatory and recommendatory documents appeared which have dealt with DDT and HCH (Guidelines For Drinking-Water Quality, [4, 5]. So, the problem of presence of the mentioned pollutants in different objects of environment is still relevant.

Till 2008 the amount of obsolete pesticides in Republic of Moldova was estimated at 7,245 t. The pesticides included also HCH (α-, β- and γ-isomers) and DDT with metabolites (DDD, DDE). Due to the Project "Management and disposal of the POPs stockpiles" of Global Ecological Fund/World Bank, about 1,296 t were disposed. About 4,000 t of the pesticides are located at dump site for chemicals (southern part of the country). Remaining 1,949 t are located in 22 central warehouses (www.moldovapops.md) [11]. Notwithstanding that the repacking and evacuation works have been performed, according to the inventory of old pesticide storages in Moldova executed by Ministry of Environment and World Bank, about 1,500 of polluted sites there were found. It also should be mentioned, that more that 15 % of the sites have extra high pollution by POPs (more than 50.0 mg/kg of soil) (http://pops.mediu.gov.md/). Therefore, the risk regarding environmental pollution by the mentioned pollutants still exists. Special concern presents the pollution of water resources [7].

There are different techniques of water cleanup from various pollutants.

In process of elimination of obsolete pesticides from water solutions adsorption plays important role [12]. Earlier it was observed adsorption of some obsolete pesticides (DDT, for example) by natural organic substances and also by mineral substances [3, 8].

Techniques of water cleanup include application of natural sorbents [12, 13]. In Republic of Moldova some mineral raw materials occur, such as diatomite, bentonite, tripolite etc [6]. These minerals can be used as potential sorbents for different cleanup processes [2, 9].

This study was aimed at exploration the possibility of use the local mineral raw materials for adsorption of DDT and HCH from water solution. For that the amount of DDT and HCH that remains in the studied mineral sorbents after applying model water solution, containing these pesticides ("spiked" solution) has been estimated.

29.2 Materials and Methods

The following pesticides have been chosen as analytes of interest:

a-HCH, b-HCH, g-HCH – alpha-, beta, gamma isomers of Cyclohexane, 1,2,3,4,5,6-hexachloro- (CAS# 319-84-6, 319-85-7, 319-86-8, correspondingly);
p,p-DDE – 1,1′-(Dichloroethenylidene)bis(4-chlorobenzene)- (CAS# 72-55-9);
o,p-DDDBenzene – 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]- (CAS# 53-19-0);
p,p-DDD Benzene – 1,1′-(2,2-dichloroethylidene)bis[4-chloro- (CAS# 72-54-0)];
o,p-DDT – Benzene, 1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]- (CAS# 789-02-6); p,p-DDT – Benzene, 1,1′-(2,2,2-trichloroethylidene)bis[4-chloro- (CAS# 50-29-3)].

In Table 29.1 are presented general properties of the studied compounds

The following minerals have been employed as local mineral materials for studies: bentonite (Otaci), tripolite (Senatovka), bentonite (Prodanesti), diatomite (Molochisul Mare). The occurrence of the minerals and their chemical properties are indicated on the Fig. 29.1 and Table 29.2, respectively.

The study included the following stages (see Fig. 29.2):

(A) The deionized water with pH value 6,08 (26 °C) and electroconductivity 5 μS/cm (26 °C) was taken under investigation.
(B) “Spike” was prepared by: (a) 20 times dilution of standard solution (Supelco inc.) (b) 50 times dilution of 20 times diluted solution. Finally, 1,000 times dilution was achieved. The data on initial concentration of compounds in standard solution are presented in Table 29.3.
(C) A solution (100 μl) containing 2,4,5-trichlorobiphenyl (2,4,5-TCB) (1.0 μg per ml of acetone) as a surrogate was added to every sample prior the extraction stage . Surrogate was used to evaluate recovery of analytes after the extraction. Solution of surrogate was prepared from standard solution of 2,4,5-TCB (Supelco inc.).
(D) Liquid-liquid extraction stage included: (a) shaking the water sample in a separatory funnel with 20 ml of n-hexane during 3 min; (b) collecting n-hexane part of water-n-hexane mixture 10 min later; (c) adding a new 20-ml aliquot of n-hexane to water part and shaking during 3 min; (d) collecting n-hexane part of water-n-hexane mixture and combining with n-hexane part collected earlier; (e) evaporation of combined n-hexane extract till 1 ml under nitrogen stream.
(E) Amount of mineral raw, added to the “spiked” solution was 0.2 g for all mineral adsorbents. Besides, for samples of tripolite the correlation between adsorption efficiency and sample amount was studied. For that scope the samples were prepared, containing 0.2, 0.4, 0.8, 1.5 and 2.0 g of tripolite.
(F) For all studied minerals shaking was performed during 1 day and settling – during one night. Besides, for samples of tripolite the correlation between adsorption efficiency and shaking/settling time was studied. With this aim,

Table 29.1 Properties of the studied compounds[a]

	HCH				DDD		DDT	
	a-	b-	g-	p,p-DDE	o,p-	p,p-	o,p-	p,p-
Molecular weight, g/mol	290.83	290.83	290.83	318.03	320.05	320.05	354.49	354.49
Solubility in water, (20–25 °C), mg/L	2	0.542	5.75	0.12	0.1	0.09	0.085	0.025
Vapor pressure (25 °C), mHG	$4.26 \cdot 10^{-5}$	$4.9 \cdot 10^{-7}$	$3.72 \cdot 10^{-5}$	$6.0 \cdot 10^{-6}$	$1.94 \cdot 10^{-6}$ (30 °C)	$1.35 \cdot 10^{-6}$	$1.1 \cdot 10^{-7}$ (20 °C)	$1.60 \cdot 10^{-7}$
Sorption coefficient, Koc	3,12	3,14	3,04	4,70	5,19	5,18	5,35	5,18
Diffusion coefficient in water, cm^2/s	$7.34 \cdot 10^{-6}$	$7.34 \cdot 10^{-6}$	$7.34 \cdot 10^{-6}$	$5.87 \cdot 10^{-6}$	$4.7 \cdot 10^{-6}$	$4.76 \cdot 10^{-6}$	$4.95 \cdot 10^{-6}$	$4.95 \cdot 10^{-6}$
Maximum admissible concentration for drinking water, mg/l	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	Total pesticides – 0.0005							

[a] http://www.gsi-net.com/en/publications/gsi-chemical-database

http://www.atsdr.cdc.gov/toxprofiles/tp35.pdf

Council Directive 98/83/EC on the quality of water intended for human consumption

Fig. 29.1 Occurrences of local mineral raw materials used for the studies. *1*.bentonite (Otaci); *2*.tripolite (Senatovka); *3*.bentonite (Prodanesti); *4*.diatomite (Molochisul Mare)

Table 29.2 Chemical properties of the studied mineral raw materials

	Components, %									
Mineral raw	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	CaO	MgO	R_xO	SO_3	LOI	LOD
1	54.20	23.24	2.58	0.32	0.50	2.26	5.36	–	–	–
2	78.42	4.24	3.18	–	2.04	0.43	0.28	0.16	8.64	2.42
3	51.84	15.59	2.06	0.40	2.92	4.12	1.03	0.04	22.1	–
4	80.20	4.46	2.80	–	2.08	0.75	0.37	0.70	6.12	2.50

for a part of the samples the shaking/settling time constituted 7 days. The pH values of water solution after adding of 0.2 g of mineral adsorbents were also measured. As a result, the following pH values have been obtained (at 26 °C): tripolite (Senatovka) – 6.78, bentonite (Otaci) – 6.84, bentonite (Prodanesti) – 6.71, diatomite (Molochisul Mare) – 6.57.

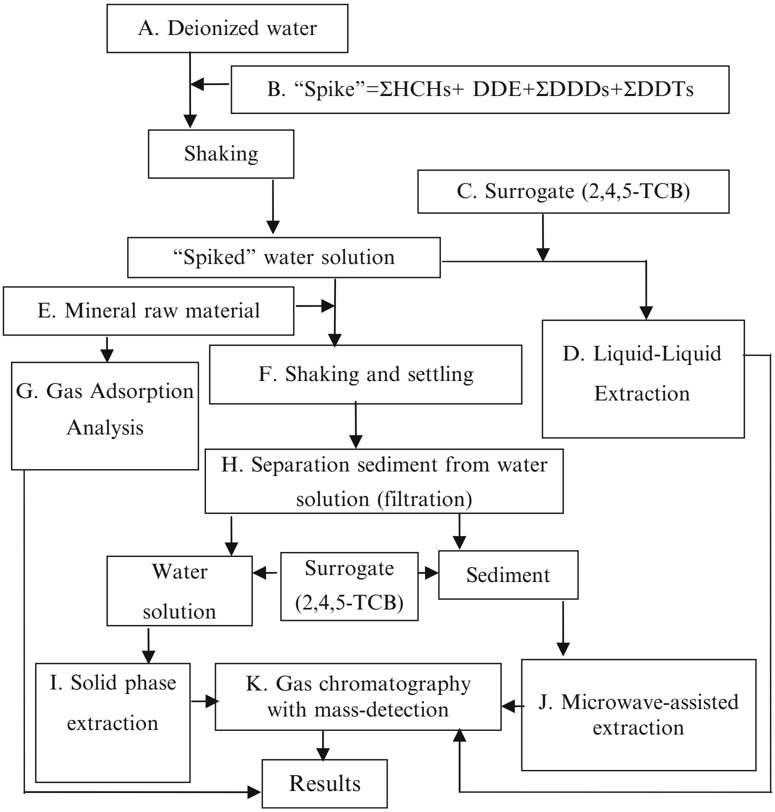

Fig. 29.2 The experiment scheme

Table 29.3 Initial concentration of compounds in standard solution

Compound name	Concentration, μg/ml
a-HCH	25.02
b-HCH	99.9
g-HCH	25.01
p,p-DDE	100.2
o,p-DDD	200
p,pDDD	190
o,pDDT	225
p,pDDT	259.8

(G) Gas adsorption analyses have been performed to determine specific surface areas and pore distribution of the studied minerals. Data were obtained from the physical adsorption of N_2 at 77 K by using automatic apparatus (Autosorb 1-MP) after out-gassing the samples at 105 °C to a residual vacuum of 10^{-5} Pa. The surface areas were calculated using the BET equations. Pore size distributions (PSDs) were calculated using non-local density functional theory (NLDFT) method assuming slit pore geometry. As a result, the following

Table 29.4 Parameters of SIM mode, used for quantitative determination of analytes

Compound	Ion mass, used for SIM mode
a-HCH	219
b-HCH	217
g-HCH	217
2,4,5-TCB	256
p,p-DDE	235 316
o,p-DDD	235 316
p,pDDD	235 316
o,pDDT	235 316
p,pDDT	235 352

parameters have been determined: surface area (S_{BET}), constant BET (C_{BET}), total pore volume (V_S), micropore volume (V_{mi}), adsorption energy (E_a) and pore radius (r).

(H) Filter paper with pore size 1–2,5 nm has been used for filtration stage.

(I) Solid phase extraction stage [10] included: (a) washing solid-phase extraction disk (C-18 disk from Supelco inc.) with 5 ml methylene chloride and drying under vacuum; (b) conditioning the disk with 5 ml of methanol, adding 20-ml aliquot of deionized water, then adding the sample; (c) passing the whole sample through C-18 disk, drying the disk under vacuum; (d) flushing the disk first with 15-ml of acetone, then with 15-ml of methylene chloride and collecting the extract; (e) adding anhydrous sodium sulfate to the extract for the elimination of water residues; (f) quantitative transfer of the extract to the concentration tube and evaporation till 1 ml under nitrogen stream.

(J) Microwave-assisted extraction stage included: (a) heating of the sample with 30-ml aliquot of acetone-*n*-hexane mixture (1:1) in high-pressure vessel under the influence of microwave radiation (power level 400 W) during 15 min; (b) quantitative transfer of the extract to the concentration tube and evaporation till 1 ml under nitrogen stream.

(K) Gas-chromatographic analyses were fulfilled using gas chromatograph Agilent 6890 with 5973N mass-detector (Environmental Monitoring System Laboratory Method 525, [13]) Quantitative determination of analytes has been performed by using Select Ion Monitoring (SIM) mode. Working parameters of SIM-mode are listed in Table 29.4.

For quantitative analysis three calibration solutions there were prepared, containing the analytes in the following ratio with regard to the "spike": 4 times less than "spike", 2 times less than "spike", "spike" and 2 times more than "spike". As a result the calibration curves with correlation coefficients from 0.988 (for α-HCH) till 0.999 (for o,p-DDT) were obtained.

Operational conditions for gas chromatographic analysis are presented below (see Table 29.5).

Reagents employed in our studies were of HPLC-grade of purity. All the used glassware was priory washed four times with aliquots of methylene chloride. During the whole experiment for all the samples three replicates there were prepared and blank samples, as well.

Table 29.5 Operational conditions for gas chromatographic analysis

Sample injection volume	2 μl
Inlet (type, ratio, temperature (°C))	Split/Splitless, 1:5, 300
Flow/pressure mode	Constant flow (1.3 ml/min), pressure 11.6 psi
Column	HP-5MS
(type, length, internal diameter, thickness of phase)	25 m × 250 μm × 0.25 μm
Carrier Gas,	Helium
Flow (ml/min)	(1.5 ml/min)
Oven programme	100 °C 1 min hold 20 °C/min till 200 3 min hold 10 °C/min till 280 5 min hold

Table 29.6 Concentration of analytes in the "spiked" solution

Analyte	Concentration in "spiked" model solution, μg/L	Recovery, %
a-HCH	0.023 ± 0.00016	90.75 ± 0.65
b-HCH	0.098 ± 0.00050	98.17 ± 0.50
g-HCH	0.027 ± 0.0013	108.55 ± 5.01
2,4,5-TCB	0.097 ± 0.00067	96.97 ± 0.67
pp-DDE	0.088 ± 0.0014	88.14 ± 1.45
op-DDD	0.20 ± 0.00052	98.36 ± 0.26
pp-DDD	0.18 ± 0.0031	94.73 ± 1.65
op-DDT	0.22 ± 0.00069	96.43 ± 0.30
pp-DDT	0.22 ± 0.0010	85.09 ± 0.41

29.3 Results and Discussions

In Table 29.6 the results of analysis of the "spiked" model solution are presented.

As it can be seen from the Table 29.6, the analytes in "spiked" model solution have concentration which does not exceed the solubility of analytes in water, being higher than maximum admissible concentration for drinking water (see Table 29.2).

Data on specific surface area, pore distribution and other parameters of the studied minerals, determined by gas adsorption analysis are presented in Table 29.7.

Results of analysis of the studied minerals after interaction with the "spiked" solution are presented in Fig. 29.3.

According to the data given in Fig. 29.3, one can conclude that the studied minerals demonstrate adsorption activity only regarding the isomers of DDT and metabolites, but not for isomers of HCH. It should also be noted, that there is no very significant difference in adsorption activity between the studied materials, but tripolites (Senatovka) show a better results. This can be related to the data on specific pore volume values that were obtained for the studied materials (see Table 29.7). Taking into account the fact that tripolites have shown maximal

Table 29.7 Results of gas adsorption analysis of the studied minerals

	S_{BET}		V_S	V_{mi}	E_a	r
Mineral raw (Fig. 29.1)	m^2/g	C_{BET}	cm^3/g	cm^3/g	kJ/mol	(method BJH) Å
1	90.14	28	0.244	0	12.82	28.03
2	73.11	205	0.331	0.010	12.96	19.47
3	82.50	660	0.171	0.016	12.63	17.09
4	59.33	200	0.110	0.015	12.77	19.50

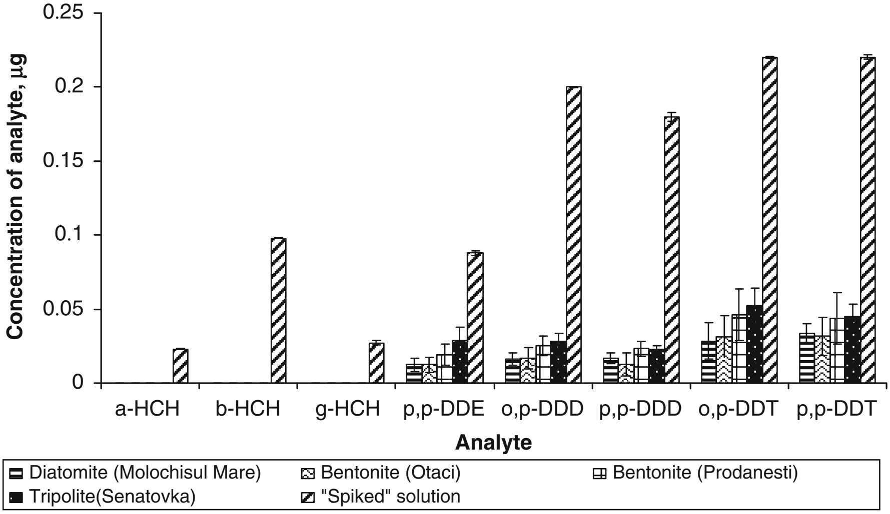

Fig. 29.3 Adsorption of analytes by the studied mineral sorbents

adsorption efficiency amongst all studied mineral raw materials; further results are related only to tripolites.

The data describing the correlation between adsorption efficiency and amount of adsorbent applied to the “spiked” solution were obtained (Fig. 29.4).

It is well seen from Fig. 29.4, that adsorption efficiency for tripolites increases with the augment of adsorbent amount, applied to the “spiked” solution, thus proving their direct correlation. But it should be mentioned, that a ten-fold increase of the sorbent amount gives no more than two, five-fold enhancement of adsorption efficiency.

Figure 29.5 depicts the correlation between contact time for adsorbent and the “spiked” solution (shaking/settling period) and adsorption efficiency.

It is obvious, that for the most cases 1 day is enough to establish the equilibrium for system “adsorbent-solution”. Therefore, there is no necessity to increase the shaking/settling period.

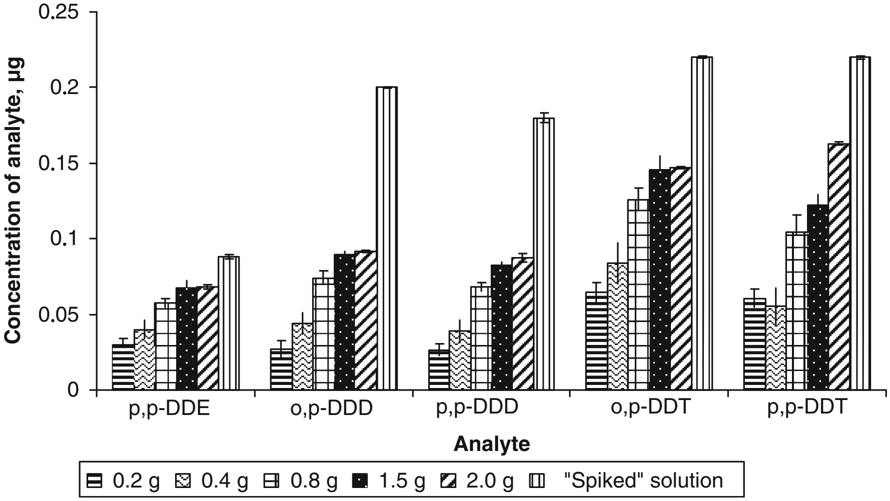

Fig. 29.4 Influence of adsorbent amount on adsorption efficiency

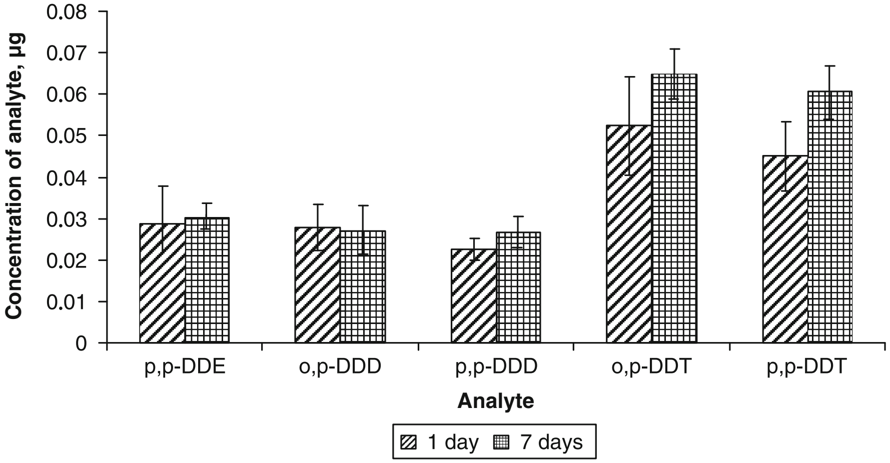

Fig. 29.5 Influence of contact time on adsorption efficiency

29.4 Conclusion

The obtained by us results have demonstrated the following:

(a) All studied mineral raw materials showed adsorption activity regarding DDT and metabolites in the "spiked" solution. No any sorption activity was noted for HCH-isomers. Though the differences between presented minerals are not significant, tripolites (Senatovka) showed the highest adsorption efficiency.

(b) Increase of the sorbent amount applied to the "spiked" solution results in augment of the sorption efficiency, but the observed correlation is not clearly linear.
(c) One day of shaking/settling time is enough for setting the equilibrium in the system "adsorbent-water solution".

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Chapter 30
Inventory and Status of Persistent Organic Pollutants – Pesticides in Croatia

Marko Vinceković, Darko Vončina, Tomislav Kos, Iva Pavlinić-Prokurica, and Darka Hamel

Abstract Persistent organic pollutants (POPs) which include some pesticides are very important environment polluters. Their harmful impact is mainly consisted of their air transmission, resistance to chemical-, photo- and bio-degradation and possibility of their accumulation in fat tissue of all living organisms. As they can be transported by air on long distances, they were found in samples on locations where they were not used. This group of pollutants includes pesticides (such as aldrin, chlordane, dieldrin, endrin, heptachlor, lindane, alpha-hexachlorocyclohexane, beta-hexachlorocyclohexane, hexachlorobenzene, mirex, toxaphene, DDT), industrial chemicals (such as polychlorinated biphenyls, PCBs) and unintentional by-products of industrial processes (such as dioxins, furans etc.). Because of their high efficacy in agriculture through the middle of twentieth century their consumption was very high. In the meantime their negative impacts appeared and their harmfulness became well known threat to the environment and to human health all over the globe. In this work an overview of inventory and status of POPs in Croatia is given. The emphasis is given on POPs pesticides. In accord with global tendencies to reduce and eliminate production, use and releases of harmful substances a gradual prohibition of pesticides (aldrin, dieldrin, hexachlorocyclohexane, DDT) usage started in Croatia 1972 and ended in 2001 with proscription of lindane and dicofol usage. Up to date all of them are banned from production and application, and no stockpiles are detected. In the period of their common usage, production of POP's pesticides in Croatia was mainly based as supplement to some fertilizers (aldrin). Today there is no need for their production, since all preparations based on POPs are now substituted with numerous toxicologically and environmentally friendlier

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active ingredients. Also there is no import and export of POPs through Croatian border, with exception for some institutions which have permission for import of small quantities used as laboratory standards for determination of residual POPs pesticides. Residues of POP's pesticides are monitored now days only in water. There is still pronounced need for adequately equipped laboratories and trained stuff for their monitoring in vegetables, animals, water, soil and human biomaterial.

Keywords Inventory • Pesticides • Persistent organic pollutants • Croatia

30.1 Introduction

Persistent Organic Pollutants (POPs) are chemical compounds which persist in the environment. All of them are to some level resistant to photo-, chemical- and biodegradation processes. They tend to be highly soluble in fat but not in water. Since they bio-accumulate in the fat of living organisms they represent a high risk to human health [2, 8]. Exposure to individual POPs has been associated with cancer, neurotoxic, behavioral, and reproductive changes. Due to their accumulation in fats they are likely to be passed to the next generations in uterus and through breast milk. They have also been found far away from places where they have been produced or used. They are able to evaporate in warm climates and can be absorbed and transported by atmospheric particles. POPs have been linked to population declines in fish and other wildlife, and also to specific disorders such as reproductive and hormone system dysfunction, eggshell thinning, metabolic changes, deformities and birth defects, cancers, behavioral changes, etc. [8]. In order to eliminate and reduce production, use and releases of POPs joined action of international community resulted in two important agreements: (i) The Protocol to the Regional UNECE Convention on Long-Range Trans boundary Air Pollution (CLRTAP) on POPs opened for signatures in June 1988 and comes into force on 23rd October 2003 and (ii) The global Stockholm Convention on POPs, opened for signatures in May 2001 and comes into force on 17th May 2004. These documents establish strict international regimes for initial lists of POPs (16 in the UNECE Protocol and 12 in the Stockholm Convention). Both documents also contain provisions for including additional chemicals into lists of prohibited compounds. Croatia has signed implemented international documents on POPs as well as 30 international conventions and agreements having similar implications. Illustration of POP's pesticides status in the Republic of Croatia is given in Table 30.1.

30.2 POPs Inventory in the Republic of Croatia

The most up to date and comprehensive overview of POPs levels and their sources in Croatia is the 2009 National Implementation Plan for the implementation of Stockholm Convention on POPs (NIP) [2]. The NIP contains a good overview of the work which has been done so far on POPs in Croatia. Unfortunately it shows

Table 30.1 List of active ingredients classified as POPs under the Stockholm Convention and the time of their prohibition in the Republic of Croatia

Active ingredient	Permitted since	Usage prohibited since
Hexachlorobenzene (HCB)	1962	11/07/1980
Toxaphene	1957	27/04/1982
Endrin	1957 (since 1971. Used as rodenticide)	29/05/1989
Aldrin	1958	1972
Dieldrin	1958	1972
Heptachlor	1956	July 1973
Hexachlorocyclohexane (HCH)[a]	1944	1972
DDT	1944	1972 (in agriculture)
Lindane	1944	July 2001
Chlordane	Data before 1995. Not known	1971 (in agriculture)
Mirex	Never allowed in the Croatian plant protection	

[a] Alpha-hexachlorocyclohexane, beta-hexachlorocyclohexane are on the list since 2009

that much of the data available is rather old or that sampling has often taken place on a one-off basis rather than consistently over time. A comprehensive monitoring of POPs in samples taken from the environment, food or people does not exist. Today, in Croatia, organochlorine pesticides (OCPs) are regularly monitored in the frame of national monitoring program only in rivers and accumulation waters. Data on POPs pesticide residues in food, sediments, soil and vegetables can be found in various projects and inspectional supervisions.

30.2.1 POPs in Surface, Ground and Drinking Waters

The studies of organochlorine pesticides and polychlorinated biphenyls (PCBs) in the surface and drinking water started in late 1970s. Concentration of OCP in ground water from eastern Slavonia was examined from 1980 to 1983, because of potential grounder water using as drink water. Data obtained during 20 years period are presented in Table 30.2.

According to data from the annual reports of the Institute of Public Health of Istria in the period from 1980 to 1984, the highest concentrations of DDT-type compounds (500 and even 8,800 ng L^{-1}) were reached in the regional rivers of Istria (Boljunčica, Mirna, Raša, Pazinčica). Values detected in the later period of time (from 1986 to 1994) were significantly reduced (<100 or even <50 ng L^{-1}) as a result of limiting 4,4′-DDT application. During the same period of time γ-HCH concentrations were also reduced in three Istria rivers (Boljunčica and Raša (ca. 50 ng L^{-1}), Mirna (ca. 30 ng L^{-1}) and Pazinčica (200 ng L^{-1})). The values are less than 10 ng L^{-1}, and in Pazinčica from 200 to 100 ng L^{-1}.

According the annual reports of the Institute of Public Health of the Split-Dalmatia county measurements performed in Croatian coastal region has shown

Table 30.2 Concentration of PCBs in river, drinking water (ng L^{-1}), river sediments (mg kg^{-1} dry sample) and potable water (ng L^{-1})

Sample (N)	Sampling period	The range of concentration/ weight ratios
	River water	ng L^{-1}
Sava (7)	1992/95	<1–25
Kupa (22)	1985	<1–52
Kupa (6)	1985/86	2–16
Kupa (24)	1988/89	<1–8
Drava (8)	1981/82	<1–7
Cetina (7)	1993/94	2–8
Jadro (7)	1993/94	3–13
	River sediments	mg kg^{-1} (dry sample)
Kupa (6)	1985/86	1–39
Jadro (8)	1993/94	2–507
Cetina (18)	1993/94	<1–7
	Potable water (*karst springs before treatment*)	ng L^{-1}
Labin	1980/90	2–48
Pula	1980/90	4–176
Buzet	1980/90	4–50
	Drinking water (*after treatment*)	ng L^{-1}
Zagreb (10)	1988	<1–5
Sisak (16)	1988/89	<1–5
Labin (10)	1989	<1–3
Drava (8)	1981/82	<1–7

that the highest concentrations of DDT-type compounds in Dalmatian rivers from 1988 to 1993 were in the range from 20 ng L^{-1} (river Jadro 1993) to 195 ng L^{-1} (Krka 1988/89). The highest concentration of γ-HCH (ca. 56 ng L^{-1}) was detected in the river Čikola 1988/1989. However, in samples of river water Jadro, Cetina, Žrnovnica and Pantano collected during 1993/1994, concentrations of HCB, α-and γ-HCH, and DDT-type compounds were reduced to 2 ng L^{-1}.

Measurements performed from 1979 to 1989 in the continental regions revealed that the highest concentrations of DDT-type compounds were below 1 ng L^{-1} (in rivers Sava, Drava, Korana, Dobra, Kupa). However, in several water samples collected near the river Kupa at Sisak area in the period 1988/89 detected concentrations of 4,4′-DDT and its metabolites were up to 6 ng L^{-1}. The highest concentrations of γ-HCH (1–20 ng L^{-1}) were also detected in the same samples. The second most frequently detected OCP in Kupa river was HCB at concentrations up to 3 ng L^{-1}. Examination of OCP in the Sava river, streams, lakes and ground water in the area of Zagreb in the period from 1992 to 1995 confirmed the frequent presence of traces of γ-HCH and the occasional occurrence of very low concentrations of other compounds. From 1980 to 1983 investigations of OCP concentrations were performed in ground water on several locations in eastern Slavonia, including the area of the city Osijek. The highest concentrations of frequently detected compounds were up to 28 ng L^{-1} for γ-HCH, up to 25 ng L^{-1} for DDT and its metabolites and up to 3 ng L^{-1} for HCB. Organochlorine pesticides were detected in drinking water. The frequency of their occurrence in tap water in Sisak was during 1988/89 similar to that of the Kupa River water. Concentrations of γ-HCH ranged from 1 to 59 ng L^{-1}. At the same time examination of OCP in drinking water in Zagreb and Labin revealed regular presence of γ-HCH traces, while other compounds was detected occasionally. In the period from 1981 to 1990 the concentrations of OCP in raw water karst springs from which drinking water is prepared in Labin was 7–574 ng L^{-1}, in Buzet 11–260 ng L^{-1}, and in the area of Pula 1–180 ng L^{-1}. Data on the levels of OCPs in river sediments in our country are deficient. In accord of high tendency in sorption and bio-concentration in these media one can expect a higher level than in water. Detected traces of HCB, α-and γ-HCH and DDT and its metabolites in sediments of Middle River (Jadro, Cetina, Pantana and Žrnovnica) may be attributed to global environmental pollution. Sources of pollution of the aquatic environment with PCBs are often results of discharged untreated wastewater and/or improper disposal of waste, primarily waste oil.

30.2.2 POPs in the Food Chain

Even a small release of POPs can have a significant impact on living organisms due to processes of bio-magnifications. There is little data about POPs pesticides in the food chain and systematic monitoring is not carried out. The Croatian Institute of Public Health has analyzed only the content of some OC compounds in food samples in the period from 1986 to 1989 year and in 1999 given in Table 30.3. From Table 30.3 it is evident that the concentrations of all compounds in all types of food decrease with time. Drastic diminishing of POPs in all food types can be ascribed to positive effects of their prohibition starting 30 years ago.

30.2.3 POPs in the Sediments and Soil

There is little data about POPs pesticides in soil and no systematic monitoring in Croatia is carried out. Studies of OC pesticides and PCBs water starting in early 1980s were expanded to river sediments and it was not until past few years that soil become included. Levels of total PCBs were measured in samples of surface soils collected in the vicinity of several industrial and power plants, around airports and in urban and rural area that are close to possible sources of contamination. Results of mentioned concentrations of PCBs in soil are presented in Table 30.4. In most of the samples collected in urban and rural areas, concentrations of PCBs were characteristic of global environmental pollution (<10 μg kg^{-1} dry sample). Higher

Table 30.3 Concentrations (mean, mg kg^{-1} fat, fish mg kg^{-1} wet weight) of OC compounds in food samples

Source	HCB	α-HCH	Lindane	DDT complexes
Fish and fish products				
1986/89 (153)	5	2	25	127
1999 (46)	0.1	0.1	0.5	4.7
Meat and meat products				
1986/89 (733)	3	2	25	75
1999 (80)	0	1	6	62
Milk and milk products				
1986/89 (438)	7	3	24	83
1999 (52)	1	1	6	35

Number of analyzed samples is indicated in parentheses
0- below the limits of determination

Table 30.4 Concentrations (mg kg^{-1} of dry sample) of PCBs in the soil

Locations	Sampling period	Range (N[a])
Airports	1994/96	3–41,327 (18)
Industrial facilities	1997	21–1,207 (7)
Substations destroyed during the war		
Konjsko (Split)	1993	7–166 (17)
Komolac (Dubrovnik)	1996	1,640 (2)
Zadar	1996	173–204,823 (6)
Šibenik	1996	470,320–2,094,151 (3)
Delnice	1996	21 (1)
Urban and rural areas	1994/97	2–39 (18)

[a] N denotes samples

values were determined in the soil near the substations, especially those destroyed during the war, airports and in industrial areas.

It can be clearly seen in Table 30.4 that the highest level of soil contamination by PCBs occurred in the Croatian karst areas damaged during the war. Risk values, caused by the spread of capacitor oil, also were recorded at several locations in the vicinity of the damaged substation in Zadar. The highest concentrations of PCBs in the soil inside airports were observed in samples collected close to the runway for aircraft. Most probably this is a consequence of some uncontrolled discharge from electrical and hydraulic systems of aircraft. The fact that in the vicinity of airports the level of PBC was regularly at the level of global environmental pollution indicates the existence of local pollution sources within airports. Polychlorinated dibenzodioxins (PCDDs) and Polychlorinated dibenzofuran (PCDFs) in soil were found only in samples taken inside the airport where the mass fraction PCBs was higher than 5,000 μg kg^{-1}, and in soils collected near the former plant chloralcalic electrolysis. The levels of total PCDDs and PCDFs in soil collected inside the airport were within the typical values for urban and rural areas (<10 ng I-TEQ kg^{-1} dry sample). Much higher values were detected in soil sampled near the former plant chloralkaline electrolysis (Kaštel Sučurac near Split). However, even in these soils, the calculated values of I-TEQ were far below the value of 10,000 ng I-TEQ kg^{-1} dry sample which is the statutory limit values in Germany over which must be carried out rehabilitation of polluted industrial areas.

30.3 Status of POPs

Currently, there are no POP compounds productions in Croatia, nor they are planned so far. Today there is no need for their production, since all plant protection products based on POPs are now substituted with numerous toxicologically and environmentally friendly active ingredients. Also there is no import and export of POPs in Croatia, with exception for some institutions which have import permission for small quantities used as laboratory standards for determination of residual POPs pesticides. The NIP [2], gives details about usage of POPs pesticides from 1962 to 1976 and about DDT usage in Forestry Institute until 1986. It must be stressed that all production, circulation and usage of POPs pesticides, whether for agriculture or other purposes, are now forbidden in Croatia [3]. Additionally, no stockpiles of POPs pesticides have been detected in Croatia.

30.3.1 Former, Present and Future Production of POPs Pesticides

In the period when the POPs pesticides were allowed for application, there were several manufacturers that used to put pesticides of different formulations on the market. It is necessary to emphasize that the amounts produced in Croatia during the former Yugoslavia were meant for use in all Yugoslav republics. INA Kutina produced NPK fertilizers from 1969 to 1972 (12:12:12) with 1 % aldrin, which was soon banned. Since 1975 and up to the year 2000 production has been substituted with another type of fertilizer (Florin 3 which didn't contain aldrin). Endrin was used in the beginning of the first applications in 1957. Due to high risk level for the appliers and the environment it was used only in small quantities since 1971 as rodenticide. Numerous products are now days registered that completely replaced the toxicologically unfavorable pesticides, including POPs. Future production of POPs pesticides is neither planned nor possible as their manufacture has been prohibited. Until the ban of POPs pesticides, the quantities used were those indicated in the respective licenses. Their elimination has not caused big problems because of substitution with less toxic, less hazardous and ecologically acceptable compounds. Before the ban, POPs pesticides were used in the control of many pests. With respect to the wide use against the pest and to the target cultures, their quantities were significant.

30.3.2 Exports and Imports of POPs pesticides

In the fact that there is no production of POPs pesticides in Croatia, there is no way of their export in any country of the world. It is permitted to import only the active ingredience from the List of active substances authorized for use in plant protection products in the Republic of Croatia. On the List of existing active ingredients are those allowed in biocidal products or finished pesticide formulations with registered use pursuant with the Register of plant protection products. In the Register of biocidal products that are allowed to be placed on the market and all other are published annually or even more often in the Official Gazette. Each import must be approved by the Ministry of Health (MH) or Ministry of Agriculture (MA) and reported to The Croatian Institute for Toxicology and Antidoping (CITA). The importer is obliged to announce the transfer of such a consignment across the national border at least 3 days in advance. Importer is also obliged to fill in the prescribed form and submit it to CITA. At listed border crossing there is a competent sanitary inspection for biocidal products or phytosanitary inspection for plant protection products. Inspection based on the permit of the competent ministry and the registry of the permitted products in the Croatia, approves the import of pesticides. Because customs control of import bases itself on the approvals of respective ministries, not many misuses can be expected in this respect. It is not likely that POPs pesticides will be illegally traded because neighboring countries have prohibited most of them, while Croatia has registered corresponding environmentally friendly substitutes. According to the regulations regarding operations of agricultural and veterinary pharmacies, only the pesticides approved by MH or MA can be placed on the market [1, 4, 5, 6, 7]. Small quantities of POPs can be imported if they were used as laboratory standards for determination of residues in food, water and other materials.

30.3.3 Current Stockpiles, Waste with POPs Pesticides, Disposal Sites and Sites, Contaminated with POPs Pesticides

The inventory of POPs pesticides has not detected any stockpiles [2]. The products including currently used pesticides do not contain POPs. Consequently, there is no risk that waste with POPs can be generated. Special disposal sites for such POPs waste do not exist up to now. If there has been any, disposed at municipal waste disposal sites, then it must have been covered by layers and layers of other waste during 40 years, making it difficult to find them. Empty packaging materials for old pesticides could be the exception. So far there has been no site identified for disposal of hazardous waste, i.e. POPs. However, the association CROCPA (Association of Producers and Representatives of Producers of Plant protection products of Republic Croatia) that has ten members decided to organize collection of empty packages of plant protection products of the companies they represent. The idea is to collect empty packages from the end users, especially small farmers. In front of agricultural pharmacies where plant protection products are sold farmer can dispose the package into the designated box. Collecting of waste is carried out by the CIAK Company that is working on ecological and environmentally friendly disposal of hazardous waste. Considering that there are other types of POPs persistent compounds requiring special disposal sites, in case of their occurrence these landfills should be foreseen for disposal of POPs too.

30.4 Conclusions

According to the available data in Croatia POPs pesticides are not manufactured, used, exported or imported. Despite the ban or restricted use of PCBs and OCPs, they are still present in the environment. However, POPs inventory has not identified any contaminated sites or stockpiles. Data collected through various projects and from the analysis of inspection samples, although not done permanently and within a national monitoring program clearly revealed significant decrease in POPs concentration with time.

Currently, in accord to legal regulations, environmental levels of POPs pesticides are monitored only in waters, whereas other environmental elements and human are monitored occasionally as part of specific projects. It is, therefore, necessary to promulgate regulations for systematic and permanent monitoring of POPs in all environmental elements and human. Also, it is necessary to establish legal obligated collection of the results and continuous monitoring of their levels at the central registry.

A problem which should be solved in the near future is to provide funding for organization of laboratories, equipment and adequately trained staff. Poorly equipped analytical laboratories and their staff without proper training require the funds that would also enable certification of these laboratories. Despite the falling trend of POPs residue in the analyzed samples of vegetable and animal origin, water, soil and human biomaterial, still reliable information are needed to know their real status.

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Chapter 31
Some Institutional Aspects and Technological Approaches to Cleaning of Polluted Territories from Obsolete Pesticides in the Russian Federation Under the Framework of Synergism Between the Basel, Stockholm and Rotterdam Conventions

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Abstract Impact on human health and environment of persistent organic pollutants (DDT, toxaphene, polychlorpinene, polychlorcaphene, polychlorinated biphenyls, hexachlorobenzene) is a serious problem requiring attention of International Community as nowadays the amount of the obsolete and prohibited pesticides in the Russian Federation exceeds 24,100 t. Also, there are some polluted by pesticides territories which require special technological approaches based on identification and definition of sequence of disinfection of the territories polluted with pesticides. For the practical solution of problem connected with withdrawal from use and liquidation of obsolete pesticides stocks as a part of persistent organic pollutants (POPs) it is necessary to develop institutional measures including economic, legal and administrative mechanisms as well as technologies allowing to choose the most safe and economically sound methods of their destruction/neutralization. The problem of destruction/neutralization of above mentioned pesticides in the Russian Federation has not been solved yet. So, it is necessary to select the best available technologies for destruction of these pesticides.

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Keywords National legal regulation • Harmonization of legislation on environmental protection • Synergism between the Basel • Stockholm and Rotterdam Conventions • Obsolete and prohibited pesticides • Best available technologies (BAT) for destruction • Neutralization of pesticides

31.1 Introduction

On base of ANO "CIP" are operating:

- The Basel Convention Regional Centre on training and technology transfer for CIS countries (since 1996);
- The Stockholm Convention Regional Centre on the capacity building and technology transfer for the Central and Eastern Europe Region (since 2011);
- Inter-agency Council (IAC) for NIP consideration and approval.
- *Pesticides are regulated by international environmental agreements*:
- The Stockholm Convention on Persistent Organic Pollutants (POPs),
- The Rotterdam Convention on Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade,
- The Basel Convention on Transboundary Movements of Hazardous Wastes and their Disposal,
- The International Code of Product on the distribution and use of pesticides.

For the practical solution of problems connected with withdrawal from use and liquidation of obsolete pesticides as a part of POPs it is necessary:

- to develop institutional measures including economic, legal and administrative mechanisms;
- to choose technologies allowing the most safe and environmentally sound methods of their destruction/neutralization.

31.2 Basic Institutional Approaches

Basic institutional approaches are included:

- Analysis of national legislation, methodologies and rules of management of obsolete pesticides, assessment of their advantages and disadvantages; and enforcement practice in the sphere of pesticide management including regulation at regional/local level;
- Analysis of international legal commitments of the Russian Federation, governmental authorities, state subdivisions and local authorities on management of obsolete pesticides;
- Analysis of state administration in the sphere of safety management of obsolete pesticides, supervision and control for renewal of production and application of

obsolete pesticides as well as storage, transportation, use and landfill of obsolete pesticides;
- Legal questions of regulation concerning recovery of polluted territories;
- Legal questions of stimulation of safety methods for management of obsolete pesticides, environmentally efficient technologies for destruction and disinfection of pesticides;
- Legal questions of use and production of alternative pesticides;
- Legal regulation of safety for labor and health protection;
- Emergency measures;
- Determination of gaps in existing legislation of the Russian Federation relating to pesticides;
- Responsibility for “warrantable” and “unwarrantable” environmental pollution provided by legislation of the Russian Federation;
- Analysis of USA, Canada, European Union, Japan and other developed countries experience in obsolete pesticides management

31.3 Main Features and Disadvantages of the Existing Legislation

- Difficulties in providing of adherence of requirements in particular connected with activity of insolvent enterprises;
- System of inflexible quantitative norms;
- Generally orientation on sewage treatment, waste disposal etc. other than on pollution prevention in technological process, rehabilitation and recovery;
- Insufficient accounting of environmental impacts of implementation of programs, plans, projects;
- Contradictions between vertical (i.e. between international, national, regional and local levels) and horizontal elements of system (between various acts at the same level).

31.4 Legal Regulation of Management of Wastes of Production and Consumption

Main principles of state policy in the sphere of management of wastes are adjusted by the Federal Law № 89-FZ from June 24, 1998 “On Wastes of Production and Consumption” according to which the following stages are specified:

- Deactivation, i.e. release from harmful contaminants;
- Utilization, i.e. such deactivation in the process of which useful products necessary for further production are released;

- Stocking, i.e. temporary placement of wastes;
- Collection, i.e. accumulation of hazardous wastes in the place of its formation (generation);
- Storage, i.e. its placement in storage facilities for further reuse or disposal;
- Landfill, i.e. permanent placement in special containers at special conditioned sites;
- Transportation, i.e. movement of wastes from sites of collection, storage, stocking to the sites for treatment or disposal, destruction;
- Disposal, i.e. collection, transportation, treatment, storage and landfill above and under the ground as well as activity on their recovery, recycling etc.

31.5 Legal Regulation of Management of Chemicals and Their Storage

Main normative documents and rules in the sphere of storage and management of hazardous materials include:

- Federal Law "On Industrial Safety for Hazardous Production Units," which is the basis for industrial safety in production, storage, transportation and use of chemicals. It was adopted in August, 1997;
- National standard (GOST) 12.1.007-76 "Hazardous Substances, Classification and General Safety Requirements";
- National standard (GOST) R 50587–93 "Safety Certificate for Substances (Materials). Main provisions Information on ensuring safety at production, application, storage, transportation and utilisation";
- General Safety Rules for Explosive and Flammable Chemical, Petrochemical, and Oil-Refining Enterprises, 1988.

31.6 Synergism of the Basel, Rotterdam and Stockholm Conventions (see Table 31.1)

Table 31.1 Synergism of the Basel, Rotterdam and Stockholm

Joint Activities	Basel	Rotterdam	Stockholm
Consumption control of chemical substances/wastes (limits/prohibits)	**X**	**X**	**X**
Import/export control	**X**	**X**	**X**
Calculation and hazard assessment		**X**	**X**
Waste management	**X**		**X**
Dangers/risks of substances	**X**	**X**	**X**
Substitution/alternatives		**X**	**X**
Reports on emissions/releases to the environment			**X**
Technical support	**X**	**X**	**X**
Financial supply	**X**		**X**

31.7 Some Institutional Aspects on Synergism Decisions

31.7.1 Organizational Aspects

- Harmonization of National legislation;
- Coordination at National level;
- Cooperation by Programs in the sphere of interests of three Conventions;
- Coordinated use of Regional offices and Centre's.

31.7.2 Technical Aspects

- National reporting;
- Mechanisms of conformance/nonconformance of reporting on each of three Conventions;
- Cooperation by technical and research questions.

31.7.3 Increasing of Information Control and Public Awareness

- Mutual informative agitation and public informing activity;
- Mechanisms on information exchange/coordination concerning public health and ecological impact.

31.7.4 Administrative Aspects

- Joint control functions;
- Mobilization of resources;
- Functions of financial management and audit;
- Joint activities on technologies of the information processing, information and legislation services.

31.7.5 Adoption of Decisions

(a) Coordinated meetings (Conferences);
(b) Extra Conferences of Parties;
(c) Joint meetings of the Secretaries.

31.8 Inventories, Labeling and Storage of Obsolete Pesticides in the Russia Federation

During POPs inventories of 2000–2011 it were found large amounts of the PCB and obsolete pesticides in the Russian Federation as shown in Table 31.2.

Total amount of absolute pesticides exceeds 24,100 t. The amounts of obsolete pesticides in Federal Districts are shown on Fig. 31.1.

Obsolete pesticides in the Russian Federation are mostly in the Southern agricultural regions and approximately 10 % in the Arctic Region.

According to the ACAP/AMAP project which was fulfilled in 2000–2009, it was carried out the inventory of obsolete pesticides in 11 selected priority regions of the Russian Federation – eight Arctic and three Sub-Arctic Regions namely:

- Arctic regions: Arkhangelsk (including Nenets Autonomous District),Komi, Krasnoyarsk Krai (including Taymyr Autonomous District), Magadan Region (including Chukchi Autonomous District), Republic of Sakha, Tyumen, Kamchatka Region (including Koryak Autonomous District), and Murmansk Region;
- Sub-arctic regions: Altai Krai (including Altai Republic), Kurgan and Omsk
- The ACAP/AMAP project resulted in the following activities:
- Improvement of temporary storage conditions (see Fig. 31.2);
  - repackaging and labeling;
  - consolidation of stocks of waste pesticides;
- Assessment and selection of the best available technologies and methods for destruction/disposal of pesticides stocks.

Table 31.2 Inventory data of PCB and obsolete pesticides

Name of POPs	Inventory data, 1,000 t	Estimation data, 1,000 t
Obsolete pesticides	24	35–40
Including chlorine-containing	5 ÷ 7	10 ÷ 15
PCB	30	22–25

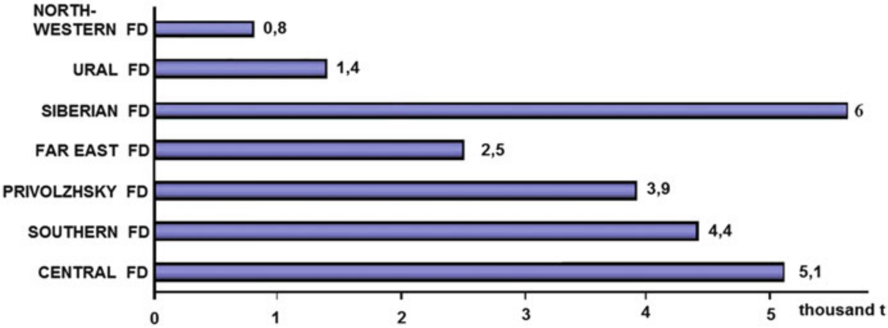

Fig. 31.1 Amounts of obsolete pesticides in Federal Districts of the Russian Federation

Fig. 31.2 Improvement of obsolete pesticides storage conditions: (**a**) initial storage conditions; (**b**) after repacking and labeling of obsolete pesticides

31.9 Identification of PCB-Contaminated Sites of Serpukhov Town (Moscow Region)

Identification of PCB-contaminated sites consisted of localization and estimate area of PCB-contaminated sites as well as the amount of soil subject to decontamination.

The territory of Serpukhov town (Moscow Region) was contaminated by PCBs and was used for many years by the local condenser manufacturing plant "KVAR". The studies undertaken right after the shut down of the plant and 10 years later showed that overall area of contamination was about 600–700 ha or 6–7 km^2. This area covers sediment ponds of the former runoff treatment system of the condenser plant (about 3 ha) with the content of 18,300 mg/kg with penetration depth of 0.5 m and agricultural lands of Zaborye district (about 200 ha) used for vegetable planting with content of up to 8.5 mg/kg. Estimated amount of PCBs contained in soil of Serpukhov is about 340 t (Table 31.3).

The PCB's contamination of the territory of the town of Serpukhov has spread far beyond the zone of "Zaborie". The proportion of contaminated territory is 82 % of the town total area.

31.9.1 Recommendations for Treatment of Contaminated Sites in Serpukhovsky District

Different technologies for treatment of highly-contaminated territory of Serpukhovsky District were assessed. According to the estimates the total amount of PCBs on the area of 600–700 ha (6–7 km^2) is 350 t. Cyclone kiln and thermal desorption technology was recommended for destruction of PCBs on the basis of "cost-effect" analysis. The reduction of PCB content in soil to the levels

Table 31.3 Percentage distribution by PCB contamination level of contaminated territory of the town of Serpukhov

No of zones	Pollution levels, multiple estimated admissible concentration of PCBs in soil is 0.06 mg/kg	The proportion of the total territory of the city Serpukhov, %
1	10	30
2	10–50	30
3	50–100	5
4	>100	14
5	>1,000	3

corresponding to environmental protection standards is possible. The recommended methodologies are suitable for treatment of contaminated sites in Serpukhovsky District; technologies for treatment of other PCB-contaminated sites on the territory of the Russian Federation should be selected and considered specifically. In each separate case thorough examination of the site should be carried out to get the necessary data for selection of the optimal technology.

On the basis of the "cost-effect" analysis the following activities were recommended for decontamination of sites in Serpukhovsky District:

- Decontamination of the soils of sediment ponds ($\approx$3 ha) of the former condenser producing enterprise with application of cyclone kiln technology;
- Decontamination of agricultural lands (200 ha) through treatment with humic mineral concentrate and biological treatment;
- Decrease of impact on human health through a number of administrative activities aimed at reduction of production and consumption of contaminated agricultural products.

All these soil rehabilitation methodologies can be implemented in Serpukhovsky District although the experience of their application is not numerous. Nevertheless, the application of these technologies might require additional data, for example, on further status of PCBs bound by humic acid in the course treatment with HMC.

The considered methodologies can be recommended only for treatment of contaminated sites in Serpukhovsky District and cannot be directly applied on other PCB-contaminated sites in the Russian Federation. In each separate case thorough examination of the site should be carried out to get the necessary data for selection of the optimal technology.

31.10 Waste Polygons in the Russian Federation

Now on the territory of the Russian Federation there are five waste polygons – four operating and one on reconstruction – which are used in Tomsky, Leningradsky, Kaluzhsky and Omsky regions for **ground disposal**:

- **Landfill "Serebristiy" (Ltd. "Zeleniy Gorod", Krasnoyarskiy Kray).** Wastes of I-III classes of danger to environment including Hg-containing waste; the PCB containing waste; not identified pesticides with expired validity;

Fig. 31.3 Pictures of landfill “SEREBRISTIY”, Ltd. “Zeleniy Gorod” in the Krasnoyarskiy Kray

- **The State enterprise “Polygon” (Tomsky region).** Wastes of I-III classes of danger to environment including Hg containing waste and the PCB containing waste; HCCB and other POPs;
- **The State landfill “Krasniy Bor” (Leningradsky region).** Wastes of I-IY classes of danger to environment including Hg-containing waste; the PCB containing waste; not identified pesticides with expired validity. Now the landfill is on reconstruction;
- **The landfill ASEKO (Kaluzhsky region, Obninsk).** Wastes of I-III classes of danger to environment, including waste of pesticides, dry waste of galvanic, delayed chemicals are placed;
- **The landfill of toxic waste ground disposal (Omsky region).** Wastes of I-IY classes of danger to environment including Hg-containing wastes; wastes of the pesticides forbidden to application and with expired validity.

The given quantity of landfills obviously is not enough. Existing conditions of ground disposal often do not correspond the requirements; there is a set of cases of outflow or passages in an environment.

On Fig. 31.3 is shown landfill “SEREBRISTIY” (Ltd. “Zeleniy Gorod”, Krasnoyarskiy Kray) which was created in 2006 and is able to save all types of wastes including:

– Prohibited and forbidden pesticides;
– Hg containing wastes;
– PCB containing wastes.

31.11 Russian Accredited Laboratories for POPs and Pesticides Analyses

There are **four accredited Russian Laboratories** using international methodology and standards for POPs and pesticides sample analyses:

– Institute of Ecology and Evolution Problems named after N.A. Severtsov, Russian Academy of Sciences, Moscow;

- NPO “Typhoon” of Roshydromet, Obninsk, Kaluzhsky region;
- FSE “Russian Science and Research Center for Emergencies” of the Federal Medical and Biology Agency, Moscow;
- The Bashkir’s Research Ecological Center of Bashkortostan Ministry for Natural Resources, Ufa, Republic of Bashkortostan.

Obviously the presented number of accredited laboratories is not enough owing to increasing needs for POPs and pesticides analysis.

31.12 Russian Technologies of High-Temperature Oxidation

On the basis of the considered **technical, ecological and economic requirements to technologies** of POPs destruction were developed four Russian technologies of high-temperature oxidation with using:

- Thermodestruction in cyclonic reactor (Author JSC “Techenergochimprom”, Moscow and Orekhovo-Zuyevo, the Moscow region);
- The rocket engine (Author – ZNIIMASH Korolev; the Moscow region, Open Society “Severstyle” Cherepovets, the Vologda region);
- The liquid rocket engine (Author– INCC of the Russian Academy of Science, Moscow, Federal State Enterprise “Voenmech”, St.-Petersburg);
- The plasmatron and a chemical reactor:
  - the traditional approach (Author -RNC “Prikladnaya Chemistry”, St.-Petersburg);
  - the new construction (Author – International Centre of Thermal Physics and Power Engineering (ICTP&PE), Novosibirsk, Russia). These Russian technologies has been described below.

31.12.1 Cyclonic Reactor of Thermal Destruction of PCB/Pesticides (Author – JSC “Techenergochimprom”, Moscow and Orekhovo-Zuyevo, Moscow Region) [1–3]

This technology for the destruction of liquid toxic organic-chlorine wastes with the capacity of 200–2,500 kg/h was designed in Scientific and Industrial Association “Techenergochimprom”. One of installations is now in operation in the sewage cleaning plant in Orekhovo-Zuevo in Moscow region.

Air for the combustion and incineration of chlorine-containing wastes as well as the fuel enters the cyclone reactor (see Fig. 31.4) with the rate of 100 m/s. The exposure time in the furnace is 0.3 s at 1,600–1,700 °C.

Twenty percent solution of caustic is introduced into the operation zone of the reactor via special nozzles and the temperature drops down to 1,250–1,400 °C.

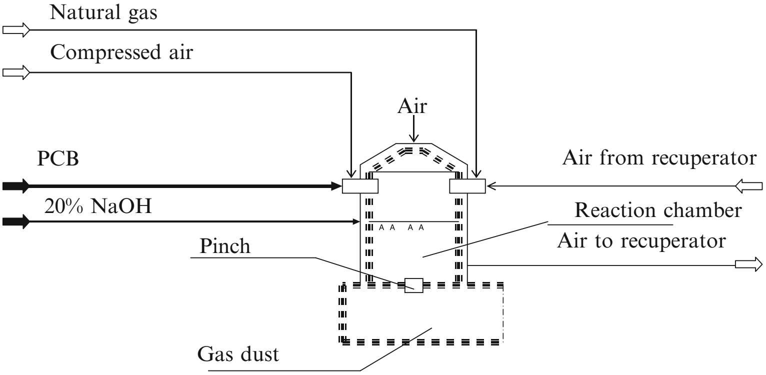

Fig. 31.4 High-temperature incineration in cyclonic reactor

Supply of caustic solution directly to the hot zone of combustion is the key point of the process. This part of the method is patented in Russia.

The set of mandatory process requirements applied to the technologies of high-temperature incineration of PCB is fully met by cyclone reactors:

- temperature in the reaction zone – above 1,200 °C;
- exposure time for PCB – above 2 s;
- turbulent flow in the reaction zone;
- 10 % (by volume) excess of oxygen against PCB;
- DRE of PCB – not less than 99.9999 %;
- a particular attention to CO concentration monitoring.

Combustion of CO is slower than the combustion of dioxins. The experience shows that when the content of CO is below 5 mg/m^3, the content of dioxins will be less than 0.1 ng/m^3. This process doesn't have a special unit for the neutralization of hydrogen chloride – it is done directly in the cyclone reactor with the generation of solid substances. This technology rather fully meets environmental and process requirements and is recommended for the feasibility study.

31.12.2 Rocket Technology (Author – Zniimash Korolev; The Moscow Region, Open JSC "Severstyle" Cherepovets, the Vologda Region) [1, 2, 4]

This process was preliminary tested at the experimental test-bench in the Scientific Institute of Machinery Construction (ZNIIMash) (Korovel, Moscow region) and is implemented on the industrial scale at OJSC "Severstyle" in Cherepovets, Vologda region. The installation is in operation since 1998 and during this time destroyed about 100 t of the products called "Sovtol-10", which is a mixture of 90 % of pentachorodiphenyl and 10 % of thrichlorbenzene.

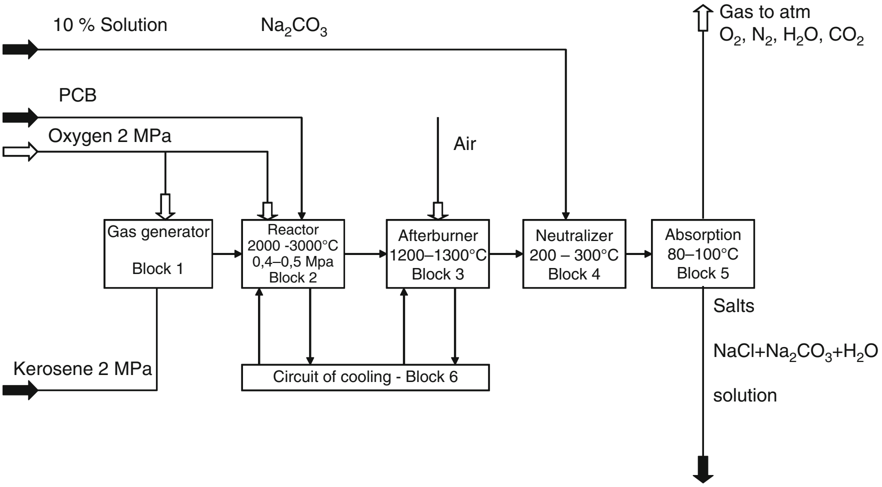

Fig. 31.5 Rocket technology of PCB incineration

The key point of this technology (see Fig. 31.5) is the application of a rocket engine. This type of the rocket engine of a standard size was designed for the orientation of space stations. The nozzle for the fuel mixing is 10 cm long and has the maximal diameter of 8 cm. Steel pipelines of a small size, about 10 mm supple the components of the reaction into the engine.

The engine is made of two parts: a high-temperature gas-generator where the fuel (kerosene) and oxygen enter into the reaction at ~3,000 °C, and a reactor, where PCB and oxygen are supplied. The operation pressure in the first chamber is about 16 bar.

Consideration of the other process parameters shows:

- temperature in the reaction zone is 2,000–3,000 °C;
- exposure of PCB is under 0.1 s, which is considerably less than the required 2 s;
- high velocity of the gas flow in the reaction zone is ensured;
- excess of oxygen against PCB is not less than 13 % by volume;
- the level of CO in the flue gases is above 5 mg/m^3, which testifies the increased level of dioxins;
- the flow chart has the stages of after-burning, quenching and the separator for the separation of flue gases from waste waters.

As far as there are not approved standards on the emissions of dioxins in Russia, the authors of the technology came to the conclusion that while DRE = 999,999 % was reached at the industrial facility it is well comparable with the similar results of the industrial facilities in Europe and the USA. This lead to the approval of this PCB destruction technology in Russia. Industrial facility with the capacity of 200–250 kg/h was constructed at OJSC “Severstyle” in compliance with all regulatory, legal, methodological and technical standards and documents, specifying the operation of the facility, instrumentation and monitoring of the technological and environmental parameters.

31.12.3 Liquid Rocket Engine (Author– INCC of the Russian Academy of Science, Moscow, Federal State Enterprise "Voenmech", ST.-Petersburg) [1, 2, 5]

This technology (see Fig. 31.6) implies the destruction of PCB in chemical reactors based on the rocket engines with liquid fuel (LRE), where the chemical process is well combined with the generation of secondary heat coming from the reaction. The chemical reactor based on LRE has three main components: block of jets, combustion chamber and block of nozzles. The block of jets supplies the mixture of kerosene with PCB and oxidizer (oxygen) to the combustion chamber where the decomposition and incineration of PCB takes place at 2,500–3,000 °C.

After combustion chamber a turbulent flow of gases leaving the block of nozzles is subject to an instant expansion with additional turbulization and intensive mixing of the main volume of gases with the gases of the thermal boundary layer. This expansion occurs in the second chamber, which is larger than the first one so the exposure time in the second chamber is by an order on magnitude longer and PCB are subject to additional destruction. Quenching is used to prevent the generation of dioxins. Examination of special requirements to the technology of high-temperature incineration showed that:

- temperature in the reaction zone is 1,500–3,000 °C;
- exposure time of PCB in the reaction zone ensures its complete transformation;
- turbulence in the reaction zone is ensure by the special design of the reactor;
- there is an excess of oxygen against the content of PCB. The designer gave this parameter a special attention;
- DRE is 99.999999 %;
- from the operation experience the concentration of CO is considerably lower than 20 mg/m^3 that, in accordance with the designers, indirectly confirms the low level of dioxins;

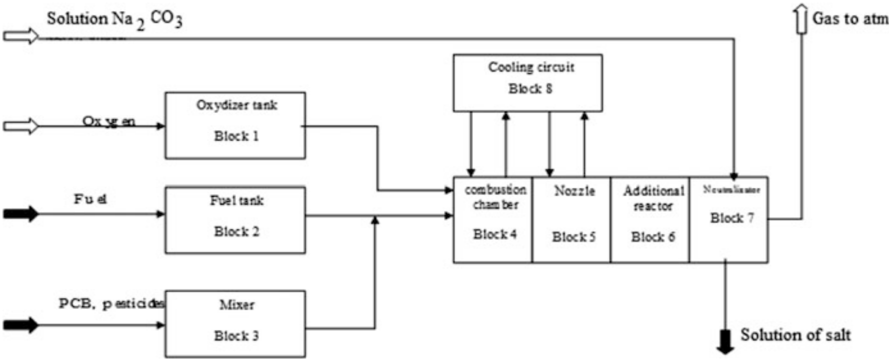

Fig. 31.6 Liquid Rocket technology of PCB incineration

– the technology implies the stage of after-burning and the supply of soda solution for the neutralization of acid gases directly into the second chamber of HTR. Solid substances are created in flue gases. This mixture is subject to the quenching in the scrubber (fast cooling by evaporation), which the separation of mineral dust. A special filter is used to catch this dust before the release of the flue gases into the atmosphere.

The process was tested not only with 20 % solution of PCB in the fuel, but also with dioxin-generating mixture consisting of 10 % of chlorobenzene хлорбензол and 90 % of low-octane petrol. These mixtures were recommended by German companies Mannesmann and Institute Fresenius. Dioxin analyses were made by Institute Fresenius and domestic company ECROS. Results showed that the content of dioxins are the first 4 h of operation was 10.8 pg/m^3, after second 4 h 9.8 pg/m^3, and after third 4 h – 9.1 pg/m^3.

Results of the analyses fully meet the European environmental requirements. DRE was 99,999,999 %. The similar results were obtained during the joint work with the Institute of Applied Studies (TNO, the Netherlands) and with the colleagues from St. Petersburg.

31.12.4 Plasma-Chemical Destruction of PCB/Pesticides (Author – RNC “Prikladnaya Chemistry”, ST.-Petersburg) [1, 2, 6]

Technological flow sheet (*see* Fig. 31.7) *description*:

The mixture of PCB and water vapor is warmed up by an electric heater up to 600 °C and is fed to the plasma-reactor (Block 1) simultaneously with a high temperature (5,000 °C) nitrogen spurt.

Pyrolysis products are fed from the plasma- reactor to the quenching and neutralization unit (Block 2) that are fed to the separator upon cooling and neutralization.

The alkali solution consumption is adjusted so that the temperature within the separator remains on 80 °C level. Upon the alkali solution drops are removed, the gaseous phase released into the atmosphere following additional treatment.

The liquid phase is fed to the centrifuge (Block 3) in the amount needed to remove the salts formed. The precipitate consisting primarily of NaCl with a minor content of soda and sooth is discharged from the centrifuge and is recycled or disposed.

The salts formed during PCB elimination could be used as a raw material for chlorine and caustic production. The used alkali solution is returned for reuse (Block 4). To maintain alkali content in the solution fresh alkali is fed to the system (Block 4).

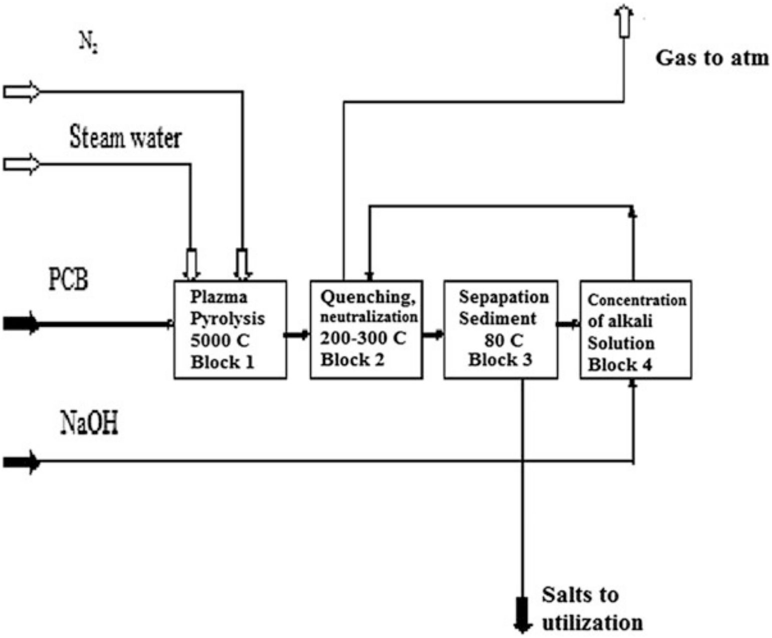

Fig. 31.7 Plasma chemical technology of PCB destruction

Tests of the plasma-chemical plant carried out in the presence of NEFCO's experts in the city of Kapitolovo (Leningradsky region), showed that DRE was 99.9993 % and dioxide content in the stack gases was about 55 ng/nm^3.

31.12.5 *Plasma Chemical Reactor for Destruction of Wastes (Author – International Centre of Thermal Physics and Power Engineering (ICTP&PE), Novosibirsk, Russia) (www.dtat.ru)*

The installation of plasma chemical reactor of new construction for destruction of wastes is shown on Fig. 31.8. Advantages of the plasma chemical reactor of new construction against the plasma chemical reactor of traditional construction (see Sect. 31.12.4) are:

- the long resource of continuous work;
- the opportunity of use steam as plasma gas;
- the high degree of processing, DRE was 99,999,999 %.

Fig. 31.8 The installation of the plasma chemical reactor of new construction for destruction of wastes (plasma torch power-500 kW; gas flow rate −50–150 kg/h; waste capacity up to 200 kg/h)

31.12.6 Mobil Installation of Plasma Destruction of Toxic Wastes (Author – International Centre of Thermal Physics and Power Engineering (ICTP&PE), Novosibirsk, Russia) (www.dtat.ru)

On base of the plasma chemical reactor of new construction was created the Mobil installation of plasma destruction of toxic wastes presented on Fig. 31.9, which is having capacity up to 100 Kg/h and supplied electric power – 250 kW.

31.13 Comparison of Destruction Methods on Technical and Economic Parameters

On the basic of economic parameters – advantage of the high-temperature oxidation technology with use of a cyclonic reactor has the less costs of creation of installation and cost of processing of 1 t of pesticides as shown in Table 31.4.

There is the **high-temperature cyclonic reactor technology** of the pesticide-POPs destruction is also recommended by Company "Synthesis" (Moscow) for using in Russia.

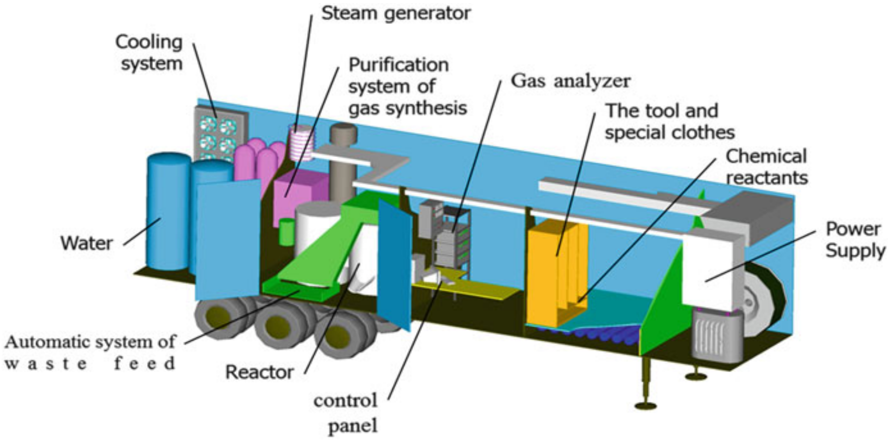

Fig. 31.9 Mobil installation of plasma destruction of toxic wastes

Table 31.4 The basic economic parameters of the Russian destruction technologies (In thousand US dollars on the prices of January, 2007)

		Cost of installation development			Expenses on destruction of 1 t of pesticides	
Name of destruction Method	Cost of R&D	Cost of design	Capital cost	Summary cost	Prime cost	Price
Thermodestruction in cyclonic reactor	100	200	1,800	2,100	1,000	1,200
The rocket engine	200	200	2,200	2,600	1,200	1,400
The plasmatron and a chemical reactor	Traditional scheme/New construction					
	200	200	3,500	3,900	1,700	2,000/ 500
The liquid rocket engine	200	200	2,000	2,400	1,200	1,400

31.14 Conclusions

For practical solution of the environmental problems having a global character the complex approach is necessary and it has to include:

- Scientific and technical measures (low-waste technologies, technologies for treatment and destruction of wastes, effective technologies for waste treatment etc.);
- Administrative and technical measures (creation relevant systems of waste recycling, creation of regional and local systems of water circulation etc.);
- Public organizational measures (environmental training and education, the environmental law, public awareness etc.);

- Mutual activities (joint management staff, joint offices on rendering services, synchronizing of budget cycles, joint audit of Secretaries accounts and etc.);
- The period from the beginning of the development of new technology and installation for POPs destruction proceeds for a long time;
- The high-temperature cyclonic reactor technology of the pesticide-POPs destruction has advantages among the high-temperature oxidation technologies.

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Chapter 32
Environmental Security Assessment Based on the Cytogenetic Estimation of Mutagenicity and Human Health in Ukraine

Iryna Klimkina

Abstract The accumulation in different environmental objects of many toxicants defines the real risks for biota and human health. An especially serious risk for all living organisms is presented by environmental mutagenes that can affect the hereditary apparatus of somatic and sex cells, resulting in cancer increases and other ecologically dependent pathologies. Therefore, an investigation of mutagenesis in cells at genetic level is very urgent and real. Biological monitoring, which includes genetic and cytogenetic monitoring, provides a useful tool for estimating the genetic risks deriving from integrated exposure to a complex mixture of chemical, physical and biological environmental agents. A positive association between occupational exposure to complex pesticide mixtures, and the presence of different cytogenetic pathologies including micronuclei (MN), has been detected in the majority of studies. The application of cytogenetic testing, using the micronucleus assay (MN-test) on exfoliated buccal cells of children, substantiates the estimation of total mutagenicity in the studied territory in which they live. A positive modifying cytogenetic influence of natural adaptogens was established.

Keywords Genotoxicity • Cytogenetic monitoring • Pesticides • Exfoliated buccal cells • Micronucleus • Natural adaptogenes

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32.1 Introduction

One of the most significant global current problems is unquestionably the issue of ecology. An accumulation in the biosphere of harmful substances, together with an increase in radio-activity in the environment, threatens the state of ecosystems, genofonds and the population's health, and limits for the potential further development of civilization [75].

As a rule, the quality of different environmental objects is controlled in accordance with the system of, for example, maximum permissible concentrations of harmful substances, and the toxicological, organoleptic, sanitary and biological indices, through the use of numeral methods. However, mutagenic properties of the environment as the main risk from pollution, have remained undefined by such research. Therefore, a significant value is acquired by taking into consideration such problems as the control in the above mentioned processes of environmental contamination by mutagenes, as well as preventing the growth of mutagen contamination. It is also necessary to understand the nature of mutagenic actions and to search for methods of protecting living organisms from their negative influence. This can be achieved by the use of indicator biotests among which the cytogenetic tests are the most informative, as they are highly sensitive and sufficient for suitable estimations [22, 32, 53].

The advantages of biotesting at the cellular level are predefined by the theory about cellular structure and its similarity for all live organisms, as well as the universality of cell's chemical composition and its metabolism. It is considered that 60–70 % of substances with an identified genetic activity have similar mutagenic and carcinogenic influences on plants, animals and human health [19]. There are many scientific results confirming the mutagenic potential of pesticides [6], heavy metals [3, 36, 42], radioactive nuclides [20, 48], or the general influence of toxicants on plant and animal organisms [25, 61].

One of the specific features of mutagenic and carcinogenic substances is an ability to display biological activity even at weak concentrations. It complicates their analytical determination in biological tissues. Therefore, for the analysis of genotoxicity of environmental agents the different test-systems are worked out on the basis of use in the cells of bacteria and other microorganisms [57, 60, 65], plants [15], animals [2, 76] including cells of human [67].

Accordingly, it is necessary to implement a well-developed system of ecological and genetic monitoring that will allow adequate estimation of genetic risks to human health. Such a system must combine investigation results from the bioindication of mutagenes presented in the different environmental items, the cytogenetic survey at the population level and direct genetic monitoring among the human population. Only on the basis of applying biological and medico-genetic research it is possible to estimate the real level of mutagenic load and the genetic risks for humans from the influence of harmful ecological factors [32]. It is necessary for substantiation of priority-oriented managerial decisions directed at a decrease in the technogenic loading, as well as improving of environmental conditions, biota and the nation's genofond [31].

32.2 Genetic Monitoring

For estimating the possible influence of mutagenic environmental factors on human health it is necessary to have a system of genetic monitoring among the population living in regions with different ecological conditions. Implementation of the genetic monitoring should be based on the complex study of potential mutagenicity of total environmental contamination *in vivo* and *in vitro*, as well as epidemiological and cytogenetic changes in the human population.

Frequency in occurrences of spontaneous changeability of chromosomes in the somatic human cells can be used as a biological characteristic at the population level and at the same time can reflect the mutagenic environmental load on genetic human health. In addition, it is expedient to conduct a retrospective analysis of morbidity and death rates among the human population according to the following indices: frequency of spontaneous abortions and malformations; infantile death rate; level of cancer diseases; frequency of the inherited illnesses; and multifactorial pathology. Such no experiment-based prognosis of genetic risks from environmental contamination allows a plan to be defined for the realization of the next stages of the genetic monitoring.

Comparative analysis the frequency of chromosomal aberrations and level of sister chromatid exchanges in the cultivated leucocytes of peripheral blood from people living on the conditionally clean territories (control groups) and persons professionally contacting with some potentially dangerous mutagenic agents, and also comparing the control groups to the residents being under influence of factors of radiation or chemical nature, is presently adequate, sensitive and widespread method recognized for estimation of mutagenic effects both occupational and environmental factors. Moreover, existing methodology for effect analysis by comparing with the spontaneous level of mutations provides with sufficient certainty to the answer to the question about radiation (on frequency of two-hit types of chromosomal aberrations; [40]) or chemicals (on frequency of one-hit types of chromosomal damages and level of sister chromatid exchanges; [24, 49, 59]) origin of mutagenic effect.

32.3 Pesticide Influence on Human Health

Acute poisoning caused by large doses of pesticides appears usually after accidents or fires on chemical enterprises. Chronic influence by measurable doses is connected, as a rule, with professional activity of workers involved in chemical production, or personnel directly engaged in the process of pesticide application.

Moreover, it is established that women living on land contaminated by pesticides suffer changes in their reproductive health [1] including spontaneous abortions [69], a high frequency of obstetrical pathologies [69], and abnormalities in the rates and terms of physical and sexual development of girls [43]. A number of pesticides, including dibromochlorophane and 2,4-D, have also been associated with impaired fertility in males [64].

It is also shown that these are significant risk factors for mothers having professional contact with pesticides, using pesticides in housekeeping, and living within 0.4 km of agricultural activities [41].

Many studies have examined the effects of pesticide exposure on the risks of cancer [74]. For example, associations have been found with leukemia and lymphoma, and cancers of the brain, kidneys, breasts, prostate, pancreas, liver, lungs and skin [26]. These increased risks occur with both residential and occupational exposures [26], for example, increased rates of cancer have been found among farm workers who apply these chemicals [47]. Mothers' occupational exposure to pesticides during pregnancy is associated with an increase in their children's risk of leukemia, Wilms' tumor, and brain cancer [26, 72].

Strong evidence links pesticide exposure to worsened endocrinic [37] and neurophysiological outcomes [23, 68]. Thus, the risk of developing Parkinson's disease is 70 % greater in those exposed to even low levels of pesticides. Indeed, people with Parkinson's were 61 % more likely to report having had direct pesticide application than were their healthy relatives. Both insecticides and herbicides significantly increased the risk of Parkinson's disease, and there are also concerns that long term exposure may increase the risk of dementia [4].

The World Health Organization [75] and the UN Environment Programme estimate that each year, three million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18,000 of whom die. According to biomonitoring results of the Centers for Disease Control and Prevention's (CDC) National Health and Nutrition Examination Survey (2005–2006), as many as 25 million workers in developing countries may suffer mild pesticide poisoning yearly [26]. This report stated that detectable body levels of about 50 pesticides have been found in a representative blood sample of the U.S. population.

Thus, against the background of deterioration in basic medical and demographic indices that characterize the population's health (such as death rate, birth rate, an increase in allergic, infectious, reproductive, oncologic and other morbidity), pesticides promote a substantial risk factor for both the population's state of health and environmental quality.

The significance of the pointed factor is aggravated by the application of pesticides on land polluted by radionuclides and/or heavy metals, and also on land with deficit/excess of different microelements (such as iodine and fluorine). This is due to the combined influences which can cause an intensification of harmful action of the indicated factors on both human organisms and biota.

32.4 Genotoxicity of Pesticides on Biota

Mutagenic activity of pesticides is one of the most dangerous displays of negative influence on all living organisms including human health and its posterities. However, because of the enormous variety of pesticides used in agriculture, according to all available data the information about pesticide genotoxicity is not sufficient.

At the UN Conference on Environment and Development, 1992, pesticides (along with heavy metals) were attributed to the prevailing contaminants in the biosphere. Therefore, research of their toxicity and long-term effects is extremely important today. Some research show the genotoxicity of pesticides; for instance, in plants treated by Basagran much of the chromosomal damage at the different stages of merosis is detected [56]. Also, Herbicide Round Up induces the formation of anaphase bridges and other mitotical defects in the cells of root meristem of *Vicia faba* [21] as well as reversion genes in prototrophic bacteria *Salmonella typhimurium* detected in the Ames-test [60]. Senkor in concentrations of 0.01 and 0.05 % causes chromosomal aberrations in cells of *Crepis capillaries L*. The spectrum of chromosomal aberrations is presented by chromatid and isochromatid deletions and micro-fragments [73].

Using the Ames-test on the strains of *Salmonella typhimurium* TA98 and TA100 a mutagenic activity based on a mixture of seven pesticides was studied: six herbicides (Round Up, Senkor, Basagran, Kusagard, Lontrel, and Setoxidim) and the fungicide Tachigaren. Also tested were complexes of herbicide of Lontrel (ML_2) with eight metals (Cu, Co, Zn, Ni, Fe, Mn, Mo, and Mg). It is established that the mutagenic indices of the investigated pesticides correlate with the value of complexing constants of these substances with DNA [60]. Similar results are obtained from the study of mutagenic potential of mixture of the following pesticides: Aktar, Senkor, Mospilan, Penkozeb and Fastak, which are widely used for treatment of potatoes in Republic of Tatarstan [38].

Studying the influence of "Decis" (deltametrine) insecticide and "Magnum" (metsulfuronmethyl) herbicide on morphological and the cytogenetic mutability of *Drosophila melanogaster*, authors concluded that specified concentrations cause damage to the stability of development and increase the frequency of puff-formation in polytene chromosomes of flies [29].

A study aimed at clarifying the effects of environmental pollution, including from agriculture, on some genetic processes of *Tilapiane* fish showed an increase in the frequency of both chromosomal structural and numerical aberrations, such as deletion, gap, end-to-end association, fragmentation, polyploidy, stickiness and monosomy [76].

Thus, for monitoring purposes bio-indicators are exposed to the environmental pollutants "*in situ*" or in laboratory tests "*in vivo*". Tests with eukaryotic cells or organisms might be more relevant for human and ecological risk assessment, but generally they are much more time-consuming. Several tests have been developed using the integrity of DNA as a non-specific endpoint of genotoxicity e.g. comet assay, alkaline DNA-elution assay, DNA alkaline unwinding assay and other. Most eukaryotic genotoxicity tests detect macro damage of chromosomes in the visible light microscope following appropriate staining (i.e. chromosomal aberration, micronucleus assay, and SCE assay). Plants, amphibians, fish and water mussels, as well as permanent mammalian cell lines such as V79, CHO or CHL, have been used as the test organisms. Newer technologies, such as transcriptomics, proteomics and metabolomics, provide the opportunity to gain insight into genotoxic mechanisms and also to provide new markers *in vitro* and *in vivo*. There is also an increasing number of animal models with relevance to genotoxicity testing. These types of models will undoubtedly have an impact on genotoxicity testing in the future [46].

32.5 Genotoxicity of Pesticides on Human Health

The relevance of research into the mutagenic properties of pesticides is defined by the fact that most mutagenic substances cause a carcinogenic effect and so represent a hazard for human health [5].

Investigation of the cytotoxicity and genotoxicity of Acephate on the peripheral blood of healthy humans under in vitro test conditions revealed that, after 2 h of exposure to the pesticide, almost 100 % cells became non-viable at a 70 μM concentration. In addition, it was established that the percentage of chromosomal aberrations and cells with DNA damage studies by single cell gel electrophoresis technique increased with the increase in the concentrations of the pesticide. At further higher concentrations there was a predominance of necrotic cells [17].

Cytogenetic investigations among greenhouse farmers exposed to pesticides showed a significant increase in sister-chromatid exchanges (SCEs) frequency in peripheral lymphocytes. The results of SCEs were expressed through two variables: (a) the mean number of SCEs per chromosome, and (b) the proportion of high frequency cells (i.e. cells with more than eight SCEs). A high correlation was found between these two variables that indicates a potential cytogenetic hazard for humans due to pesticide exposure [62].

A study of structural and numerical chromosomal aberrations (CAs) in workers of a plantation of flowers located in Quito, Ecuador, South America, exposed to the Aldicarb and Fenamiphos pesticides concluded that workers exposed to these pesticides showed an increased frequency of CA compared with the control group. In addition, the authors determined the level of erythrocyte acetylcholinesterase which was below the optimal in 88 % of exposed individuals. This clearly shows the negative effect of organophosphate pesticides on human health [52].

Several studies have shown a decline in human semen quality with respect to the effects of pesticide exposure *in vitro* and *in vivo* and increased risks of male subfertility. In epidemiology studies clear effects on male fertility have been demonstrated for some pesticides (e.g. dibromochloropropane and ethylene dibromide) [9].

As for realization of mass cytogenetic and other monitoring surveys it is difficult, even sometimes impossible, to use invasion methods, and so it is extremely important to use non-invasion methods for the prognosis of origins of genetic human abnormalities.

32.6 Use of the Micronucleus Test for Assessment of Genotoxic Influence

In recent years, in the conditions of the permanent worsening of ecological situation, it is important to assess the level of population health and the identification of potentially high-risk groups of people.

Damage to the genome is probably the most important and fundamental cause of the development of anomalies and degenerative diseases. It has been established that genomic damage is produced by exposure to (a) genotoxic substances, (b) medical procedures (such as from radiation and chemicals), (c) micronutrient deficiency (from folic acid), (d) life styles (relating to alcohol, smoking, drugs, and stress, for example), and (e) genetic factors (such as defects in metabolism and/or in the repair of DNA). Hence, it is essential to perform biomonitoring with minimally invasive markers. The micronucleus trial on exfoliated cells of the buccal mucosa is a potentially excellent biomarker candidate for monitoring studies [35]. In addition, the micronucleus test (MN-test) in buccal mucosa cells is one of the less invasive methods to measure DNA damage in humans.

Cells of buccal mucosa in the oral cavity are comfortable objects for estimating the physiology state of the organism [35, 39] and the influence of environmental factors such as: pesticides [7, 8, 16, 50, 51]; automobile exhaust-gases [13, 63]; oil products [18]; chemotherapy [11, 71]; genotoxicity agents [10, 12, 14, 16, 27, 28, 44, 45], as well as lifestyle habits [54, 55, 58].

As a criterion for estimation of negative influence of different environmental agents is presence and frequency of occurrence of cytogenetic abnormalities: cells with micronuclei (MN), binucleated cells, pycnosis, karyorechsis, karyolysis and other nucleus anomalies [35, 66, 70].

High correlation is set between the increase of number of chromosomal aberrations, activity of mitosis process and induction of micronuclei [51]. Micronuclei are formed from acentric fragments or whole chromosomes because of abnormalities of processes of cell division. The presence of micronuclei in cells is considered to be as the marker of genetic instability [7].

Considering the above mentioned, we have used the MN-test for assessment of mutagenic influence on genome of children living in the industrial regions with a high technogenic load.

32.7 Research Subjects

The subjects of the research were the cytogenetic status of human organism and the general mutagenicity in the Dnepropetrovsk region which has cities of different types and technogenic load levels, namely Marganets, Zholtyye Vody (Yellow Waters), Nikopol and Dnepropetrovsk. These cities are characterized by high levels of development of such branches of industry as mining, metallurgical and chemical manufacturing, ore production and uranium ore dressing. In the capacity of a local “control” an area with a low technogenic load was chosen, namely the area of the medically-improving complex “Solyony Liman (Silted Estuary)” located in the Novomoskovsk part of the Dnepropetrovsk region.

32.8 Materials and Research Methods

In a group used in the cytogenetic survey the healthy and practically healthy children of 5–7-years-old were selected by a special questionnaire [35, 66].

Subjects were required to rinse their mouths with water before sampling. Exfoliated epithelial cells of buccal mucosa were obtained by scraping the middle part of the inner cheek with a wadded tampon on a spatula. The epithelial cells collected from buccal mucosa were smeared onto clean microscope glass slides which were then air-dried and fixed with a mixture of ethanol and glacial acetic acid (3:1) within 1 h. Then the slides were stained with aceto-orcein.

A light microscope "Olympus" using 100-times magnification on coded slides was used for MN analysis. At least 1,000 cells per child were analyzed to determine MN frequency.

The scoring criteria used are mainly based on those originally described by Tolbert et al. [70]. Normal differentiated cells have a uniformly stained nucleus which is oval or round in shape. They are distinguished from basal cells by their larger size and by a smaller nucleus-to-cytoplasm ratio. No other DNA-containing structures apart from the nucleus are observed in these cells. These cells are considered to be terminally differentiated relative to basal cells because no mitotic cells are observed in this population group [66].

The micronuclei are round or oval in shape and their diameter should range between 1/3 and 1/16 of the main nucleus. Also, they have the same staining intensity and texture as the main nucleus. Most cells with MNi will contain only one MN but it is possible to find cells with two or more MNi.

MN-index was calculated in accordance with frequency of cells with MNi. The received experimental data were used for the calculation of conditional indices of damage (CID) for biosystems. On this basis an estimation was made of the ecological situation on the mutagen background [32, 33]. Using statistical analyses all the data were expressed as the mean ± standard error of the mean and results with $p < 0.05$ were considered significant.

32.9 Research Results

Analysis of results of cytogenetic survey of pre-school age children living in the technogenic loaded cities of the Dnepropetrovsk area (Table 32.1) showed an increase in frequency of micronuclei in buccal cells that is 3.5–4.8 times that found in those who live in the "conditionally clear" territory of the medically-improving complex "Solyony Liman".

It should be noted that, although during the period 2005–2009, there is a positive dynamic in the change of the studied index in the investigated cities, but these differences are not significant ($p > 0.05$). In addition, increases were observed in minimum and maximal values of MN-index ($p < 0.01$) during 2005–2009 in

Table 32.1 Results of micronuclei testing in buccal cells of pre-school age children living in cities of Dnepropetrovsk region ($p < 0.05$)

	MN-index, per cell; 1999–2004		MN-index, per cell; 2005–2009	
City	Average	Min – Max	Average	Min – Max
Dnepropetrovsk	0.077 ± 0.006 (n = 127)	0.024 ÷ 0.100	0.080 ± 0.007 (n = 98)	0.040 ÷ 0.120
Zholtyye Vody	0.111 ± 0.008 (n = 85)	0.053 ÷ 0.160	0.092 ± 0.007 (n = 75)	0.080 ÷ 0.170
Nikopol	–	–	0.089 ± 0.003 (n = 52)	0.056 ÷ 0.142
Marganets	0.085 ± 0.010 (n = 30)	0.045 ÷ 0.130	–	–
Local control "Solony Liman"	0.023 ± 0.004 (n = 53)	0.001 ÷ 0.042	0.026 ± 0.002 (n = 35)	0.001 ÷ 0.050

comparison with 1999–2004. This testifies the tension of ecological conditions in the investigated cities as a result of presence and accumulation of environmental pollutants having mutagenic properties.

In accordance with the methodology used [31, 32, 33], the calculated conditional indices of damageability (CID) of child organisms for cytogenetic parameters (considering the minimal (P comfortable) and maximal (P critical) values of the investigated parameter) testify that the total mutagen background in the Dnepropetrovsk region has to be considered "unsatisfactory" on the basis of a "threatening" condition for children's organism, and that the level of damage to their cells is "above average". Of the four investigated cities in the Dnepropetrovsk region the greatest index of micronuclei in epithelial cells of children is defined in the center of the uranium-extractive and uranium-processing mining industry, namely the city of Zholtyye Vody. In the control area there was defined a "low" level of genetic damages in epithelial cells and a "safe" condition of organism in respect of the cytogenetic status. It has allowed considering as "favorable" the ecological condition of the control territories of the medical-and-health improving complex "Solyony Liman".

In the investigated cities there were defined groups of children with an increased genetic risk having values of the MN-index equal to or exceeding 0.100. In Zholtyye Vody an elevated risk group included 48.4 % of the examined children, while in Dnepropetrovsk it was 35.9 %, in Marganets 40 %, and in Nikopol 36.5 %. As to the 'control' area, there are undetectable representatives with an elevated level of genetic disorders in somatic cells.

32.10 Rehabilitation/Prevention Measures

The rehabilitation program held at the base of Pulmonary Sanatorium of Dnepropetrovsk included the combined oral administration of humic substances (humics), carotene oil (pro-vitamin A), enterosorbents (pectin), and probiotics (acidophilus). All were officially approved and previously tested for food supplements. A humic food additive in the form of a 0.05 % solution of humic acid was used for 21 days according to instructions from the Pharmaceutical Committee of the Ukraine Ministry of Health.

Treatment was provided for 2 months to the 37 children suffering recurring bronchitis. All food supplement dosage levels were age-appropriate and reassessments were conducted at the end of the 2-month treatment period.

An anti-mutagenic effect was observed in 87.5 % of cases. The number of cells with MN decreased from 0.071 ± 0.008 to 0.037 ± 006 (i.e. $p < 0.01$). In addition, experiments displayed normalization of the immune system condition of child organisms and a reduction in the level of respiratory diseases by 1.3 times within the next autumn and winter period.

32.11 Conclusions

Under the influence of harmful environmental factors in human organism it is observed that there is an increase in the frequency of occurrence of cells with micronuclei. However, the condition of one's organism can be improved on the basis of using special rehabilitation courses with natural adaptogenes. The obtained data present a theoretical basis for the formation of rehabilitation programmes for population health status in technogenically-loaded areas.

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Chapter 33
Method Validation for Pesticides Identification

Camelia Drăghici, Elena Galan, and Maria Georgeta Stoian

Abstract Method validation for identification of some pesticides by gas chromatography coupled with mass spectrometry (GC-MS) was subject of internal validation procedure. Method performance criteria were investigated on standard sample measurements: selectivity, linearity, precision, limit of detection, and robustness. The validated method will be further used to identify the pesticides of interest in forensic analysis.

Keywords Method validation • Pesticide analysis • Carbofuran • Cipermetrin • Dinoseb • Dimetoat • Lindan • 2,4-D ester

33.1 Introduction

The modern society requires quality in chemical measurement results, in such a way to make them acceptable everywhere. Therefore over the last decade, studies and initiatives have been taken at international level to ensure that measurement science issues are applied in a systematic way. There are two ways to ensure the quality of chemical measurements: quality management systems and accreditation [28], but recently the principles of measurement science (metrology) were also applied to chemical measurements (metrology in chemistry – MiC) as a complementary tool for quality assurance (QA).

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Metrology in chemistry is assuming that chemical analysis is based on sampling and measurements, both stages contributing to the measurement uncertainty. The International Vocabulary of Metrology (VIM), gives the definitions of the concepts and terms used in MiC, especially since chemical measurement is also covered in the last version of VIM (2007) for the first time [7]. Considering the need for training the chemists in metrology in chemistry, initiatives aiming to set-up education and training modules in metrology in chemistry (TrainMiC) in order to disseminate knowledge and awareness on the use of metrological principles in chemical measurements [21]. Metrological principles are important for all analysts who deal with chemical measurements [27], the most relevant pillars of MiC being: traceability, uncertainty of measurement results, method validation, the use of certified reference materials (CRMs) and interlaboratory comparisons (ILCs).

This study aims to give an overview of the methods and methods validation principles used for pesticide determination, as well as to present a validation procedure for some pesticides identification.

33.2 Quality Assurance in Chemical Measurement Results

There are different levels of measures aiming to ensure quality assurance for the chemical measurements results, as presented in Fig. 33.1. At the minimal level, the laboratories have to demonstrate that they use methods validated prior to use. Then, they may implement internal quality control (IQC) procedures, or may participate to proficiency testing (PT) schemes together with other similar laboratories. For higher level quality assurance, laboratories may apply for accreditation, based on ISO/IEC 17025 standard, and establishing traceability of the results of their measurements.

In order to ensure the quality in chemical measurement results, in 1999 was launched the ISO/IEC 17025 accreditation standard, designed as guidance for general requirements for the competence to carry out tests and/or calibrations, including sampling, with standard methods, non-standard methods, or even laboratory-developed methods. ISO/IEC 17025 is based on the following aims:

- to provide a basis to be used by accreditation bodies when assessing the competence of laboratories;
- to establish general requirements for demonstrating laboratory compliance to perform specific tests or calibrations;
- to assist in the development and implementation of a laboratory's quality system.

Accreditation allows the laboratory to demonstrate that the results are defensible to a recognized standard and do not change when either laboratory personnel or circumstances are changing. The 2005 version (ISO/IEC 17025:2005) is applicable to any organization performing tests and/or calibrations. It is used by: (i) laboratories accredited or in accreditation process; (ii) accreditation bodies; (iii) regulatory authorities.

Fig. 33.1 Levels of quality assurance (Adapted after Taverniers et al. [20])

In order to obtain the accreditation, the laboratory has to provide information related to the origin of method (standard or no-standard), comparison with the standard methods they replace (if applicable) as well as the validation of data procedure.

33.3 Method Validation Procedure

The international science community is recognizing the need of method validation, both in research laboratories and in the accredited ones. Therefore international associations related to chemical analysis, like International Standardization Organization (ISO), International Union of Pure and Applied Chemistry (IUPAC), and Association of Official Analytical Chemists (AOAC) have started collaborating, aiming to agree on specific protocols or guidelines [22].

Method validation is dealing with the question whether the analytical purpose of the method is achieved, in order to obtain analytical results with an acceptable uncertainty level, with an acceptable standard of accuracy. According to the EURACHEM Guide [26], method validation is the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires. Validation is also considered to give the confirmation by examination and provision of objective evidence that the particular requirements of a specified intended use are fulfilled, or fit to purpose. A method should be validated whenever a change in the analytical process occurs, and the laboratory has to demonstrate that its performance parameters are adequate for its use for a particular analytical problem, meaning:

– new method was developed;
– the existing method was revised;

Table 33.1 Method performance criteria dependent on the analytical purpose

Method-performance parameter	Identification test	Impurity test Limit impurity test	 Quantitative impurity test	Assay test
Selectivity and specificity	+	+	+	+
Precision	–[a]	–	+	+
Trueness	–	–[a]	+	+
Linearity and linear range	–[a]	–	+	+
Limit of detection (LOD)	–[a]	+	–	–
Limit of quantification (LOQ)	–[a]	–	+	–
Robustness and ruggedness	+	+	+	+

Adapted to Taverniers et al. [20]
[a]May be performed

- method is changing with time;
- method is used in a different laboratory, or with different analysts or different instrumentation;
- the method is equivalent with another one, for example a standard one.

To validate a method is compulsory to demonstrate the following method performance criteria: specificity/selectivity, precision, trueness, linearity and linearity range, limit of detection (LOD), limit of quantification (LOQ), and ruggedness/robustness. As presented in Table 33.1 not all the method performance parameters are required for any validation procedure. They depend on the analytical purpose: identification tests; impurity tests (limit of impurity test or quantitative impurity test); assay tests.

Selectivity and specificity are measures which assess the reliability of measurements in the presence of interferences. A method is selective if it produces responses for a group of chemical entities or analytes which can be distinguished from each other, while the method is specific if it produces a response for a single analyte only. Whether or not other compounds interfere with the measurement of the analyte will depend on the effectiveness of the sample preparation stage (isolation, purification, clean-up) as well on the selectivity/specificity of the measurement stage.

Accuracy gives the degree of spread of data and is studied as two components: precision and trueness. Trueness of a method is a measure of how close the mean of a set of results (produced by the method) is to the true value, and is an expression of the systematic deviation of the measured result from the true result. Due to the fact that the true value is always not known, it is replaced by a reference value, and estimated by mean value. Therefore, practical assessment of trueness relies on comparison of mean results from a method with reference values, obtained using a (certified) reference material (CRM, RM). When (C)RM are not available, mostly used is the recovery test (R), based on spiking a blank sample with a known quantity of the pure and stable compound of interest (reference value), and calculated with Eq. 33.1. Precision of the method is a measure of how close results are to one another, and relates to the random error of a measurement system. Precision may be evaluated as: repeatability precision; intra-laboratory reproducibility precision or intermediate precision; inter-laboratory reproducibility precision. Usually, precision is expressed by measures such as standard deviation (s or SD), dispersion (s^2), or relative standard deviation (RSD%), according to the Eqs. 33.2 and 33.3:

$$R = \frac{C_F - C_I}{C_A} 100 \tag{33.1}$$

$$s = \sqrt{\frac{\sum_{1}^{n} (\overline{X} - X_i)^2}{n - 1}} \tag{33.2}$$

$$RSD = \frac{s}{\overline{x}} \cdot 100 \tag{33.3}$$

where: C_F is the final concentration of the spiked sample; C_I is the initial concentration before spiking; C_A is the concentration of the added spiking solution; X_i are discrete measured values, $\overline{X}$ is the average value of n repeated measurements.

Linearity and linearity range give information about the ability of the method to produce signal response in a linear relationship to the analyte concentrations (or quantity). At the lower end of the concentration range the limiting factors are the values of the limits of detection and/or quantitation.

Limit of detection (LOD) represents the lowest content of the analyte that can be measured with reasonable statistical certainty. It gives a "qualitative" information and may be expressed as concentration (c_L) or quantity/amount (q_L), derived from the smallest signal (x_L) which can be detected with reasonable certainty for a given analytical procedure. **Limit of quantification** (LOQ), or **limit of quantitation**, represents the lowest content of an analyte that can be determined with acceptable accuracy under the stated conditions of the test. It gives "quantitative" information and may be also expressed as concentration (c_L) or quantity/amount (q_L).

Robustness and ruggedness are measures of the ability of the method to produce unaffected results by variations interviewing either at the method parameter (robustness), or at the operation conditions (ruggedness).

33.4 Analytical Methods and Validation Procedures Applied for Pesticides Determination

Taking into account the structural and physic-chemical properties of the pesticides, there are several methods adequate for pesticide qualitative and quantitative determination. The most applicable are the chromatographic method, with gas chromatography (GC) and liquid chromatography (LC) techniques, as well capillary electrophoresis (CE), with micellar electrokinetic chromatography (MEKC) technique. GC is the mostly used one, but is limited to volatile pesticides. LC is applicable for water soluble pesticides, while CE also requires water soluble pesticides, which should also be electrically charged. For this reason, pesticides not being charged compounds, the only capillary electrophoresis techniques that can separate pesticides is the micellar one (MEKC). LC and CE with nonpolar (water) system are less applied. All these separation techniques use different detection systems to put in evidence the separated compounds.

There are available several combinations for separation-detection techniques for pesticides determination, as follows:

- for GC separations: nitrogen phosphorus detector (GC-NPD); flame ionization detection (GC-FID); electron capture detector (GC-ECD and GC-μECD); flame ionization detector (GC-FTD); electrochemical detection (GC-ELCD), Atomic Emission Detection (GC-AED); mass spectrometry (GC-MS); tandem mass spectrometry/mass spectrometry (GC-MS/MS); inductively coupled plasma mass spectrometry (GC-ICP-MS);
- for LC applications: UV detection (HPLC-UV); diode array detection (HPLC-DAD); mass spectrometry (LC-MS); tandem mass spectrometry/mass spectrometry (LC-MS/MS);
- for micellar electrokinetic chromatography separations: diode array detection (MEKC-DAD).

All these analytical techniques are used as standard methods, non-standard methods, or laboratory-developed methods. For example, the United States Environmental Protection Agency (US-EPA) has more than 90 standard methods for pesticides analysis, from different chemical calsses, from different matrixes, like GC-ECD, GC-ELCD, GC-AED, GC-MS, HPLC/UV are [16].

Separation techniques used for the pesticides determination are presented in Table 33.2. In order to show the interest for method validation in research activity, a selection of recent research publication is given, only from those that also included the method validation procedure, as applications for specific pesticides, from different matrixes, with information about the validation criteria that have been used.

From the selected studies, all of them (20) tested the method linearity (as linearity range and/or calibration curves), almost all of the studies tested the method repeatability (18), trueness by recovery tests (19), and LOD (19). Surprisingly, not all of them (14) applied LOQ performance criteria, while some of them were also interested by the interim precision. Even if selectivity and robustness are the only ones performance criteria to be tested in all validation procedures (according to Table 33.1), from the selected studies presented in Table 33.2, these to parameters were less applied. It might be considered that chromatographic methods are selective, implicitly demonstrated by the separation resolution, while robustness was considered the less relevant validation criteria.

On the other part, routine laboratories are also implementing standard, non-standard, or laboratory-developed methods. For example, in Romania, at this moment,

Table 33.2 Validation procedures applied for pesticides determination in different sample matrixes

				Validation criteria								
	Pesticides	Matrix	Analytical technique	Selectivity	Repeatability	Interim precision	Recovery	Linearity	LOD	LOQ	Robustness	Reference
1	Hexazinone, tebuthiuron, diuron	Soil	HPLC-UV		√	√	√	√	√	√		[14]
2	Organochlorines (bromocyclen enantiomers)	Fish tissue	GC-ECD; GC-ICP–MS		√			√	√			[9]
3	11 pesticides residues	Red wines	MEKC-DAD		√	√	√	√	√	√		[19]
4	Eight organophosphorus	Baby food	HPLC-UV	√	√	√	√	√	√	√		[3]
5	Coumaphos, coumaphosoxon, dichlorvos	Bovine milk and liver	LC-MS/MS	√	√	√	√	√		√		[11]
6	27 pesticides	Grapes, musts, wines	GC-MS; LP-GC/MS		√		√	√	√	√	√	[6]
7	Strobilurin fungicides	Baby foods	GC-MS	√	√		√	√	√	√		[23]
8	Heterocyclic insecticides	Waters (tap, lake fountain)	HPLC-DAD		√		√	√	√			[13]
9	Pyrethroid pesticides residues	Grapes	GC × GC-FID; GC × GC-μECD		√	√	√	√	√	√		[17]
10	Eight fungicides; Two carbamates; Two organophosphorus	Sludge, suspended materials	LC-MS	√	√		√	√	√	√		[2]
11	36 pesticides	Olive oil	GC-NPD; GC-ECD		√		√	√	√	√		[1]

(continued)

Table 33.2 (continued)

				Validation criteria								
	Pesticides	Matrix	Analytical technique	Selectivity	Repeatability	Interim precision	Recovery	Linearity	LOD	LOQ	Robustness	Reference
12 Insecticides (isocarbophos, isofenphos-methyl)	Insecticides (isocarbophos, isofenphos-methyl)	Peppers	GC-MS; GC-MS/MS		√	√	√	√	√			[15]
13	12 pesticides	Fruit juices	LC-MS		√		√	√	√	√		[18]
14	Organochlorines; pyrethroids; herbicides	Fish	GC-MS		√		√	√	√			[5]
15	16 pesticides	Surface and groundwater	GC–MS		√	√	√	√	√	√		[10]
16	Procymidone	Leeks, soil	GC–MS		√		√	√	√	√		[4]
17	Organophosphates	Water	GC–MS		√		√	√	√	√	√	[8]
18	Dimethomorph	Pepper, soil	GC-ECD		√		√	√	√	√		[12]
19	Fenpropidin	Wheat, soil	LC–MS/MS; GC-MS				√	√	√			[25]
20	Diafenthiuron	Pakchoi, soil	HPLC–MS				√	√	√			[24]

Other abbreviations: *GC* × *GC* two dimentional GC, *LP-GC/MS* low pressure GC mass spectrometry

Table 33.3 Analyzed pesticides in the accredited laboratories in Romania

Matrix	Pesticides class	Analytic technique
Atmospheric air	Organchlorines	
Gaseous effluents (emission)	Organophosphates	
Water (surface, groundwater, wastewater)	Carbamates	GC-ECD
Soil	Fenoxiacetic acids	GC-FTD
Wastes/leaches/sludge	Hidroxibenzonitrils	GC-MS
Food of animal origin	Pyrethroids	
Food of vegetal origin	Triazines	HPLC-UV

there are 27 laboratories, accredited by the national accreditation body (RENAR), which have validated and implemented typical chromatographic methods for different pesticides analysis, from different matrixes, as presented in Table 33.3 [29].

33.5 Method Validation for Pesticide Identification by Gas Chromatography Couppled with Mass Spectrometry

The analytes of interest were pesticides, most commonly found in forensic investigations, from different chemical classes and with different uses, as presented in Table 33.4.

33.5.1 Experimental Conditions

The method validation was performed at the laboratory of the Forensic Institute, Bucharest, Romania. A gas chromatograph (THERMO–FOCUS GC) coupled with mass spectrometry ionic trap (THERMO–POLARIS Q), equipped with autosampler (THERMO–TRIPLUS) was used. The carrier gas was He (source), capillary column (DB5), 30 m × 0.25 mm (source), solvents (methylene chloride, toluene (source)) were of HPLC purity and the data were processed according to the Romanian EURACHEM Guide, 1998 version. The validated method is further used for samples of soil or food matrixes. Pesticides were certified standards of analytical grade ($>99\ \%$) (source).

According to Table 33.1, the validation parameters tested for pesticides identification were: selectivity, precision (as repeatability and interim precision), linearity, limit of detection (LOD) and robustness. The only parameter not tested is LOQ, of no interest for the forensic analysis

33.5.2 Results and Discussions

Selectivity of the GC-MS method was evaluated by mean of the chromatographic resolution values (R_S) of consecutive separated pesticides (see Fig. 33.2.)

Table 33.4 Standard pesticides used for method validation

Pesticide	Chemical class	Pesticide use
Carbofuran	Carbamate	Insecticide, nematicide
Cipermetrin	Piretroid	Isecticide
Dimetoat	Organofosforic	Insecticide, acaricide
Dinoseb	Nitroderivative	Insecticide, herbicide
2,4-D ester	Ariloxiacids	Herbicide
Lindan	Organochlorurate	Insecticide, insectofungicide

Table 33.5 Selectivity, repeatability, interim precision and linearity tests results for the investigated pesticides

Pesticide	2,4-D ester	Dimetoat	Carbofuran	Lindan	Dinoseb	Cipermetrin
t_R (min)	18.77	22.56	23.10	24.06	25.54	36.84
R_S	–	8.71	1.74	3.15	4.77	37.05
$RSD_r\%$ (t_R) (<1 %)	0.08	0.05	0.01	0.03	0.07	0.01
$RSD_r\%$ (A) (<9.5 %)	1.18	2.69	3.55	2.52	4.68	4.66
$RSD_{ip}\%$ (t_R) (<1 %)	0.06	0.20	0.18	0.03	0.02	0.04
$RSD_{ip}\%$ (A) (<11.3 %)	0.92	5.56	1.87	1.33	4.20	1.31
r^2	0.9983	0.9998	0.9961	0.9993	0.9999	0.9990

calculated with Eq. 33.4, than compared with the accepted chromatographic limit for a good resolution, $R_S > 1.5$. The results are given in Table 33.5.

The obtained resolution values are lower than 1.5, indicating that the pesticides were separated at the base, meaning that the chromatographic method is capable to detect and separate selectively the investigated pesticides.

$$R_s = 2\ (t_{R2} - t_{R1})\ /\ (w_1 + w_2) \tag{33.4}$$

where: t_{R1} and t_{R2} are the retention times of two consecutive eluted compounds (1 and 2); w_1 and w_2 are the respective peaks widths at the peak base.

Repeatability of the GC-MS method was evaluated using standard pesticides solutions of different concentrations (0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 μg/mL), injected ten times each. RSD_r % was calculated according to Eq. 33.2, with both sets of values of retention times (t_R) and peaks areas (A), than compared with the accepted RSD_r % limits (see Table 33.5). **Interim precision** of the GC-MS method eas evaluated with standard pesticides of 1.0 μg/mL solutions, injected six times each, during three different days. RSD_{ip} % was calculated with both sets of values of t_R and A, than compared with the accepted RSD_{ip} % limits, as presented in Table 33.5.

Reproducibility and interim precision RSD% values, calculated with both retention times and peaks areas are lower than the accepted ones, demonstrate that the GC-MS method is precise.

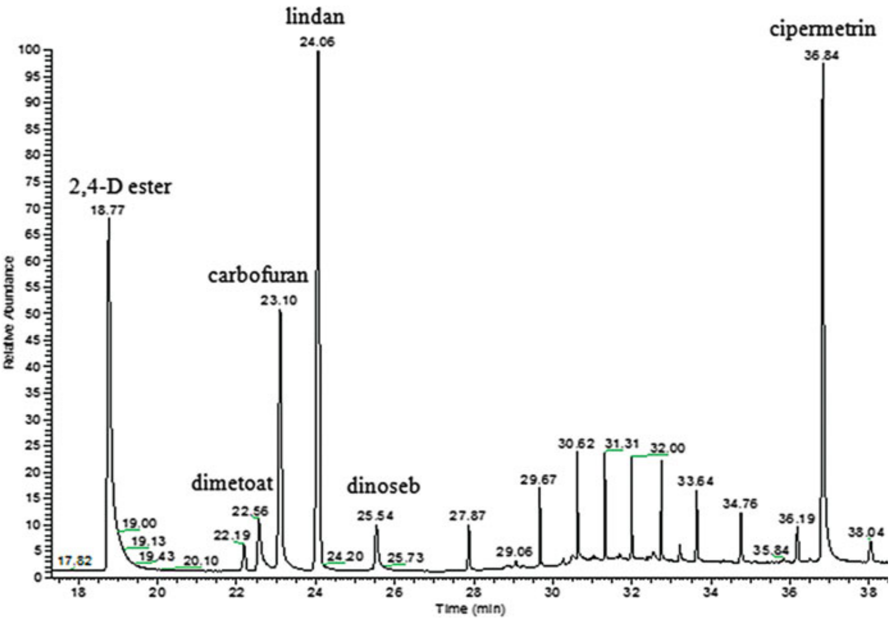

Fig. 33.2 GC-MS chromatogram of the standard pesticides (chromatographic conditions given in text)

Table 33.6 Limit of detection for the tested pesticides

Pesticide	2,4-D ester	Dimetoat	Carbofuran	Lindan	Dinoseb	Cipermetrin
RSD%	3.86	2.23	4.01	3.45	3.34	3.18
Concentration (μg/mL)	0.025	0.25	0.025	0.01	1	0.05

Linearity of the teste method was demonstrated using standard pesticides solutions of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1 mg/L. Calibration curves were plotted for each pesticide and the correlation coefficients (r^2) were determined. Figure 33.3 presents an example of the calibration curves (2,4-D ester).

The correlation coefficients determined for all pesticides are given in Table 33.5. The obtained values of $r^2 > 0.9950$ are showing that the detector response is liniar on the studied concentration range.

Limit of detection (LOD) was tested on standard pesticides solutions of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 μg/mL, injected six times each. RSD% was calculated and LOD was evaluated as the lowest concentration for which RSD % is <5 % (Table 33.6.)

Robustness was tested on standard pesticides solutions of 1.0 μg/mL, in analytical conditions slightly modified from the initial ones: gas flow, initial temperatura, temperature gradient, injector temperatura, ionic source temperature (see Table 33.7). RSD% values were determined with both retention time and peak area values (Tables 33.8 and 33.9).

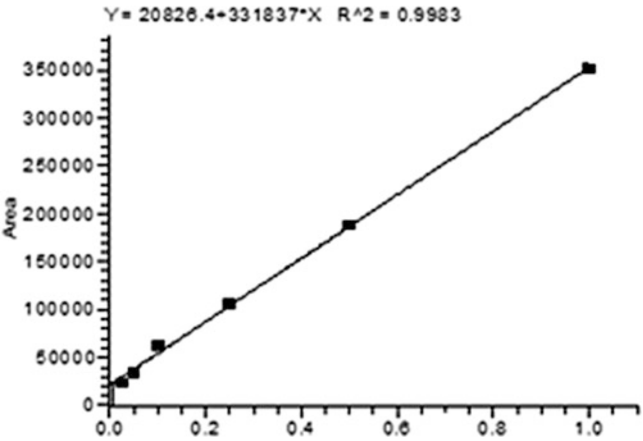

Fig. 33.3 Calibiration curve of 2,4-D ester

Table 33.7 Initial and modified experimental conditions

Experimental modified parameters	Gas flow (mL/min)	Initial temp. (°C)	Temp. gradient (°C/min)	Injector temp. (°C)	Transfer line temp. (°C)	Ionic source temp. (°C)
Initial values	1	80	10	250	280	250
Modified values	0.9	81	11	260	270	230

Table 33.8 Robusteness evaluation by retention times RSD %

t_R with initial or modified parameters values	2,4-D ester	Dimetoat	Carbofuran	Lindan	Dinoseb	Cipermetrin
t_R with initial parameters	19.00	22.65	23.09	24.04	25.61	36.84
t_R with modified parameters						
gas flow (mL/min)	18.95	22.11	22.85	23.84	25.75	36.63
Initial temperature (°C)	19.01	22.61	23.08	24.04	25.60	36.84
Temperature gradient (°C/min)	18.99	22.64	23.09	24.05	25.62	36.85
Injector temperature (°C)	19.02	22.62	23.09	24.05	25.60	36.84
Transfer line temperature (°C)	19.02	22.64	23.09	24.06	25.60	36.85
Ionic source temperature (°C)	19.00	22.64	23.09	24.05	25.61	36.85
RSD% (by retention times)	0.14	0.93	0.42	0.36	0.23	0.24

To be noticed that RSD% calculated with retention time values are lower than 1 %, while the RSD% calculated with peak area values are lower than 8 %, demonstrating that no relevant changes are registered when altering the operational parameters of the method, GC-MS being a robust one for pesticides determination.

Table 33.9 Robusteness evaluation by peak areas RSD %

Robustness evaluation	2,4-D ester	Dimetoat	Carbofuran	Lindan	Dinoseb	Cipermetrin
t_R with initial parameters	376,428	21,287	25,690	81,791	89,766	3,952
t_R with modified parameters						
Gas flow (mL/min)	356,012	19,250	25,031	81,077	86,916	4,220
Initial temperature (°C)	356,364	18,775	24,323	82,525	88,121	4,145
Temperature gradient (°C/min)	352,040	18,240	24,837	82,212	96,396	3,895
Injector temperature (°C)	351,043	22,336	24,332	80,714	93,789	4,684
Transfer line temperature (°C)	361,423	20,493	22,431	82,975	86,249	4,172
Ionic source temperature (°C)	360,191	21,338	23,676	81,767	83,758	4,699
RSD% (by retention times)	2.38	7.51	4.32	0.97	4.96	7.58

33.6 Conclusions

An overview on the importance of accreditation and method validation was presented, together with analytical methods available for pesticides determination. The necessity of method validation in different required conditions was underlined, for both research and routine laboratories. A validation procedure was also presented, to be used for pesticides identification from soil and food matrixes, of interest for forensic laboratories. The results demonstrated that GC-MS is an adequate method for pesticides identification, being validated by the required performance criteria: selectivity, precision, linearity, limit of detection and robustness.

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Chapter 34
Analytical Study of Obsolete Pesticides Stockpiles in the Republic of Moldova into Nato Science for Peace Project "Clean-Up Chemicals – Moldova"

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Abstract In order to assist in the environmental risk assessment, ultimate obsolete pesticides disposal, and eventual restoration of the dump sites into ongoing projects in the Republic of Moldova, NATO awarded a Science for Peace and Security Project (Top-Down EAP SfPS 981186 "Clean-up chemicals – Moldova") with basic aims focused on the establishment of a laboratory infrastructure with sophisticated equipment for analysis and characterization of obsolete pesticides, including persistent organic pollutants (POPs) into the various waste dumps, training of personnel for the characterization, and elaboration of a comprehensive database of the composition of the waste dumps and their toxicological evaluation. The results showed that from more than 70 types of detected pesticides, 49 of them have been prohibited or restricted for use in agriculture, in Moldova since 1970, while the use of 24 substances was prohibited in the European Union countries. In all sampling sites were detected DDT and its isomers/metabolites (DDE and DDD), alpha-, beta-, gamma-HCH, which are defined as POPs in the Stockholm Convention due their persistence and accumulation in the fatty tissues of most

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living organisms. They are also prone to long rang trans-boundary transport and are likely to cause significant adverse human health or environmental effects.

Keywords Obsolete pesticides • Persistent organic pollutants • Gas chromatography-mass spectrometry • High-pressure liquid chromatography • Ion sensitive electrode measurements

34.1 Introduction

NATO's Political Affairs started a project under its Trust Funds Program to assist the Government of the Republic of Moldova with the destruction of pesticides and hazardous chemicals stockpiles of amounting to 3,250 ton. The project is implemented in four phases which have been supported by NATO/PfP-OSCE/ENVSEC: (i) repacking and centralization, (ii) chemical analysis (the Science for Peace and Security Project 981186), (iii) destruction and (iv) remediation of contaminated sites [4].

The NATO Partnership for Peace (PfP) Trust Fund as prepared by the NATO Maintenance and Supply Agency (NAMSA) defined the needs for chemical analysis as follows: "*A programme of analysis will be necessary to confirm the nature of the chemicals involved. This could be critical, as the condition of the storage of the pesticides has resulted in mixtures of compounds, which have yet to be fully identified. The extent of the analysis that is necessary will be dictated by the nature of the destruction method eventually selected*".

In order to assist in the ultimate waste disposal NATO awarded a Science for Peace Project SfP 981186 "Clean-up of Chemicals Moldova" in January 2005 for a period of 5 years with basic aims of the establishing laboratory infrastructure and training of personnel for the characterization of obsolete pesticides (OPs), including persistent organic pollutants (POPs) in various waste dumps in Moldova; the elaboration of a comprehensive database of the composition of the waste dumps and their toxicological evaluation [4].

In the project a central laboratory was created and equipped in terms of man-power and instrumentation to perform the full scale characterization of the pesticide stocks. In the first phase of the project equipment was installed for the analysis of volatile (non-polar) type pesticides and other chemicals on the basis of gas chromatography-mass spectrometry (GC-MS) and in the project extension was added instrumentation for the determination of more polar organic compounds with high-pressure liquid chromatography (HPLC). Ion sensitive electrode measurements (ISE) were used for the determination of a number of cations and anions.

During a prolongation for 1 year (in period 01 February 2009 – 31 January 2010) the project activities focused on environmental analysis of pesticides residues in soils close to the pesticides landfill of Cismichioi, Gagauzia Territorial Autonomy where has been buried more than 4,000 ton of pesticides, including 654.1 ton of DDT during '70–'80. The site was selected because it is considered as one of the national priority sites and requires urgent attention in order to eliminate acute risks for the public health and environment. The site is only a few km away from the Ukrainian and Romanian borders and close to watersheds discharging in the Prut River and the Danube near to its estuary. Under the Action Plan for Implementation of Stockholm Convention on Persistent Organic Pollutants, were conducted research studies around the site in the NATO Science for Peace Project "Clean-up chemicals – Moldova" and showed migration at least ten dangerous pesticides (atrazin, simazine, alpha-HCH, beta-HCH, Gamma-HCH, delta-HCH, dazomet, prometryn, triflualin, DDTs) and sulphur containing compounds characterized in the mass spectra through six, seven or eight sulfur atoms [2, 3]. Thus, it will contribute in solving the most pressing issues related to environmental quality and human health, the integration of environmental concerns in national economy sectors and promotion of sustainable development.

The project was recognized by the Moldovan Ministry of Environment as most important for the country. The NATO-data base has been assisted the Global Environment Facility/World Bank Project for the Management and Disposal of Persistent Organic Pollutants stockpiles on evacuation and destruction in France of 1,296 ton of obsolete pesticides from 12 regional central storages, the ongoing Project "Remediation of environmental burdens by pesticides waste in Moldova" funded by Czech Development Agency, aims to destroy in Germany 200 ton of pesticides waste from three storages, and ongoing NATO/PfP Trust Fund Project for destruction of 1,269 ton of obsolete pesticides from 15 regional central storages. In addition the project aims at a gradual build-up of the competence level in the country for the analysis and the evaluation of environmental and toxicological risks of dangerous organic and inorganic contaminants in different compartments of the environment (soil, water, air) and in various agricultural products e.g. wines as the country's important export product [1]. Also, this data base was a useful tool on the eventual restoration of the dump sites.

The project provided a comprehensive database of the composition of the waste dumps and their toxicological evaluation. In the project emphasis is placed on compliance with general laboratory safety standards for containing solvents and reagents. Quality criteria were put in place to arrive at high quality data for the chemical characterization.

An overview was obtained of the nature and composition of 3,245 ton of pesticides stored in two municipalities, 32 districts and the Territorial Autonomy Gagauzia from Moldova. Five thousand nine hundred and eighty samples were analyzed (3,479 samples analyzed with GC-MS: 2,141 samples – identified and 1,338 samples – non-identified; with HPLC – 1,331 samples were tested: identified – 249 and non-identified – 1,082; with TLC – 120 samples; with ion selective electrode analysis – 1,050 samples: identified – 816 samples, non-identified – 191 samples), which provided up to now the identification rate of various chemicals in ca 93 % of the samples.

The sites from which samples were collected for analysis follow the location of pesticide storage sites defined in the NATO PfP Trust Fund project for the destruction of pesticides and dangerous chemicals in Moldova. The number of samples per sampling site was chosen as a function of the overall complexity of the dumps.

Also, in the project we identified the nature of pesticides, which were found additional buried or stored in 33 locations of 13 districts of the Republic of Moldova.

The proportion of analysis of pesticides samples by GC-MS, HPLC, ISE methods of analysis is also given in Table 34.1.

34.2 Materials and Methods

34.2.1 Gas Chromatography-Mass Spectrometry Method of Analysis

The combination of gas chromatography (GC) and mass spectrometer (MS) into a single GC-MS system constitutes a powerful instrument that is capable of separating mixtures into their individual components, identifying them and then providing quantitative and qualitative information on the amount and chemical identity of each compound present in the sample by comparison of the data with a computer library information (NIST library). Qualitative analyses of samples of unknown pesticides were analyzed using a gas chromatograph (GC Agilent Technologies 6890N) connected with a mass selective detector (MSD Agilent Technologies 5973) equipped with a split/splitless programming. The capillary column used is HP-5MS (30 m × 0.25 mm × 0.25 μm).

The standard operating procedures (SOPs) for the analysis were established with essential support of the University of Antwerp, Belgium, as follows.

Weigh with analytical balance 20 mg sample and solubilise into 10 ml mixture of dichloromethane (DCM)-acetone (Ac) 9:1. mix 1 min (Biovortex Biosan V1) with filtration through anhydrous sodium sulfate. The front inlet and detector (MS Quad and MS Source) temperatures were 275, 150 and 230 °C respectively. The samples were injected (1 μl) by applying the SPLIT-SHORT – method in ratio 1: 50. To avoid some reaction or converting of samples into another substance, helium is used as the carrier gas. Helium with high purity 99.9999 % is used at 10.48 psi and a constant flow of 37 cm^3/s constant flow of 1.0 ml/min and total flow rate of 7.5 ml/min.

The temperature of the oven was programmed from 120 °C (hold for 0.50 min) to 280 °C at the rate of 10 °C/min (hold for 6.0 min). If there remains a residue of the sample a mixture of methanol (MeOH)-ethyl acetate (EtOAc) (1:9) is used.

In order to confirm the information about detected pesticides were used the NIST library as well as for the identification of isomers and metabolites of some pesticides were used the analytical standards like as Atrazine, Simazine, DDT, DDE and DDD, *alpha*-, *beta*-, *gamma*-HCH, Dicofol (from Russian origin), Diazinon, Promertryn (“Mackteshim Agan”), Chlorpyrifos (“Dow AgroSciences”), Metribuzin, Triadimefon (“Bayer Crop Science”), Acetochlor, and Metolachlor (“Syngenta”).

Table 34.1 Overview of the characterization of the sampling sites by GC-MS, HPLC and ISE methods of analysis

				GC-MS			HPLC			ISE			
Sampling site	Responsible for repacking	Quantity of Ops (ton)	Disposal status	Total	Id.	Not	Total	Id.	Not	Total	Id.	Not	% id
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Anenii Noi	NATO/GRM	47.68	Disposed 20.6	62	53	9	9	0	9	9	9	0	100
Basara-beasc	NATO/GRM	47.268	–	87	46	41	41	1	40	40	33	7	92
Briceni	GRM	132.0	132.0	258	155	103	103	10	93	93	48	45	83
Cahul	NATO/GRM	77.519	–	95	52	43	43	10	33	33	28	5	95
Calarasi	NATO/GRM	132.13	–	22	18	4	4	0	4	4	4	0	100
Cantemir	GRM	12.211	12.211	100	40	60	60	5	55	55	35	20	80
Causeni	GRM	141.71	141.71	62	53	9	9	3	6	6	6	0	100
Ciadir-Lunga	GRM	76.81	–	131	82	49	49	14	35	35	24	11	92
Cimislia	GRM	170.00	170.0	100	64	36	36	2	34	34	0	0	66
Comrat	NATO/GRM	139.17	–	244	96	148	148	94	54	54	43	11	95
Criuleni[a]	NATO/GRM	205.87	–	221	163	58	58	12	46	46	40	6	97
Dondu-seni	NATO/GRM	54.875	–	39	23	16	16	1	15	15	9	6	85
Drochia	GRM	23.468	–	28	26	2	2	0	2	2	2	0	100
Edinet	GRM	9.199	–	13	10	3	3	0	3	3	2	1	92
Ialoveni	NATO	123.44	–	76	28	48	48	22	26	26	26	0	100
Falesti	NATO	200.19	–	83	44	39	39	5	34	34	28	6	93
Floresti	GRM	155.00	155.0	100	74	26	26	4	22	22	21	1	99
Glodeni	NATO/GRM	38.870	–	103	58	45	45	12	33	33	27	6	94
Hincesti	Milieukont	104.50	104.5	112	96	16	16	4	12	12	12	0	100
Leova	NATO/GRM	56.771	–	110	64	46	46	2	44	33	20	13	78
Nispo-reni	GRM	54.00	54.00	160	98	62	62	7	55	55	49	6	96
Ocnita	NATO/GRM	16.000	16.00	120	76	44	44	4	40	19	7	3	80
Orhei	NATO/GRM	57.449	–	96	62	34	34	0	34	34	23	11	89
Riscani	NATO	65.94	65.94	53	42	11	11	0	11	11	8	3	94

(continued)

Table 34.1 (continued)

				GC-MS			HPLC			ISE			
Sampling site	Responsible for repacking	Quantity of Ops (ton)	Disposal status	Total	Id.	Not	Total	Id.	Not	Total	Id.	Not	% id
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Rezina	GRM	34.120	–	50	36	14	14	0	14	14	12	2	99
Singerei[b]	NATO/GRM	195.35	–	150	64	86	86	9	77	77	77	0	100
Soroca	NATO	34.446	–	55	36	19	19	0	19	19	18	1	99
Soldanesti	GRM	35.00	35.00	105	81	24	24	0	24	24	23	1	99
Stefan-Voda	GRM	215.4	215.4	80	65	15	15	0	15	15	15	0	100
Straseni	NATO	58.20	58.20	71	51	20	13	0	13	13	11	2	97
Taraclia	NATO/GRM	190.7	–	196	88	108	108	26	82	82	68	14	93
Telenesti	GRM	305.50	305.5	100	61	39	39	0	39	39	39	0	100
Ungheni	GRM	97.015	–	107	89	18	18	0	18	18	10	8	93
Vulca-nesti	NATO/GRM	34.894	34.89	90	47	43	43	2	41	41	39	2	98
TOTAL				**3,479**	**2,141**	**1,338**	**1,331**	**249**	**1,082**	**1,050**	**816**	**191**	**93**

[a] The Criuleni site contains pesticides from the municipality of Chisinau (139.2 ton), the Criuleni district (45.7 ton) and the Dubasari district (21.008 ton)

[b] The Singerei site contains pesticides from the Singerei district (130,322 ton) and the municipality of Balti (32,516 ton)

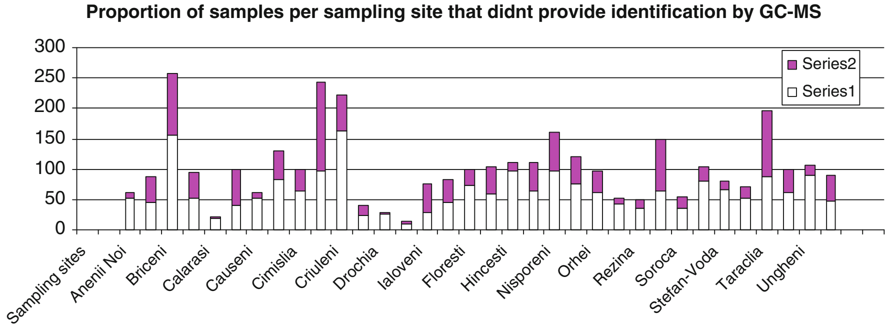

Fig. 34.1 Success rate of detection of pesticides in each site

A total of 3,479 samples from 2 municipalities in 32 districts of Republic of Moldova and the Autonomy Territory of Gagauzia were analyzed using GC-MS method. In 2,141 of these one or more pesticides were identified while detection of any pesticides failed in the other 1,338 samples. Figure 34.1 show the proportion of samples that provided a positive identification of pesticides.

From the start of the project a quality assurance and quality control procedures were used regularly for producing defensible data of known and precision through two procedures.

- A GC-MS tuning check was used to verify the performance of the GC-MS system before samples are analysed and after every ten analysed samples;
- The instrumental blank was measured after every ten samples and in special after high concentration of samples in organic analysis or organochlorine pesticides analysis;
- Laboratory control of sample was done to control the quality of analysis through repeated analysis of duplicate samples. Therefore, larger samples homogenized and split in several aliquots.

Quality assessment ensued that the quality control measures are implemented correctly. We studied equipment blanks (to verify if the equipment blank is contaminated or if the solvent used in blank preparation, or if the GC-MS system is contaminated, or if laboratory contamination may have taken place) and we evaluated the correlation between the results of the primary and the duplicate samples prepared in the field. Quality control through duplicate samples, control samples and blank samples confirmed that the laboratory personnel respected the rules and maintenance of the procedures during the analysis period but permanent attention is maintained to check regularly the quality of the data.

With the instrumentation available in the laboratory (and in the country) it is not possible to obtain compositional information on those samples that do not give rise to the detection of pesticides with the GC-MS instrument.

34.2.2 Thin Layer Chromatography

A total of 120 samples which could not be identified through the GC-MS method and have a non-crystalline appearance were analyzed supplementary by TLC in different systems of eluent: hexane-acetone (3:2), chloroform-acetone (9:1), ethyl acetate-citric acid (95:5), benzene-citric acid (4:1), on the plate Silufol, Merck, Sorbfil (UV at 254 nm).

The following reagents were applied: *o*-tolidine, bromphenol blue + $AgNO_3$, alpha-naphtol, *p*-diethylamino-benzaldehyd, diphenylamine, *p*-nitro aniline + KOH, iodide, copper chloride, and palladium chloride for detection of the following functional groups: urea, NH_2-, NH-, OH-, halogen, sulfur, unsaturated links, and coordinative compounds.

The results of this analysis were negative with the exception of six samples, which were positive to “bromphenol blue + $AgNO_3$” – reagent and visible in the UV. Further identification of these pesticides is possible by using the standards of pesticides of older generations that are presently not applied anymore for agriculture in the country.

TLC was applied originally but proved to be too difficult to apply in routine work.

34.2.3 High Pressure Liquid Chromatography

The HPLC-DAD system was intensively used for the characterization of samples which could not be identified by GC-MS. The HPLC-DAD equipment was used for routine analysis instead of TLC starting in 2009.

The mass spectral database of the GC-MS instrument contains information on a large number of organic compounds, including degradation products. However, a number of pesticides may not be suitable to be analyzed by GC-MS, because of their polar or less volatile nature or because their instability and degradation at higher temperatures.

The Library data base PESTICID UVL was optimized on HPLC for the analysis of 36 active ingredients of pesticides, which cannot be analyzed by GC-MS: 2,4-D, difenoconazole, propiconazole, penconazole, benomyl, dicamba, lenacil, carbofuran, desmedipham, phenmedipham, etofumesat, fenarimol, diflubenzuron, folpet, diquat dibromide, clorsulfuron, tribenuron, flusilazol, florasulam, azoxistrobin, acetamiprid, imidaclorprid, tiram, carboxin, rimsulfuron, chlorosulfuron, nicosulfuron, trifensulfuron-methyl and diflubenzuron, for which standards were also available.

A summary of the measurement conditions is presented in Table 34.2.

An overview of the success of identification of samples per sampling site by HPLC is provided in Fig. 34.2.

Table 34.2 Measurement conditions for the Library PESTICID UVL with HPLC

Type of compounds	Type of column	Eluent	Flow rate eluent (ml/min)	λ (nm)	Aliquot (μl)	Retention time (min)
1	**2**	**3**	**4**	**5**	**6**	**7**
EXPRES,M (different chemical groups)						
Tiram	Zorbax C_{18}	75 % ACN: 25 %H_2O	1	233	5	12.60
Carboxin	Zorbax C_{18}		1	254	5	12.65
Rimsulfuron	Zorbax C_{18}	30 % ACN: 70 % H_2O, at pH-3,0 with H_3PO_4	1.5	254	10	9.46
Clorsulfuron	Zorbax C_{18}		1.5	254	10	9.45
Tifensulfuron-metil	Zorbax C_{18}		1.5	254	10	6.02
Nicosulfuron	Zorbax C_{18}		1.5	254	10	3.87
Diflubenzuron	Zorbax C_{18}		1.5	254	10	
2,4-D	Zorbax C_{18}	40 % ACN: 60 % H_2O, at pH-4,2 with H_3PO_4	1.5	230–360	10	8.54
Dicamba	Zorbax C_{18}		1.5	230–360	10	5.53
Tiram	Zorbax C_{18}		1.5	230–360	10	8.42
Lenacil	Zorbax C_{18}		1.5	230–360	10	4.42
Benomil	Zorbax C_{18}		1.5	230–360	10	1.22
Diflubenzuron	Zorbax C_{18}		1.5	230–360	10	35.68
Clorsulfuron	Zorbax C_{18}		1.5	230–360	10	6.12
Carbofuran	Zorbax C_{18}		1.5	230–360	10	6.03
Desmedipham	Zorbax C_{18}		1.5	230–360	10	18.27
Fenmedipham	Zorbax C_{18}		1.5	230–360	10	18.37
Etofumesat	Zorbax C_{18}		1.5	230–360	10	33.54
Fenarimol	Zorbax C_{18}		1.5	230–360	10	18.46
Diquat dibromid	Zorbax C_{18}		1.5	230–360	10	1.33
Difenoconazole	Zorbax C_{18}		1.5	230–360	10	1.44
Propiconazol	Zorbax C_{18}		1.5	230–360	10	37.57
Penconazole	Zorbax C_{18}		1.5	230–360	10	29.35

(continued)

Table 34.2 (continued)

Type of compounds	Type of column	Eluent	Flow rate eluent (ml/min)	λ (nm)	Aliquot (μl)	Retention time (min)
1	**2**	**3**	**4**	**5**	**6**	**7**
Tribenuron methyl	Zorbax C_{18}		1.5	230–360	10	12.24
Flusilazol	Zorbax C_{18}		1.5	230–360	10	32.27
Florasulam	Zorbax C_{18}		1.5	230–360	10	6.58

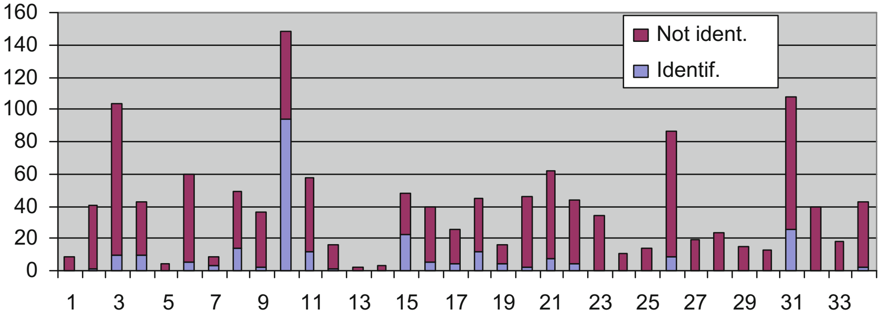

Fig. 34.2 Success rate of identification per sampling site by HPLC methods of analysis

34.2.4 "Tetratest Kits" for Cations and Anions

At beginning of the project, test kits were used for the detection of a number of common cations and anions present in fertilizers. Ten samples from three sampling sites were analysed with spot tests for a number of cations and anions (Tetratest kits) of which three pointed to a composition indicating a fertiliser. A procedure based on the spot tests described below was used throughout the experiments. The procedure relied on the following:

- Observation of general appearance (color, form, particle size and crystalline appearance);
- The sample is dissolved in water and the insoluble fraction is separated. The insoluble fraction is dissolved in dilute HCl solutions and in the soluble fraction NH^{4+} is identified after the treatment of the sample with NaOH and heating with paper filter imbibed with $Hg_2(NO_3)_2$ solution;
- Cations of group I-V are separated from those of group VI (acid-basic analyzed method) in fraction soluble with Na_2CO_3 on heating;
- K^+ is identified with $Na_3[Co(NO_2)_6]$, $Na_2[Cu(NO_3)_6]_2$, $NaHC_4H_4O_6$;

- Ca^{2+} is identified with H_2SO_4 (micro-crystallic reaction) or with $(NH_4)_2C_2O_4$ in NH_4OH;
- Zn^{2+} is identified with Na_2S in dilute acidic medium, $K_4[Fe(CN)_6]$ or with dithizone;
- SO_4^{2-} is identified with Ba^{2+} in the presence of the HNO_3;
- PO_4^{3-} is identified with $(NH_4)_2MoO_4$ in HNO_3 medium using heating or with magnesia solution ($MgCl_2$, NH_4OH, NH_4Cl);
- In the fraction soluble in water NO_3^- is identified with diphenylamine, or by reduction to NO_2 with metallic Zn in CH_3COOH medium and the identification of NO_2^- with KI;
- For the identification of S^{2-} in organic compound, the solutions, obtained by dissolving of the samples in HCl is treated with NaOH 6 mol/l until basic medium, then Br_2 is added after which the solution is acidified with concentrated HNO_3 and Ba^{2+} is added.

Later this method was changed by the application of ion selective electrodes (ISE).

34.2.5 Ion Selective Electrode Analysis for Determination of Cations and Anions

To obtain information on inorganic substances, 1,050 samples from 33 sampling sites were analyzed with ISE, which did not provide a positive identification of pesticides with GC-MS and HPLC with the intention to determine a possible inorganic composition. In 816 of these samples were detected inorganic compounds and 234 samples could not be detected.

34.3 Results and Discussion

A concise evaluation of the results was obtained with the different instrumental analytical techniques as GC-MS, HPLC, TLC, and ISE.

The NATO/PfP Trust Fund Project for the destruction of pesticides and dangerous chemicals in the Republic of Moldova defined 110 different pesticides. Amongst these products more than 70 active ingredients and metabolites were detected. They can be summarized and categorized as follows:

(a) Inorganic fungicides: S_6, S_7, S_8, Mancozeb,
(b) 1,2,3-Triazines: Simazine, Atrazine, Propazine, Prometryn,
(c) Organochlorine: *alpha*-HCH, *beta*-HCH, *gamma*-HCH, *delta*-HCH, DDT and metabolites, Heptachlor, Tetradifon, Propachlor,
(d) Isothiocyanate precursor: Dazomet,
(e) Derivatives of nitrophenols: Dinobuton, Dinoseb,

(f) Amide: Diphenamid, Propyzamide,
(g) Chloroacetanilide: Acetochor, Metolachlor,
(h) Thiocarbamate: EPTC, Dicofol, Ziram,
(i) Urea: Linuron,
(j) Organophosphate: Diazinon, Parathion-methyl, Clorpiryfos,
(k) Triazinone: Metribuzin,
(l) Uracil: Lenacil,
(m) Acylalanine: Mefenoxam
(n) Others: Trifluralin, Carbaril, 2,4-D, Dicamba, Tiram.

These pesticides stockpiles-waste must be considered as class I of toxicity waste and they present high risks for the environment and for public health.

Among the identified compounds 49 of them have been prohibited or restricted for use in agriculture, in Moldova since 1970, while the use of 24 substances was prohibited in the European Union countries.

Although their use was prohibited since 1970, the POPs defined in the Stockholm Convention such as DDT and its isomers/metabolites (DDE and DDD), alpha-, beta-, gamma-HCH were found in all sampling sites. The dispersal of these compounds needs to take into consideration, because their toxic characteristics are related to the persistence and the accumulation in the fatty tissues of most living organisms. They are also prone to long rang trans-boundary transport and are likely to cause significant adverse human health or environmental effects.

Although their use was banned since 1990 in the country, the most abundantly detected pesticides belong to the group of triazine herbicides (Smazine, Atrazine, Prometryn, Propazine) and their degradation products. The degradation products are more toxic than their active ingridients and characterized by a high stability in the environmental compartments (water, soil). A detailed list of identified products of degradation of analyzed pesticides is provided in the Table 34.3.

Active ingredients of old pesticides and fertilizers were identified in about 93 % of the total number of pesticide samples by different methods of analysis as GC-MS, HPLC, TLC and ISE.

Recently, commercialized pesticides such as Carboxin, Vinclozolin, Mephenoxam (Metalaxyl) and Triadimefon were only very occasionally detected.

A number of products that were detected such as Ethylthioourea, Dimethylthiourea and Trimethylthiourea are not active ingredients but are possibly decomposition products of sulfur- and urea-containing pesticides during storage e.g. through the degradation of Mancozeb-containing formulations. It cannot be excluded, however, that some of these might also result from reactions at high temperature in the injector of the GC-MS.

HPLC instruments provide an analysis of only 19 % from samples, which could not be identified by GC-MS. This is because the instrument is not provided with a library and automation and is more difficult to operate without MS detector.

Circa 77 % of the tested samples that do not provide identification with GC-MS and HPLC show the presence of the inorganic compounds. The main parts of the

Table 34.3 Detected products of degradation of obsolete chemicals

Nr,	Active ingredient	Products of degradation
	Simazin ($C_7H_{12}ClN_5$, MW:: 201)	Major metabolite I: 1,3,5-triazin-2-amine, 4,6-dichloro-N-ethyl-; $C_5H_6Cl_2N_4$, MW: 190; Metabolite II: 1,3,5-triazine-2,4-diamine,6-chloro-N-ethyl-; $C_5H_8ClN_5$, MW: 173; Metabolite III: 4,6-Bis(ethylamino)-1,3,5-triazine-2-carboxamide; $C_8H_{14}N_6O$, MW: 210,
	Dinobuton ($C_{14}H_{18}N_2O_7$, MW: 326)	Dinoseb: phenol, 2-(1-methylpropyl)-4,6-dinitro-; $C_{10}H_{12}N_2O_5$, MW: 240,
	Parathion-Methyl ($C_8H_{10}NO_5PS$, MW: 263)	Metabolite: phenol, 4-nitro; $C_6H_5NO_3$, MW: 139,
	Linuron ($C_9H_{10}Cl_2N_2O_2$, MW: 248)	Major metabolite I: Swep; $C_8H_7Cl_2NO_2$, MW: 219; Metabolite II: benzene, 1,2-dichloro-4-iso cyanato-; $C_7H_3Cl_2NO$, MW: 187; Metabolite III: benzenamine, 3,4-dichloro-; $C_6H_5Cl_2N$, MW: 161,
	Diphenamid ($C_{16}H_{17}NO$, MW: 239)	Metabolite I: benzeneacetic acid, *alpha*-phenil, methyl ester; $C_{15}H_{14}O_2$, MW: 226; Metabolite II: ethene, 1,1-diphenyl-2,2-dichloro-; $C_{14}H_{10}Cl_2$, MW: 248,
	p,p′-DDT ($C_{14}H_9Cl_5$, MW: 352)	o,p′-DDE: $C_{14}H_8Cl_4$, MW: 316; p,p′-DDE: $C_{14}H_8Cl_4$, MW: 316; DDD: $C_{18}H_{20}Cl_2$, MW: 308; DDMU: $C_{14}H_9Cl_3$, MW: 282,
	Mancozeb ($[C_4H_6MnN_2S_4]_xZn_y$, MW: 271)	Ethylenethiourea: $C_3H_6N_2S$, MW: 102 Tetrazene: 2-tetrazene, 1,1,diethyl-4,4-dimehyl-; $C_6H_{16}N_4$, MW: 144; Sulfur-6: MW: 192; Sulfur-7: MW: 224; Sulfur-8: MW: 256,
	Metribuzin ($C_8H_{14}N_4OS$, MW: 214)	Two non-identified metabolites
	Methyl niclate ($C_6H_{12}N_2NiS_4$, MW: 298)	Metabolite: Nickel, bis(dimethylcarbamodithioato-S,S')-, (SP-4-1)-, $C_6H_{12}N_2NiS_4$ MW: 298,
	Dazomet ($C_5H_{10}N_2S_2$, MW: 162)	Metabolite: 1,3,5-trimethylhexahydro-1,3,5-triazin-2-thione, $C_6H_{13}N_3S$, MW: 159,
	Chlorthal-dimethyl (DCPA) ($C_{10}H_6Cl_4O_4$, MW:330)	Metabolite I: 1-(2,3,4,5,6-pentachlorobenzoyl)pyrrole; $C_{11}H_4Cl_5NO$, MW:341; Metabolite II: phthalide, 4,5,6,7-tetrachloro-; $C_8H_2Cl_4O_2$, MW:270,

components of the samples consist of fertilizers (Ammophos, Thomas slag, potash, and superphosphate) as well as pesticide (Tsineb) and $CuSO_4$ based fungicides.

For ca 23 % of the samples the presence of the inorganic constituents was not detected. We suppose these samples contain organic products that cannot be identified by GC-MS and HPLC. Analysis by HPLC-MS might identify organic products but such an instrument is not available in the country.

Among the single-component systems the more encountered one is Tsineb, but this compound is tentatively identified on the basis of the presences of Zn^{2+} in association with S^{2-} as a counter-ion. Other prominent compounds are Ammophos in 9.2 % of samples and Thomas-slag in 4.9 % of them.

Three- and tetra-component systems contain always Ammophos. This product seems to be the main constituent of the analysed samples in mono- bi- three- tetra- compounds.

In the poorest equipped warehouses from the initial 424 storage places, the pesticides stockpiles were kept together with mixture of fertilisers. Heavy metals such as copper and mercury were sometimes detected. Therefore, there is a risk of pollution of the environment (soil, water) with these elements.

34.4 Conclusions

Over 6,000 samples from 32 waste dumps have been analysed to determine the composition of the pesticides through GC-MS, HPLC, TLC and ISE methods of analysis with elements leading to identification in ca 93 % of the samples.

The most abundantly detected pesticides belong to the group of triazine herbicides (Simazine, Atrazine, Prometryn, Propazine) and their degradation products. These and DDT and related products might be the target for systematic analysis in environmental samples.

The results gained from the Science for Peace project will contribute to a more effective decision-making for the management of the destruction of the obsolete pesticides which will be organized by a NATO/PfP Trust Fund in the near future and is crucial for appreciation of the method of remediation of the contaminated sites. Another important issue is to focus attention on the presence of heavy metals and fertilizers in mixture with pesticides in repositories at the disposal phase to avoid the creation of dioxins during the burning process.

As is shown above a large number of samples provide information on the composition of the actual and historical waste dumps. Further work with analytical instruments of analysis need to focus on characterization of POPs and the triazines group pesticides in the various compartments of the environment (sediments, soil, biological materials, etc.) and public health (breast milk).

During the last project year a study was carried out centred on the measurement of the dispersal of the most prominent pesticides from a major dump site into the environment. This study was concentrated on the analysis of soil samples for a number of obsolete pesticides and provided a methodology for the study of the accidental dispersal from the dump sites.

The project increased the technical capabilities (analytical chemistry of organic chemicals and its application in waste analysis and database management). With its equipment and scientific experience the NATO funded laboratory will remain a centre of competence in the country.

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Chapter 35
Definition of the Tasks for the Eu-Phare Danube Regional Pesticide Study Project 1995–1997

Biana G. Simeonova, Gyula Dura, and Lubomir I. Simeonov

Abstract The present chapter provides a description of the objectives of the Danube Regional Pesticide Study Project, initiated during 1995–1997 under the frame of the Danube Applied Research Programme, which is managed by the Danube Programme Coordination Unit in Vienna. The ultimate goal of the project was to achieve an agreement between the Danube countries on the list of pesticides that are allowed, conditions under which pesticides may be applied especially related to protection of ground and surface water, criteria for allowance of pesticide use with regard to ecotoxicological aspects. The tasks of the project were defined to cover: inventory of pesticide application, production, formulation and distribution, and emission sources; real and estimated concentrations in Danube river water; identification of the authorities responsible for pesticide regulation and criteria used; ecotoxicological and toxicological risk assessment of the identified agents; action plan to ensure the protection of the key functions of surface water in the Danube basin and proposal of documents for harmonization of the policy. This chapter is made with educational purposes to generally assist young scientific researchers, who intend to formulate future project applications in the field of environmental sciences.

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Keywords Pesticides • Ecotoxicology • Economic reforms • Risk prediction • Management framework • Risk assessment • Aquatic life • Drinking water • Ecosystems • Environmental contamination • Adverse effects on environment and human health

35.1 Introduction

In the European Community Programme of Policy and Action in relation to the Environmentally Sustainable Development special attention is dedicated to agriculture. The objective is to decrease the input of chemicals to the point that none of the basic natural processes is affected.

In the Strategy action plan for the Danube river basin 1995–2005 agriculture is recognized as an important source of pollution of surface and ground water through agrochemicals.

The Danube Regional Pesticide Study Project as a part of the Strategy action plan for the Danube river basin was initiated during 1995–1997 with the aim to evaluate the risk of pesticides application for human and aquatic life in the region and to recommend a legal, policy and management framework, which would enable governments to develop risk reduction programmes. The economic reforms in most Danube countries have resulted in declined pesticide use patterns. On the other hand after decentralization the distribution and the use of pesticides have been dispersed among many units and farmers, which lead to the increaser of the number of point pollution sources. The situation is believed to be temporary due to the expected changes in agricultural policy introducing of new technology, increase of economic stimulators for crop protection, etc. This imposes a careful evaluation of the present status, taking in account the past situation and the future perspectives.

The Danube Regional Pesticide Study Project was funded by the EU-PHARE under a Service Contract 95.0100, PHARE ZZ 9111/0106. The project was executed in three phases, 8 month each, starting in May 1995. The Consortium included three countries: Bulgaria as a leading country, Hungary and the Slovak Republic, and consultants from the other eight Danube region countries, listed in alphabetic order: Austria, Croatia, Czech Republic, Germany, Moldova, Romania, Slovenia and Ukraine.

The members of the Consortium are from recognized nationally and internationally leading institutions in the field of environmental sciences, both in theoretical and applied research, as well as in elaboration of standards and regulation for toxic substances in the environment. Pesticide evaluation have been a part of their activities since decades. The members of the Consortium have qualified staff with relevant experience in this field.

The scope of a number of projects, conducted by the members of the Consortium, covered issues as toxicological and epidemiological studies and proposals of standards, methods for multiresidue analysis, criteria for complex evaluation of pesticides in the registration procedure, guidelines for water protection zones, exposure tests, preparation of information and training materials, education and training of trainers in the field of safe user of pesticides.

35.2 Field of Applied Research

The lack of data and reliable studies makes it difficult to access the problems of contamination of water bodies with pesticides and related environmental and health impact.

There are many general statements on these issues, but little verified information.

The main aim of the project was to evaluate the risks of pesticide application in the region for human and aquatic life and to recommend a legal, policy and management framework, which would lead to the reduction and elimination of this risk.

The work was performed in three phases, as it was mentioned above, with 14 tasks. The objectives in the first phase were related to the inventory of the existing situation in connection with the so called "life cycle" of pesticides from production to disposal and evaluation of the laws and practice of pesticide registration and regulation of their use. The second phase covers inventory of exposure data, modeling investigation for risk assessment of adverse effects on aquatic organisms and human health. The third phase uses the results of the first and second phases and the experience in the countries of EU and other countries to prepare action plan with recommendations and measures for reduction of the adverse impact of pesticides use in the Danube basin.

The expected overall output was formulated as a contribution to the improvement of the legislation, management and environmental protection in pesticide use in the Danube basin, which will reflect positively on human health and aquatic life.

35.3 Definition of Tasks

35.3.1 Phase 1

35.3.1.1 Task 1 Qualitative and Quantitative Inventory of Pesticide Application, Transport and Storage for All 11 Riparian Countries

Identification of the existing positive lists of pesticides and quantities used in each country, the variety of crops cultivated in the Danube basin, principal pests, requiring treatment with pesticides, methods of application, storage and disposal, plant protection policy, the trend of pesticide application.

35.3.1.2 Task 2 Inventories of Pesticide Production, Import, Export, Number of Producers, Formulation of Companies and Suppliers as well as Main Users

Lists of producers and produced and formulated pesticides in each riparian countries and export of pesticides between those countries. Lists of the imported pesticides. Practice of the pesticide trade and distribution. Packing and labeling. Special training requirements, licensing.

35.3.1.3 Task 3 Identification of the Responsible Authorities for Pesticide Regulation and Laws

Identification of the principle organizations responsible for pesticide regulation and enforcement. Inventory of the role of the Ministry of Agriculture, Ministry of Health, Ministry of Environment and Ministry of Internal Affairs in pesticide regulation and laws. Interagencies collaborating activity. The role of non-governmental ecological organizations. Who is authorized to have a right for “veto” in decision-making. Implementation and control.

35.3.1.4 Task 4 Economic Parameters Underlying the Usage Patterns of Pesticides

Identification of the potential loss related to plant diseases, pests and weeds. Costs of the pesticides and their application. Cost/benefit analysis. Inventory of the crops cultivated and crop production loss from non-treated crops. Study on the existing experience in the countries.

35.3.1.5 Task 5 Current Legislative Procedures and Criteria Related to Pesticide Registration

Inventory of the current legislation in relation with pesticide registration. Agricultural, health and environmental authorities duties. Health and environmental classification and registration criteria. Enforcement procedure. Registration criteria and practice. Non-authorized usage. List of the documents. Usage categories in relation with the license requirement. Sanitary standards and safe use recommendation in the registration phase.

The results are used for the harmonization of the existing legislation and practice of pesticide registration in respect of health and protection in Danube region.

35.3.1.6 Task 6 Overview of the Most Significant Emission Routes

Inventory of the sources of pesticide water pollution. Information on the priority sources of pollution. The results were used for preparation of recommendation on the policy in pesticide use.

35.3.1.7 Task 7 Classification of the Active Agents

Classification of pesticide active ingredients in connection to water pollution and ecotoxicity hazard. Data on health hazard, ecotoxicity, solubility, transformation rate, extent of sorption, persistence in the environment, leaching in soil, etc. were summarized using international specialized databanks. Classification of pesticides in several categories based in EU and other classification schemes was performed.

35.3.2 Phase 2

35.3.2.1 Task 1 Risk Assessment for Humans and Aquatic Organisms

Available data from existing monitoring systems, control laboratories, etc. was provided by Consortium members and consultants from the 11 Danube countries. Identification of analytical methodology was performed in order to assess the obtained information from different countries. Exposure models for determination of predicted environmental concentrations were used in case of lacking of exposure data. Available information from international specialized databanks concerning the dose-effect relationship for aquatic ecosystems (fish, crustaceans and algae) was summarized. Hazard assessment was performed.

35.3.2.2 Task 2 Water Quality Standards

Overview and comparative list of existing water quality standards – EU, WHO and the national standards of the Danube countries for drinking water and aquatic life was prepared.

The results may be used for harmonization of standards of riparian countries.

35.3.3 Phase 3

35.3.3.1 Task 1 Formulation of Common Procedures and Criteria for Admission of Active Agents in the Products for Sale

Common criteria based on ecotoxicity, behavior and persistence in the environment, in order to protect aquatic ecosystems and drinking water. General approaches for common procedures for pesticide registration

35.3.3.2 Task 2 Positive List for Active Ingredients

Classification of pesticides in relation with established criteria and risk estimation. Recommended list of pesticides.

35.3.3.3 Task 3 Measures for Reducing the Environmental Impact of Approved Active Substances

Development of model legislation with special requirements for pesticide use in the Danube river basin. Recommendation of legal policy and management framework, required to improve the environmental situation in concern to the pesticide contamination in the Danube river basin. Preparation of a manual with recommended measures to be used by farmers.

35.3.3.4 Task 4 Strategy for Pesticide Use in Danube River Basin

Existing OECD/EU approaches were used and adapted to Danube situation with respect of elimination of all pesticide active ingredients, which pose a threat to the sustainability of key functions of surface water in the Danube river basin: drinking water, aquatic ecosystems and fisheries.

Inventory of the quantities of banned pesticides in stock was performed. Recommendations for the most effective, safe and inexpensive methods for disposal in rural conditions was prepared. Lists of banned or not registered pesticides were proposed.

35.3.3.5 Task 5 Immediate and Long-Term Action Plans

Preparation of an action plan model for immediate and long-term actions in order to reduce the adverse effect of pesticide use in the region.

The prepared documents will be used for harmonization of the future steps in the riparian countries in environmental protection of the Danube river basin.

35.4 Methods/Methodologies

The Applied Research Programme is based mostly on inventory of the existing data in 11 riparian countries, concerning the pesticide regulations and use as well as the contamination of the water in the Danube river basin. Some of the countries are with changing agricultural economic structures, others have traditionally established organizations and practices. This situation has its advantages and disadvantages.

The disadvantages reflected on the preparation of the first phase report due to the diversity of data concerning existing practice and types and quantities of pesticide used.

Exposure data from existing monitoring systems and other analysis differ considerately in connection with the type of pesticides analyzed, methodology used and the way of data presentation, due to poor existing collaboration between these countries concerning pesticide environmental contamination.

The advantages reflected the development of guidelines on effective policy recommendations, management and decision tools and practices, required to improve the environmental situation in the Danube river basin.

European Union documents and approaches as well as those of the international organizations served as a basis for the evaluation and proposals.

35.4.1 Data Collection

The data collection processes are fundamental in this applied research. The two aspects have a priority importance:

- To obtain enough reliable data;
- To obtain as much as possible uniform information from the 11 countries, in order to facilitate evaluation and comparison of the data.

For this reason draft models of tables for each type of inventory were proposed and discussed. They included the studied parameters, possible sources of information and period to be covered. Several sources for each inventory were used in order to acquire reliable data.

35.4.2 Models for Prediction of the Exposure Levels

According to the Annex III of Directive 91/414/EEC [1] a great importance is given to the calculation of Predicted Environmental Concentrations ((PEC) as a basis of assessment of adverse effect of pesticide son non-target organisms. PEC calculations using a suitable model are required for all compartments, including the aquatic one. All types of surface water have to be considered – stream, river, lake, etc. The main relevant routes such as spray drift, run-off and drainage have also be considered.

The most commonly used scenario for risk assessment of pesticides in surface water in Europe is spray drift to a 30 cm deep stagnant pond. Assumptions include spray drift equal to 5 % of the application rate, residues uniformly distributed through the water, no losses due to volatilization or adsorption to sediment or suspended particles, and residue levels from multiple applications are additive. In UK for instance, spray drift is included as a contamination of surface water in PEC calculations based on worst case with progress of overspray: the ditch itself is sprayed with the same amount of pesticide as the field.

The need for harmonization of approaches on community level led to the establishment jointly by the European commission and European Crop protection Association (ECPA) Forum for the coordination of pesticide fate models and their use (FOCUS) with three working groups – for leaching, for surface water and for soil. After inventory and assessment of existing models the Surface water working group concluded that:

- There is no model available describing all input routes and behavioral aspects of plant protection products in the European Union;
- Standard European standards are lacking.

Large number of exposure models exist in USA as well, but only a small number of the models are validated or certified in actual field studies. For the purpose of this study the following model systems and models have been used: USES – Uniform System for the Evaluation of Substances [4], HESP – Human Exposure to Soil Pollutants [3] and Mackay (PED) Level I – Predicted Exposure Distribution [2].

The predicted quantity of pesticide use was taken into consideration for selection of pesticides for detailed evaluation and risk characterization: in the first phase for the selection of 100 pesticides using as one of the criteria the amount of pesticides in tons and in the third phase by number of countries using the particular pesticides.

35.5 Conclusions

This chapter is made with educational with general purpose to assist young scientific researchers, who intend to formulate future project applications in the field of environmental sciences.

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Chapter 36
The Danube Regional Pesticide Study Project 1995–1997: A Brief Synthesis of the Results

Biana G. Simeonova, Gyula Dura, and Lubomir I. Simeonov

Abstract The chapter is a continuation of the previous chapter in this book and provides a description of the objectives in respect to the synthesis of the results of the Danube Regional Pesticide Study Project, initiated during 1995–1997 under the frame of the Danube Applied Research Programme, which is managed by the Danube Programme Coordination Unit in Vienna. The ultimate goal of the project was to achieve an agreement between the Danube countries on the list of pesticides that are allowed, conditions under which pesticides may be applied especially related to protection of ground and surface water, criteria for allowance of pesticide use with regard to ecotoxicological aspects. The main issues under discussion include: Qualitative and quantitative inventory of pesticide application, transport, and storage; Inventory of pesticide production, formulation, import, export, and supply; Responsible authorities for pesticide regulation and laws; Economic parameters underlying the use of pesticides; Current legislative procedures and criteria related to pesticide registration; Overview of the most significant emission routes; Soil resources in the Danube river catchment area and problems of pesticide pollution; Inventory of existing laboratory data for pesticide concentrations in Danube and tributaries; Overview of existing and proposed international water quality standards for pesticides; National water quality standards of pesticides in Danube river countries;

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Estimating environmental concentrations of pesticides, using exposure models. This chapter is made with educational purposes to generally assist young scientific researchers, who intend to formulate future project applications in the field of environmental sciences.

Keywords Pesticides application • Pesticide production and formulation • Pesticide regulation and laws • Pesticide registration • Emission routes • Pesticides pollution • Danube river basin • Inventory for pesticide concentrations • Water quality standards • Exposure models

36.1 Qualitative and Quantitative Inventory of Pesticide Application, Transport, and Storage

The climatic conditions in the Danube river countries do not differ drastically from country to country. There are few differences in the types of cultivated crops depending mostly on the nutritional habits in each particular country. The most important cultivated crops are wheat, maize, sugar beet, potatoes and sunflower. Fruit and vegetables represent also an important part of cultivated crops.

The existing pests (insects, diseases, and weeds) are almost similar, in particular, weeds being the same in all Danube countries.

Pesticides are mainly used in agriculture. The control of economically significant pests covers the biggest part of their use. A limited part of insecticides and rodenticides is also used in public health for vector control of human diseases.

A cumulative list of the existing pesticides in all 11 Danube countries has been prepared. The total number of pesticide active substances used in the region is 452 (1995).

The active ingredients from the following chemical groups are mostly used:

- Organophosphorous – 62;
- Carbamates and thiocarbamates – 38;
- Synthetic pyrethroids – 24;
- Urea compounds – 35;
- Triaines – 18;
- Anilides and amide derivates – 37;
- Chlorophenoxy compounds;
- Dithiocarbamates – 10;
- Triazoles – 27;
- Copper compounds – 10;
- Others – 178.

The total quantity of pesticides used in the basin to control the pests on principal crops is approximately 118 828 tons of formulated product per year (data valid for the period 1993–1995). The use of herbicides (in tons) in the region is considerably higher than the total amount of both insecticides and fungicides.

Integrated Pest Management (IPM) was partly introduced in the region (data is for 1997). Alternative biological pesticide had still a limited use in according to data in the period 1995–1997.

The major application method is spraying with ground equipment. Seed treatment with fungicides and/or insecticides is also applied. Aerial spraying by aircraft had limited significance in the Danube river basin region during 1995–1997.

Storage of pesticides has been regulated in all Danube basin countries. Requirements and control of storehouses are almost similar in the particular countries. Disposal of pesticides, even regulated, creates problems in some of the countries. Thermal destruction is a method of choice in n most countries but still not available in some of them.

The economic reforms and new agricultural structures in down-stream Danube countries lead to a considerable decline in pesticide use after 1989. The decline during this 5–6 year period (until 1995–1997) was approximately 3.5-fold in Hungary, 2.5-fold in Slovakia, 2.6-fold in Bulgaria, 3.9-fold in Moldova and 2.9-fold in Ukraine. Some increase of the pesticide use for the next several years was expected in 1997, but the long-term trends are to reduce the agrochemicals, to increase the use of biopesticides, to introduce new technologies including pest-resistant crops.

The 1997 forecast was that less toxic and less persistent compounds are more likely to be used as chemical pesticides.

36.2 Inventory of Pesticide Production, Formulation, Import, Export, and Supply

Most of the Danube countries produce and/or formulate pesticides. Germany is one of the leading countries in pesticide production. In the other 10 Danube countries the production is limited and they rely mostly on import of pesticide active ingredients or formulations.

The pesticide registration is a primary requirement for import, production and distribution. During the period of centralized economy in Central and Eastern Europe, the import was monopolized by the relevant state organization. By the end of the 1980s many private companies and minor distributors were involved in import and distribution of pesticides. This provoked changes in regulations and required the training of much more persons, handling pesticides.

In all Danube countries packing and labeling of pesticides are approved by respective responsible authorities. The primary requirement is the product to be distributed in original packages with labels in the language of the country user.

Most of non-EU member countries in the region have already introduced the requirements for labeling and packaging covered by the Counsel Directives No.92/32/EEC [3].

36.3 Responsible Authorities for Pesticide Regulation and Laws

Multiagency preparation in the registration procedures is the usual practice with the leading role of the Ministries of Agriculture, Health, Environment or equivalent organizations. In most cases interministerial committees evaluate information supplied by the producer. A full data package is required for assessment of the risks for human health and environment. The list of approved pesticides is published annually.

Interagency collaboration exists also in the elaboration and enforcement of the regulations and laws concerning pesticides. The collaboration with the international organizations dealing with pesticides, such as FAO, WHO, IRPTC, CCPR, OECD, IUPAC, ECPA, supports the development of the legislative basis of pesticide production, sale, transport and use. The main trend nowadays is the harmonization of non-EU member counties legislation to that of EU.

36.4 Economic Parameters Underlying the Use of Pesticides

Analysis of data presented by the Danube countries showed that the production of principal crops in tons/ha differs from country to country. Pesticide use is not the principal reason for these differences. Obviously the agricultural practice, the climatic conditions, fertilizers use, soil types, and many other social and economic factors may influence the production. Nevertheless the pesticide use support the quality and quantity of yield. The losses due to pests and diseases vary from 3 to 100 % with the most common figures about 20–50 %. They are comparable to the most common figure of 40 %.

Rough cost/benefit analyses in Danube countries show that pesticide use is profitable for the principle crops. The positive effect of pesticides on crop yield and quality of production is obvious and there are no effective alternatives available to replace them in a short-term period.

36.5 Current Legislative Procedures and Criteria Related to Pesticide Registration

Data requirements for registration of pesticides are more or less similar in Danube countries. They are in accordance with internationally agreed guidelines such as FAO Guidelines on basic registration criteria and requirements as well as registration procedures.

Nine of 11 participating in the study Danube countries in 1995–1997 were not members of the European Union. They develop new legislation instruments concerning pesticides, harmonized with the EU directives. The leading principle in pesticide regulations in all Danube river basin countries is that regulatory decisions should be based on high protection for humans and environment.

36.6 Overview of the Most Significant Emission Routes

The most significant source of pesticide pollution of soil and water is the agricultural use of pesticides. Production and formulation factories, aircraft landing places, big storehouses, incinerators, etc. are also potential sources of pollution especially in emergency situations. Charts for the most important of the above mentioned point sources have been prepared for each country.

36.7 Soil Resources in the Danube River Catchment Area and Problems of Pesticide Pollution

Soils are of importance for the environmental fate of pesticides and in particular for the movement and deposition in ground water as well as for runoff and pollution of surface water. Soil resources in the Danube river catchment area are assessed based on the soil map of the world of FAO/UNESCO.

Bearing in mind the combined effect of pH, texture, organic matter content and temperature regimes on the biological activity in soils, a conclusion can be drawn that the region of Mollic soils provides the most intensive processes of chemical product decomposition and lowest hazard of pollution. The hazard increases in the following order: Luvisols, Heavy Textured soils and Acid soils.

Additional degradation processes like salinization, alkalinisation, gleyification, water-lodging, acidification, soil crusting and compaction contribute to the detoxification of pesticides.

Washing of chemical products from the surface horizons and their depositing in deeper horizons and ground water is a typical process for Gleyic soils, Saline-Alkaline soils and Fluvisols.

Washing of pesticides from the surface by runoff water takes place most intensively in soils with high water-erosion index. These are arable soils of medium texture, scanty in humus, low permeability and occurring on sloping grounds with frequent intensive rainfalls. This process is characteristic of the grounds with frequent intensive rainfalls and in the region of Luvisols, Acid and Weakly Developed soils, but is observed also in regions with steeper ground.

36.8 Inventory of the Existing Laboratory Data for Pesticides Concentrations in Danube and Tributaries

Pesticide concentrations in Danube river and its tributaries show significant differences between countries in the number and the types of pesticide analyzed. The cumulative number of analyzed pesticides is 76. Residues of only 36 pesticides and metabolites have been detected. The most frequently detected pesticides are organochlorine compounds and triazines. Organophosphorous pesticides are also occasionally detected. Only DDT and metabolites, HCH and isomers and atrazine and metabolites are found in more than 50 % of samples.

It appeared that Danube river at the middle and down stream part, if compared with the upper stream, is more polluted by:

- Atrazine and metabolites;
- Lindane;
- Simazine;
- DDT.

36.9 Overview of Existing and Proposed International Water Quality Standards for Pesticides

The EC Drinking Water Directive 80/778/EEC requires the Member States to ensure compliance with the Directive by 1985 [2]. The Maximum admissible concentrations (MACs) in drinking water are required to be 0.1 μg/l for individual pesticide and 0.5 μg/l for total pesticides.

Counsel Directive 75/440 EEC provides limits of 1 μg/l for individual and 5 μg/l for total pesticide contents in surface waters intended to use as drinking water [1].

Although the criticism expressed for unscientific considerations, the EC Drinking Water Directive is incorporated in EU member states legislation.

The USA Health Advisory Levels (HALs) for individual pesticides in water are calculated based on ADI and assumption of 2 l consumptions of water per day.

36.10 National Water Quality Standards of Pesticides in Danube River Countries

Regulatory documents for drinking and/or surface water exist in all Danube countries. They differ significantly in relation to number of pesticides regulated and the accepted limits. Some of the countries use levels for drinking water accepted by EU directives (Croatia, Germany, Romania) or WHO guidelines. Extensive list of pesticide limits in fish-household reservoirs is used in Ukraine.

36.11 Estimating Environmental Concentrations of Pesticides, Using Exposure Models

Predicted Environmental Concentrations (PEC) were calculated by USES model – Uniform System for the Evaluation of Substances [6] for 275 pesticides and by HESP model – Human Exposure to Soil Pollutants [5] for 99 of them. Due to the specific approaches of these models the results differ considerably. HESP model gives higher concentrations corresponding to the more polluted soil, just after the spraying. The results from USES have been only evaluated in the risk assessment.

Comparison of PEC and real concentration of detected pesticides demonstrated that PEC exceeded measured concentration of almost all pesticides detected in Danube and tributaries.

The results from PED (Predicted Exposure Distribution) calculation programme present more interest for accident situations, when the immediate distribution of pesticides in environmental media is of great importance [4]. Calculation of PED was performed for 307 pesticides (123 herbicides, 72 fungicides and 112 insecticides). It may be concluded from the data that 80 % of herbicides have the potential to be present in water in more than 50 % of applied dose, as well as 76 % of fungicides and 52 % of insecticides.

Similarly pesticides may have an air polluting potential due to their volatility properties estimated according to the distribution to air exceeding 2 % of applied quantity. In such a comparison 2 fungicides, 25 insecticides and 132 herbicides of 307 total investigated pesticides have a tendency to be present in the air for a time.

Such kind of initial exposure assessment of pesticides can be used for a categorization of pesticides, whether or not they are of concern for further investigation and the acceptability of a pesticide use at a large regional level on the base of potential exposure.

36.12 Conclusions

The purpose or this chapter is educational with the intention to assist young scientific researchers, who intend to formulate future project applications in the field of environmental sciences.

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Chapter 37
Principles and Application of the Integrated Pest Management Approach. Biological Pesticides

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Abstract Protection of crops from pests and from ravenous animals was long accomplished with the use of ‘natural’ means, before the introduction of ‘modern’ synthetic chemicals as pesticides in the twentieth century. The widespread use of synthetic organic chemicals as pesticides in the last several decades has contributed to the ‘background’ contamination of all environmental and biological compartments with trace amounts of small organic compounds, the combined effect of which on the health of humans and on the general well-being of complex ecological systems is far from being understood. In particular, it is claimed that the presence of trace amounts of such compounds in human food may contribute to impair the health of sensitive individuals, especially in the developmental and early-age stages of life, although there is not a clear-cut consensus on this problem. The request from consumers, mainly in developed Western countries, of food produced under perceived healthier conditions than those of mass-production, has prompted a backslash of interest in traditional, lower-yield techniques such as ‘organic farming’ and thus the necessity to protect consumers from unsubstantiated claims of food quality through the issuing of voluntary codes by producers and of guidelines such as *Codex Alimentarius* at the level of international Organizations. Another driving force into a renaissance of ‘traditional’ farming techniques is understanding that they can be successfully merged to ‘modern’ ones to achieve better productions with lower environmental impact, lower consumption of selective but expensive Plant Protection Products, lower contamination of food with residues. Another trigger to seek alternatives to

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the conventional means of pest fighting is acknowledgement that later generations may develop resistance to current pesticides in a continuous ‘chemical war’ between human scientific intellect and natural evolution of organisms. Traditional agricultural wisdom long recognized the power of coenobioses and used co-cultivation of different crops as a means to deter parasites by exploiting the natural emission of insect repellents and mutual fight between natural hosts of crops and invading organisms. Of course, since even ‘natural’ substances long employed to fight parasites of plants and food are intrinsically toxic not only to target species but also to several others, including the human, risk assessment of their use is mandatory to fully benefit of their strength without unnecessary risk for producers, consumers and the global environment. The recent exploitation of natural as well as of engineered organisms and of their toxic products as pesticides is another means the reach and limitations of which need to be fully understood. Examples of how these seemingly conflicting requirements have been or can be harmonized will be presented and discussed.

Keywords *Bacillus thuringensis* toxin • Biological agriculture • *Codex alimentarius* • Fenoxycarb • Methoprene • Neem oil • Nicotine • Pyretrins • Rotenone • Spinosad • Ecdysteroids

37.1 Introduction

It is now more than 5,000 years that selective growth of plant biomass is the main strategy through which mankind has sought its sustainment. Agriculture is an intrinsically non-sustainable activity which depletes unscathed soil of its stored chemical nutrients, which need to be replenished by fertilization, and an artificial ecosystem in which natural prey-predator equilibrium is modified.

Both issues were solved, often separately, across the history of humankind by different means which gradually entailed increasing levels of complexity to yield the agro-industrial complex of present times. *Cut-and burn*, determining the short-term release of stored mineral nutrients into the ground was the first, and still practiced attempt to exploit natural forested areas for agriculture. *Culture rotation* allowed replacement of exhausted reservoirs of organic nitrogen through the use of the nitrogen-fixing symbionts of leguminosae. *Chemical fertilization* was chronologically the last step, which uses agricultural land as the physical support of plant growth by feeding externally produced nutrients: nitrogen, phosphorus, potassium.

The long-term consequences of land exploitation were well-known deep in the past and were the underlying reasons of most human history. Depletion of local resources due to exhaustion of land fertility led to economical crises, to degradation of food quality and availability, down to famine, which in turn was the cause of epidemic, chronic and long-term diseases [42]. Desertification drove to mass migrations to unexploited areas or to conflicts with earlier dwellers. The Biblical image of the Four Knights of Apocalypses materializes the escalating effect of environment perturbations on the fragile equilibrium of pre-industrial Europe [9].

In the Euro-American global system starting with the Industrial Revolution these concerns were apparent and led to a chase for technological solutions which are at the root of current economic development and of its backslash. As early as 1898, Sir William Crookes warned: "[...] *England and all civilized nations stand in deadly peril* [...] *As mouths multiply* [...] *food sources dwindle* [...] *Any drop in wheat production will threaten* racial starvation" [15], and in 1912 the German chemist Fritz Haber developed the industrial synthesis of ammonia from atmospheric nitrogen for which he was awarded the Nobel Prize for Chemistry in 1919 [24].

The artificial ecological system of mono-culture is much more sensitive to the effect of crop-spoiling organisms than natural coenobioses. To ensure high crop yields the use of chemical pesticides was exploited as early as the mid-nineteenth century, when the efficacy of copper sulphate against downy mildews (*Peronospera*), a fungus pest of vineyards, was serendipitously discovered by Pierre-Marie-Alexis Millardet and the use of the Bordeaux Mixture was popularized. It was only after the expansion of specialty organic chemistry production that organic pesticides were developed, starting from DDT, the discovery of which in 1939 earned Paul Hermann Muller the 1948 Nobel Prize in Physiology or Medicine [25]. The introduction of rationally selected high-yield cultivars of staple food crops was the third pillar of the Green Revolution which flourished especially in the post-World War II and decolonized extra-European world, an achievement for which one of its pioneers, Norman Ernest Borlaug was awarded the Nobel Peace Prize in 1970 [26].

Supply of water and food to the ever-increasing population of large urban areas developing in the mid-to-late nineteenth century as industrial centres called for substantial advancements both in technology (in 1908 Chicago was the first city in the world to adopt chlorination disinfection for drinking water) and in the perception of the conflicting roles of private enterprise and of public interest. In his 1906 bestseller fiction novel *The Jungle* Upton Sinclair denounced the poor conditions of the U.S. meat packing industry. The public indignation raised was instrumental in passing, a few months later, of the 1906 Pure Food and Drug Act and the Meat Inspection Act in the U.S.A.

In the Old Continent, opposition to the perceived excesses of industrialism in farming spurted philosophically-grounded opposition, culminating in Rudolf Steiner's 1924 *Lectures on Agriculture*, which heralded Biodynamic Agriculture, with its elaborate techniques, the outcome of which was however mostly anedoctal and not rationally improvable, differently from the scientific approaches pioneered in the U.K. by Sir Albert Howard (the "father of organic farming") and by Lady Eve Balfour and by J.I. Rodale in the United States. The Ivy League Cornell University manages in the USA the oldest estate dedicated to research on organic farming within its Agricultural Experimental Station [6, 7].

Among the tenets of organic farming are the use of manure for fertilization, the availability of which is limited by that of agricultural land for pasturing of herds; the use of crop rotation to reconstitute organic nitrogen reservoirs, which also limits agricultural land available for human food crops and the exclusive use of naturally-available chemicals, such as plant secondary metabolites to fight crop pests.

37.2 Plant Secondary Metabolites as Pest Control Agents

Most plant secondary metabolites are the natural response of plants to parasites and plant-eating organisms – not only insects – but also birds and terrestrial animals.

As an example, *Colchicum spp* is a typical flower on mountain slopes. To avoid being eaten by wild animals, the plant bio-synthesizes in its tissues (flower, stem and root) colchicin, a cytotoxic anti-tubulin secondary metabolite. As a consequence, also cattle (cows, sheep, goats) grazing in mountain pastures naturally avoid pasturing near the flowers and teach the young individuals not to. The same pharmacological properties of colchicin which lead to toxicity are also traditionally exploited in human medicine to treat several diseases, such as gout.

37.2.1 Chemical and Toxicological Aspects of Plant Secondary Metabolites as Pest Control Agents

A number of plant secondary metabolites have been traditionally employed with more or less awareness by traditional farmers to fight pests and to deter ravaging animals from orchards and cultivated fields.

Tradition agricultural practices long identified what are also known as ‘lucky pairs’ of green vegetables and co-cultivated specific plants together with economically valuable ones (***companion planting***), with the rationale that one plant deter pests from feeding on nearby plants of a different species. In traditional practices, co-cultivated plants are each individually useful as food and can be cultivated in association, in order that those which contain or emit into the environment pest-deterrent secondary metabolites also protect the others.

- Garlic (*Allium sativum*) and onion (*Allium cepa*) rows in green gardens deter most insects and some mammals such as rabbits through the biosynthesis of an array of sulphur-containing secondary metabolites derived from cysteine and from methionine, which are also responsible for their strong taste and possibly also of their healthy-food properties;
- Tomato deters insects pests such as cabbage maggot (*Delia radicum*) and asparagus beetle from feeding through the emission of strong odorants, which are also sensed by neighbouring plants, which in turn elicit preventive anti-feedant responses;
- even exotic plants first introduced to Europe from the New World in the sixteenth century were initially more appreciated by gardeners for their unusually bright flowers and as insect deterrent also to other ornamental plants in Italian gardens than for their use as staple food, as in the case of tomato, tobacco and potato.

Companion planting as a pest-deterring strategy has advantages and disadvantages. Among the former are:

- no need for preparation of products and for repeated application on cultured areas;
- little to no cost for farmer or even some products may also have an independent food or commercial value.

This practice also faces some limitations and disadvantages, among which:

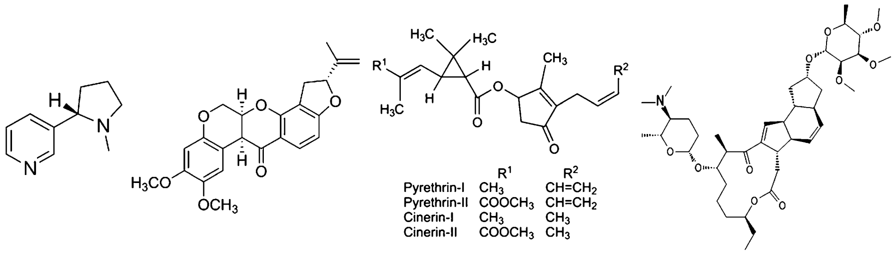
- companion plants need extra space in orchards and may consume additional fertilization;
- some plants may have different agricultural needs: a different watering schedule, may entail further work, may be inadequate for climate or
- may be harmful to farm animals due to the content of toxic compounds which are those needed for their use as pest control agents; this is also true for children who currently perform jobs in traditional farms;
- some may be regulated due to the production of pharmacologically active secondary metabolites whose effect on humans are socially undesired in specific environments.

In order to exploit the best opportunities, products may be purposely prepared from their natural sources to be applied on cultures without the need of cultivating the source plant in the same place where its effects are needed. Among the simplest traditional forms is maceration in water of exploited stems of tomato or of faded ornamental geranium and use of the water to irrigate small cultivations, such as ornamental flowers.

There are selected advantages in the large-scale application of this practice, which may grow into an independent economic activity:

- supply can be made independent from local need and would not consume local resources;
- the dose of the pest-controlling agent can be adjusted to actual need, even in the case that it is higher than that allowed by the natural content in the co-cultivated plant;
- there may be further economic advantages, such as marketing opportunities for peripheral economies (some valuable products grow in otherwise unproductive tropical areas), process wastes can be valorized (such as in the case of tobacco nicotine, *see* below) and use of local plant-derived products can reduce dependence of some regions from import or from corporate policies.

While several traditional practices only have local diffusion and most have been superseded by the availability of synthetic chemicals as pest control agents, a few examples of the sustained use of secondary metabolites are still of practical importance. A resurgence of once-practiced treatments and even the setup of new ones have been encouraged by the rise in popularity of ‘organically-produced’ food especially in some ‘First World’ areas. The merits and demerits, sustainability and hazard of the rising demand of such products will not be discussed. To protect



	R¹	R²
Pyrethrin-I	CH_3	$CH=CH_2$
Pyrethrin-II	$COOCH_3$	$CH=CH_2$
Cinerin-I	CH_3	CH_3
Cinerin-II	$COOCH_3$	CH_3

Nicotine **Rotenone** **Natural pyretrins** **Spinosad**

Fig. 37.1 Chemical structures of the main plant protection products of natural origin

consumers from the consequences of unsubstantiated claims and the reputation of responsible producers, voluntary codes of good practice in the production of different denominations or commercial brands of such agricultural and dairy products have been agreed upon by producers' associations. In most cases pest control with chemical substances can only be accomplished with the use of few substances, all being secondary plant metabolites.

Those agreed upon by the generality of protocols are: nicotine, rotenone, natural pyretrins, spinosad, neem oil and *Bacillus thuringensis* toxin. The chemical structures of some important products are reported in Fig. 37.1.

37.2.1.1 Nicotine

Nicotine is the major alkaloid of the tobacco plant (*Nicotiana tabacum*). Its biosynthesis occurs in the roots and the substance is transported to the leaves, where it acts as an anti-feedant due to its fairly high concentration (0.6–3 % of dry leaf weight) and to its activity as a cholinergic agonist. Its neuroactive properties were exploited by the native Americans and soon copied by the European explorers and settlers who imported the habit in their home Countries since mid-late sixteenth century. Its success as a leisure consumer product prompted the rise of the first non-food mono-culture at first on the east coast of North America (Virginia) and later also in Europe and in the Middle East, where tobacco cultivation afforded a revenue also from poor soils. The health hazard posed by tobacco smoking was well known as early as the seventeenth century: King James Ist of England wrote in 1604 the pamphlet '*A Counterblaste to Tobacco*' and authorized to levy the first tobacco tax to counter its use.

The insecticidal use of tobacco juice precedes the isolation of the tobacco alkaloid in 1836 and its use as a homicidal poisoning (a dose $<$ 10–60 mg is fatal to a child or a man, respectively) was at the root of analytical toxicology since the late eighteenth century. Nicotine sulfate is a side-product from tobacco industry (current production is in the 2,500 tons/year) as the alkaloid is recovered from the process of wetting tobacco leaves to soften them after curing and before manufacturing tobacco products such as cigars and cigarettes.

To mimic and enhance the insecticidal effect of nicotine while minimizing its toxic effect on humans and animals, a class of synthetic pesticides (neo-nicotinoids) was developed in the 1980s and 1990s. Important products of this class are: Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam. Imidacloprid is currently the most widely used insecticide in the world. Most neonicotinoids do show much lower toxicity in mammals than insects, but some breakdown products showed to be toxic to mammals. Recently, the use of some members of this class has been restricted in some countries due to evidence of a connection to honey-bee colony collapse disorder, although the topic is still somewhat controversial.

37.2.1.2 Rotenone

Rotenone is a secondary metabolite classified into a specific sub-family (rotenoids, around 60 different molecules identified) of isoflavones. It is bio-synthesized in the leafs and transported to the other tissues such as seeds and stems of several tropical and sub-tropical plants of the genera *Derris* (in Asia), *Lonchocarpus* and *Tefrosia* (in Sud America). Although it was first discovered in *Lonchocarpus nicou*, by the French botanist Emmanuel Geoffroy in the late nineteenth century, its ethno-botanical use as piscicide by local hunter-gatherers may be ancient, as early explorers noted that Peruvian natives used crude extracts of the Cubé plant to stun fish for eating. Rotenone has been used commercially as a garden insecticide since the middle 1800s, primarily in the UK, as a non-toxic alternative to the lead- and arsenic based pesticides then in common use.

Rotenone is still currently widely used as a garden insecticide, and in organic farming as a natural, botanical insecticide to kill potato, cucumber and flea beetles, cabbage worms, raspberry and asparagus bugs, as well as most other arthropods. In California, about 200 kg of rotenone is used per year as an insecticide for organically grown lettuce and tomatoes. In Vietnam rotenone is currently studied and applied in agriculture and pisciculture. It has been recently reported that rotenone was identified in two cultivated species, *Derris elliptica* Benth and *Derris trifoliata* (about 10,000 ha in Soctrang and Binhduong provinces) and that rotenone-based preparations are used in Mekong Delta to kill fish in shrimp aquaculture.

In the ground rotenone has a half-life of *approx.* 6 days, due to photochemical oxidation, while in water it can persist at piscicidal concentration for *approx.* 6 months.

Its insecticidal activity, by contact and by ingestion, and its piscicidal activity by absorption through the gills, is due to its pharmacological properties as a selective inhibitor of the mitochondrial electron transport chain from iron-sulfur centers in complex I to ubiquinone. This interferes with NADH during the creation of usable cellular energy (ATP).

Due to its very poor absorption by the gastrointestinal tract of humans its lowest lethal dose for a child is 143 mg/kg, which is unlikely to be reached accidentally, since the compound is a strong irritant and has a very bad taste. Only one fatality due to suicidal ingestion has been reported in the literature.

In the last decade concern has been raised that rotenone can cause a toxic Parkinson disease in rats and in 2011, a US National Institutes of Health study showed a link between rotenone use and Parkinson's disease in farm workers [40]. Currently in the U.S.A. rotenone is no longer allowed in organic farming.

37.2.1.3 Pyrethrins and Pyrethroids

Pyrethrum is the dried powder obtained from the flowers of white Chrysanthemum (*Chrysanthemum cinerariifolium* and other similar species) in West Africa. Pyrethrin is the name of the natural product extracted from pyrethrum. Chrysanthemum has no relationship with *Anacyclus pyrethrum* DC, an Asteracea which does not contain pyrethrin but has other uses.

Chrysantemum has a long tradition of ethnobotanical use as insecticide, possibly starting in Persia around 400 BC, although the employed species, *C. roseum* Adam and *C. coronarium* Linn. have a much lower content of pyrethrins. Details of its diffusion from West Africa and in Near East to the Balkans (Dalmatia) by the late nineteenth century are not clear, however it is known that its insecticidal properties were serendipitously (re)discovered by a German lady in Dubrovnik who started marketing a dried flower powder, the use of which reached the U.S.A. by 1860. Commercial plantations were established in British West Africa (Kenya) and in India in the early 1930s in Kashmir Valley, Palami and Nagili Hills. Yields in commercial plantations is of 200–600 kg of dried flowers per hectare, with a content of 1–1.3 % by weight of pyrethrin. In 1972–1974 the total production of pyrethrum dried flowers was 27,000 tonnes and by now three East African countries, Kenya, Tanzania and Rwanda produce *approx*. 80 % of total [27].

Pyrethrum works against a very wide range of insect pests and development of resistance is not frequent [27].

The active principles of Pyrethrum flowers, pyrethrins, are fast-acting (knock-down) contact poisons which disrupts the nervous system cause paralysis of the insect. The electrophysiological studies by demonstrated that the highly lipid soluble pyrethins distribute in the lipid bilayers of the nerve cell membrane and exert their action on sodium channel proteins. The rising phase of the action potential is caused by sodium influx (Na^+ activation), while the falling phase is caused by sodium activation being turned off, and an increase in potassium efflux (K^+ activation). Pyrethroids interfere with the changes in sodium and potassium ion currents by locking the Na^+ channel in the 'open' position, so that nerves cannot de-excite. Activity on the corresponding ion channels of warm-blooded animals is much lower, thus making these compounds safe as insecticides.

Pyrethrins also degrade very quickly in sunlight, moisture and oxygen, thus presenting little environmental concern but also having a short-lasting activity which requires repeated applications. Since pyrethrins are also bio-degraded by target animals, their potency can be enhanced by co-formulating with piperonyl-butoxide, a semi-synthetic inhibitor of P450 enzymes, which stops their bio-inactivation by insects.

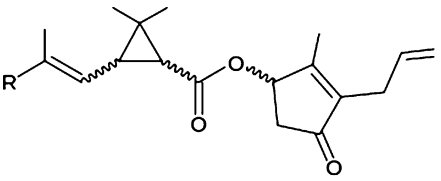
The structures of pyrethrins were identified starting in the 1920s by the Dalmatian-born, Swiss-naturalized chemist Lavoslav (Leopold) Ružićka who studied the insecticidal compounds of a plant of his native Croatia while working at ETH in Zurich with his mentor Hermann Staudinger (Nobel Prize in Chemistry 1959). He became the founding father of modern terpene chemistry and biochemistry and of rational perfume chemistry (acting as a consultant of Firmenich) and was awarded the 1939 Nobel Prize in Chemistry.

Following his studies, it was understood that pyrethrum extract is a mixture of three natural esters of chrysanthemic acid (Pyrethrins I: pyrethrin 1, cinerin 1 and jasmolin 1) and three esters of the related pyrethric acid (Pyrethrins II: pyrethrin 2, cinerin 2 and jasmolin 2), two fairly complex compounds, which feature a cyclopropyl ring in their structures and three stereogenic centres. The use of pyrethrins was expanded by a non-corporate British research institution, Rothamsted Research, starting with the development of a total synthesis of all-racemic pyrethrin I, which could only be accomplished with 1 % yield but was in principle independent from the natural supply coming from the East African colonies and from India, which could be limited by interruption of sea trade at wartime.

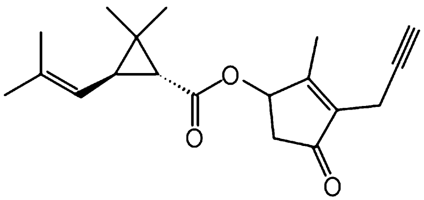
Further steps led to the preparation of synthetic analogues involving simpler chemical synthesis and seeking an improved photo-resistance (pyrethroids). In the 1960s a first group of derivatives of pyrethrin I and II were prepared by keeping the portion of chrysanthemic acid. These derivatives were still too photo-sensitive but suitable changes in the alcohol group with respect to the natural products led to an enhancement of insecticidal activity and to an even lower mammal toxicity. A further step was taken by altering also the structure of the chrysantemic acid fragment so that the new products now showed even less similarity to the original structures of the pyrethrins (Fig. 37.2).

37.2.1.4 Spinosad

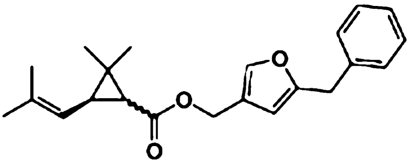
Spinosyn and its analogs are a family of over 20 natural fermentation products produced by a recently discovered soil micro-organism, *Saccharopolyspora spinosa*. They are polyketide-derived tetracyclic macrolides with a 21-carbon, 12-membered tetracyclic lactone structure, to which are attached two deoxysugars, tri-*O*-methylrhamnose and forosamine [38]. They are the active ingredients in a family of insect control agents with a *novel neurotoxic mode-of-action*. Spinosoid binding disrupts acetylcholine neuro-transmission, likely at the nicotinic receptor, but also acts as a GABA receptor agonist. The resulting effect is a hyper-excitation of the nervous system which kills the insect. Spinosad is so far non-cross-resistant to known insecticides. One main advantage of spinosyns is that, although they are slower than pyrethroids to penetrate the insect larvae cuticle, they are not metabolically deactivated and thus only low doses are needed for having the effect. As an example, Spinosad was first registered as a pesticide in the United States for use on crops in 1997 at a labelled use set at 1 ppm (1 mg a.i./kg of grain) while its toxicologically established Maximum Residue Limit (MRL) is of 1.5 ppm, *i.e.*,



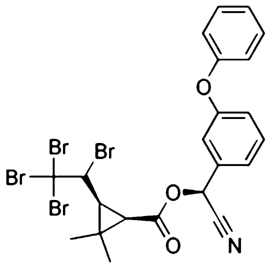
Allethrin
(all-racemic pyrethrin I)



Prallethrin



Resmethrin



Tralomethrin

Fig. 37.2 Chemical structures of some synthetic analogues of natural pyrethrum insecticides

higher by 50 % than the useful dose. To exploit the chemical diversity allowed by this new bioactive scaffold, over 1,000 *synthetic* spinosoids were also developed and tested [8]. Spinosyns are readily degraded in moist aerobic soil (half-life, 0.3–0.5 day) by photolysis or a combination of metabolism and photolysis [20].

37.2.1.5 Neem Oil

Neem (*Azadirachta indica*) is a fast-growing evergreen mahogany-like tree ranging in height from 12 to 24 m, belonging to family Meliaceae. While native of India, Neem trees are widespread in tropical and subtropical regions of the world, including semiarid and wet- tropical regions [21]. Neem oil is obtained by grounding Neem seeds are into a powder that is soaked overnight in water and sprayed onto the crop. Neem acts on plants as an anti-feeding, repellent, and egg-laying deterrent since it also suppresses hatching of pest insects. Insects starve and die in a few days. To be effective, it is necessary to apply at least every 10 days. Plant extracts contain several bitter compounds, among which the triterpenoids nimbin, nimbinin and nimbidin, which were discovered in the 1940s and following years by the Pakistani organic chemist Salimuzzaman Siddiqui [35], who had earlier isolated the alkaloid ajmaline from *Rauwolfia serpentina*. Another compound is the limonoid azadirachtin.

In 1995, the European Patent Office (EPO) granted a patent on an anti-fungal product derived from neem to the US Department of Agriculture and to the

W. R. Grace and Company. The Indian government challenged the grant, claiming that the long local use of the plant product (Foreign Prior Art) for agricultural use hampered issue of patent protection. This controversy is one important case-history in the 'biopiracy' polemic [32–34] on the commercial exploitation of natural substances for pharmaceutical or for other industrial use on which the 1993 Convention on Biological Diversity (CBD, presented at the United Nations Conference on Environment and Development in Rio de Janeiro in 1992) attempts to recognise and formalise the value of traditional knowledge and to ensure that holders of traditional knowledge are compensated accordingly [1]. After a 10-year litigation, the appeal from Grace, raised after, in 2000, the EPO had ruled in India's favour in 2005, was lost and the EPO at last revoked the Neem patent.

Bacillus thuringensis

B. thuringiensis (Bt) is a Gram-positive, soil-dwelling bacterium was first studied as a microbiological curiosity, as the cause of a disease of silkworm in Japan (by Shigetane Ishiwatari in 1901) and of the *Schlaffsucht* disease in flour moth caterpillars (by Berliner in 1911). It is closely related to *B.cereus*, a soil bacterium, and the *B.anthracis*. Further studies showed that a small crystallisable protein of the micro-organism is responsible for the toxic effect of the infection in the target species. Upon sporulation, Bt forms crystals of proteins the sequence of which is encoded in plasmids, the δ-endotoxins, also called crystal proteins or Cry proteins. The crystals are aggregates of a large protein (about 130–140 kDa) that is actually a pro-toxin. The crystal protein is highly insoluble in normal conditions, so it is entirely safe to humans, higher animals and most insects. However, it is solubilised in reducing conditions of high pH (above about pH 9.5) – the conditions commonly found in the mid-gut of lepidopteran larvae and is thus amenable to degradation by proteases present in the insect gut. The proteolytic process yields the Cry toxin, a pore-forming sub-unit which assembles into the insect gut cell membrane, forming a pore, which in turn causes in cell lysis and eventual death of the insect.

B. thuringiensis is active against moths and butterflies, flies and mosquitoes, beetles, wasps, bees, ants and sawflies and nematodes but has little or no effect on humans, wildlife, pollinators, and most other beneficial arthropods since these lack appropriate receptors.

The first formulation based on Bt was developed in France in 1938, under the name "Sporéine", mainly to kill flour moths, although the first well-documented industrial procedure for producing a Bt-based product dates from 1959, with the manufacture of "Bactospéine" under the first French patent for a biopesticide formulation. Commercial formulations of Bt consist of spore/crystal preparations obtained from cultures in fermentors; the preparations are dried and used in a granulated or wettable powder formulation for use as a spray [31]. B. thuringiensis-based insecticides are often applied as liquid sprays on crop plants, where the insecticide must be ingested to be effective [2].

A boost in the use of Bt came in the mid-1980s when genetic engineering techniques were developed at industrial level and allowed to encode the DNA for the pro-toxin directly into the genome of the crop to be protected (trans-genic *or* TG crops,), in order that the protein is expressed by the plant itself, without need for application of the pesticide. There are several advantages in expressing Bt toxins in transgenic Bt crops, namely: (a) the level of toxin expression can be very high, thus delivering sufficient dosage to the pest; (b) the toxin expression is contained within the plant system, hence only those insects that feed on the crop perish and (c) the toxin expression can be modulated by using tissue-specific promoters, and replaces the use of synthetic pesticides in the environment. The latter observation has been documented.

Major crops in which Bt was engineered are Bt-tobacco (1985), Bt-potato and -corn (approved by EPA in 1995), Bt-maize and Bt-cotton (1996) [23, 39].

It has been calculated that the use of Bt crops has since saved the use of 35,000 ton of pesticides. However, concern on long-term safety of crops and crop-derived products, such as meat and dairy from animals raised on Bt-maize have been since raised [18, 28]. Moreover, the claim that resistance to Bt-crops was unlikely to develop has been challenged by observation of first resistance in Bt cotton in India in November 2009. Another source of concern is the surge of non-sensitive ‘sucking’ pests in China, which eroded the advantage of using Bt-crops.

37.3 Integrated Approaches

It was as early as 1965, just a few years after the publication of ‘*Silent Spring*’ that ‘integrated pest control’ was the subject of FAO symposium held in Rome [12] which stated: “*Integrated pest control is a pest population management system that utilizes all suitable techniques in a compatible manner to reduce pest populations and maintain them at levels below those causing economic injury*.” (our emphasis). The concept of ‘Integrated Control’, originally limited to the combination of chemical and biological control methods, was greatly expanded in that symposium, and redefined to become synonymous with what we presently consider Integrated Pest Management (IPM). It is important to notice that the concept was framed by FAO in food protection rather than in prevention of parasitic diseases and thus called at control of pest populations rather than at eradication of disease vectors.

In fact, the ever-increasing use of pesticides to protect food and commodity crops from spoilage started to present the seemingly unavoidable drawback of an ever-increasing presence of residues in food, in water and in the environment, the significance of which in the broader eco-toxicological terms is still unpredictable. This awareness led to understand how to lower pesticide consumption without compromising the efficacy of crop protection. Current practices of pesticide application in extensive agriculture spread 95 % of pesticide dose off-target, causing a waste of expensive product, toxicity to non-target organisms and environment contamination. Moreover, with indiscriminate application practices even active

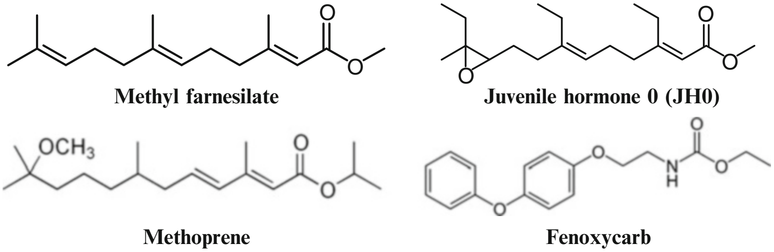


Fig. 37.3 Chemical structures of natural juvenile hormones and of synthetic analogues

useful products need to be limited or banned due to unacceptable human health hazard. Among strategies developed to improve homing of necessary dose to right target one is the use of '*medicated*' baits, such as a sweetened polymer impregnated with a hazardous organophosphate (such as Dichlorvos), which remains localized and thus with much less risk of unwanted exposure, especially in confined environments, which span from warehouses to dwellings. To enhance safety, less toxic pesticides, such as pyrethroids can be used. This approach, however, is far from being a general one and thus complementary strategies are sought for.

Starting with the discovery of ecdysones, the steroid-like hormones responsible for the periodic change of the exo-skeleton of insects and of other arthropods by Karlson [19] and following with Röller's discovery of 'juvenile hormones' of insects (Fig. 37.3) and Williams' proposal [43] to exploit this target to selectively control specific populations without interfering with others, several approaches have been devised and met with some commercial fortune. This approach was applied as early as 1974, following Carl Djerassi's foundation of the start-up company Zoecon and the development of the product Altosid IGR (isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate or methoprene; Fig. 37.3) as a growth regulator of the housefly (*Culicidae* spp.) [11]. Methoprene does not kill adult insects, but acts as a growth regulator, inhibiting pupae to mold to adult insects. This breaks the biological life cycle of the insect preventing recurring infestation.

Among the many applications of methoprene and of its analogues are the prevention of fly infestation of cattle, of fly proliferation in cattle dung and in drinking water cisterns to control mosquitoes which spread dengue fever and malaria [46]. Another agricultural application of the same product was protection of crops and commodities in warehouses, such as tobacco leaves and pineapples. In the household, methoprene and other analogues such ad hydroprene are used to fight bed bugs. A fairly exotic application of methoprene is to increase the length of the pupal stage of silkworm (*Bombyx mori*) to yield a higher production of silk.

Among the characteristics of JH analogues are lack of residues in the environment (since the molecules are completely mineralized) and a short persistence, which calls for modes of application different from conventional spraying techniques. To enhance the persistence of the products, from the molecular scaffold of methoprene a much more stable diphenylether-carbamate derivative, fenoxycarb (Fig. 37.3), was

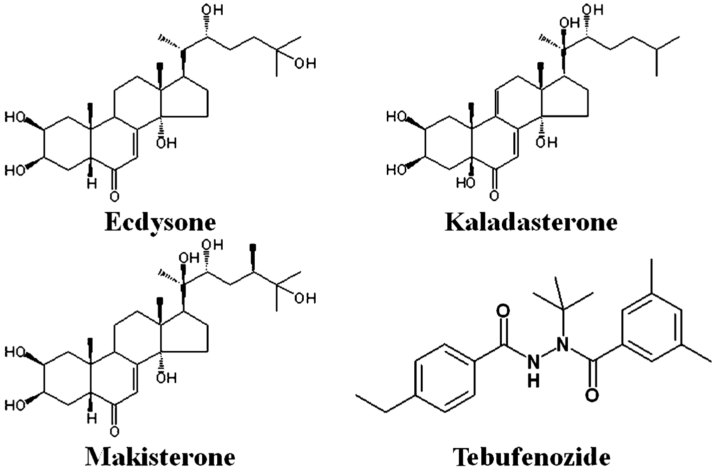


Fig. 37.4 Chemical structures of some ecdysteroids and of the non-steroidal analogue tebufenozide

elaborated. In 1989 in Northern Italy silkworm production was almost destroyed with considerable disruption of the downstream textile industry and economic loss. Studies carried to understand the cause demonstrated that silkworm had been reared on mulberry leaves which had been contaminated by traces of fenoxycarb used far elsewhere to protect fruit trees and carried by the wind [29].

Ecdysteroids, a class of steroid derivatives characterized by a *cis*- fusion of rings a and B and by the presence of several hydroxyl groups in the structure, were isolated in arthropods starting in the mid-1950s and in plants (Phytoecdysteroids) starting from the mid-1960s, just after the structure of the first ecdysteroid, ecdysone (E), isolated by Butenandt and Karlson in 1954 from silkworm pupae, was finally elucidated in 1965. Currently more than 200 plant steroids (Fig. 37.4) related in structure to the invertebrate steroid hormone 20-hydroxyecdysone are known [41]. The only truly viable hypothesis for the function of phytoecdysteroids in plants is that they serve to deter phytophagous invertebrate predators, either by being toxic on ingestion and/or by having an anti-feedant effect. However, ecdysteroids possess a number of properties (polarity, chemical complexity, metabolic and environmental lability) that make them per se generally unsuitable as pest control agents. Unexpectedly, they later found a limited use in molecular biology to promote expression of gene elements and, for the same reason, as growth promoters in cattle rearing.

Bis-acylhydrazines such as tebufenozide (Fig. 37.4) showed activity as non-triterpene analogs of ecdysones and could be developed into insecticides by Rohm & Haas, starting in 1983, which cause premature lethal molts in susceptible intoxicated insects. The low degree of environmental concern is due both to their intrinsically toxicity and to the fact that their environmental breakdown products are just substituted benzoic acids totally devoid of any toxicological interest [44, 45].

However, this approach did not fully flourish, mainly due to the difficulty of researching on selective agents for major pests of a public concern without a strong financial support, within a regulatory system based on stringent requisites for new active substances (see [22]) which apply well to synthetic chemicals but may prove scientifically unreasonable for biologically active natural substances such as the mediators of chemical communication between invertebrates developed as sexual-attractant-based methods of biological control [10]. A particularly striking early example was methoprene, a so harmless compound that a DL_{50} dose could not found even guzzling rats at 35 g/kg of body weight.

In 1975 the EPA-NIEHS-Federal Working Group and Pest Management held a Conference on Human Health Effects of New Approaches to Insect Pest Control at Durham (August 4–6), the Proceedings of which were published in the April 1976 issue of Environmental Health Perspectives. The covered topic which elicited most contributions was that of biological pest control, as both the use of natural predators of pests and of species-selective insect chemo-attractants to attract target organisms towards insecticides while sparing non-target ones.

37.4 The Global Food Trade

While what is currently known as Global Food Trade is a long-known process, which may be traced at least as back in time as the Egyptian-Roman wheat trade of the imperial age, the European colonial era which developed starting in the sixteenth to nineteenth centuries set in motion an extractive relation between Europe and the rest of the world, as the colonies were converted into supply zones of food and raw materials to fuel European capitalism, disrupting long-time established local economies. From the 1980s onwards, ‘free trade’, not state intervention, became the dominant policy paradigm. Consequent liberalization policies have deepened the conversion of the global South into a ‘world farm’ for a minority of global consumers, concentrated in the global North and in strategic states and urban enclaves of the South.

Important technological achievements were reached since the nineteenth century to improve transport, conservation and use of food, namely Sterilization, Pasteurization (the first canned food factory was inaugurated in England in 1813) and blossomed in the twentieth century, with the advent into Western markets of Frozen foods (1940s), of Freeze-dried, pressure-cooked foods (1960s), of Microwave cooking of ready-to-consume foods (1980s).

This process, in which economical and technological aspects are deeply intertwined also brings opportunities (*pros*) and threats (*cons*) the distribution of which among global actors: producers, traders, consumers involves cultural issues and cannot be solved only on a technological or scientific basis.

Higher production of food and commodity crops is possible in specific regions of the Earth, due to geographical and climatic characteristics. Global trade affords a wider variety of crops to be available to an increasing population. Wider market opportunities are an incentive to development of peripheral regions and groups. Whether diffusion of previously ‘exotic’ food to populations over much wider areas and the growth of a global culture can contribute to increase a multi-cultural approach and to lower the potential of conflicts is a still debated point but such end-point has been reached in several areas.

Among perceived and often demonstrated drawbacks of the global food trade are the necessity of applying intensive monoculture, which causes loss of biodiversity since only some cultivars, especially of food crops, may be requested by the market. Loss of local culture can be a consequence, since change of production modes also modifies social structures in unpredictable directions. Crop transportation over long distances is a major factor in the spread of pests and of diseases and the environmental cost of transportation may add to the global anthropogenic burden on the Planet.

The contemporary Global Food Trade needs to equilibrate among the conflicting pressures by social actors with perceived different interests. A crucial one is that which can be summarized in the apparent antinomy of *Quality vs. Quantity*: high yields and product homogeneity are necessary when food production aims at feeding very large populations, such as those of the Western ‘*baby boom*’ at a decreased economic cost, in order that savings can be addressed to new types of consumption, such as leisure goods [16, 17]. To achieve this results, local crop varieties are lost in favour of those selected by Agropharma, which need hi-tech care (fertilizers, pesticides) to grow to commercial value. Loss of biodiversity of crops puts at higher threaten by pests, as historically experienced several times, from the 1845to 1851 Irish potato famine caused by an airborne fungus (*Phytophthora infestans*) originally transported in the holds of ships traveling from North America to England and carried to Ireland by northerly blowing winds to the *Peronospera* vineyard epidemic carried to Continental Europe from the U.S.A. 20 years later.

Another side of the problem is the impact of new food regulations based on toxicological knowledge on several traditional foods, which contain appetizing substances deriving from added spices or deriving from specific processing, and for which some degree of toxicological hazard has been demonstrated. On this regard, safeguard of consumers *vs.* safeguard of traditions need to be accurately balanced in scenario-based risk assessments. Traditional food is often produced from starting materials and under conditions which may not be deemed ‘*consumer-safe*’ according to the current perception of food safety, yet they have been produced and consumed for centuries without yielding any substantial contribution to the global disease burden of the populations. The use of plant products as spices is long known as a way to give flavour to food and often to protect from spoilage; yet most chemicals which impart to spices and herbs their useful properties are cytotoxic allelo-chemicals intended to preserve the organism’s living niche from neighbours and predators by harming them. Also some traditional processes of food manufacturing, such as controlled fermentation and colonization by selected micro-organisms, may exploit their biochemical processes to modify texture and nutrient content, or use the natural production of antibiotics to preserve food from spoilage.

There are many examples of controversial issues due to toxicological concern on traditional food spanning from Italian pesto sauce (which contains methyl-eugenol, a weak carcinogen; [37]) to French and Italian fermented cheese (Roquefort and Gorgonzola, respectively, which contain roquefortins, the cytotoxic antibiotics produced by the mould; [14, 36]).

37.4.1 Codex Alimentarius

The *Codex Alimentarius* (Latin for '*Book of Food*') is a collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to foods, food production and food safety which are agreed upon at UN-level for production, transformation, wise use of food resources [3–5, 13]. Since protecting the purity of the nation's food supply has ever been a function of governments, elementary rules regarding the composition and purity of main foods was incorporated in religious codes (such as *kasherut*, among the oldest requiring a quantitative assessment of the maximum allowed level of a contaminant, the *one-in-sixty* rule; Islam's *halal-haram* and other approaches in large and small religious groups) [30] and later in secular laws, explicitly grounded on an increasing body of scientific knowledge.

The discipline of Merceology developed between the late eighteenth and the nineteenth century, by applying the recent breakthroughs of analytical chemistry to determine the composition of commodities with the aim of levying custom taxes. The different sets of standards arising from the spontaneous and independent development of often conflicting and contradictory food laws and standards by different countries had the effect of creating inevitable barriers to food trade, as recognized in a statement of the first meeting of the Joint FAO/WHO Expert Committee on Nutrition in 1950.

The devastation of the Second World War, especially in Europe, convinced politicians and economists that improved agricultural trade would be essential for rapid reconstruction and the ability to feed people. Following the creation of the Food and Agriculture Organization (FAO) in 1945 and of the World Health Organization (WHO) in 1948, in the early 1950s the two organizations independently began work on requirements and analytical procedures for determining the purity of fruit juices. The idea of a Europe-wide Codex Alimentarius -based on an already existing, albeit little-known outside the German-speaking world, the *Codex Alimentarius Austriacus*- was actively pursued by Hans Frenzel of Austria between 1954 and 1958 and culminated in the creation of the Council of the *Codex Alimentarius Europaeus* in June 1958 under the joint sponsorship of the International Commission on Agricultural Industries and the International Bureau of Analytical Chemistry. The First FAO Regional Conference for Europe, meeting in Rome in October 1960, recognized that international agreement on minimum food standards and related questions (including labelling requirements, methods of analysis, etc.) is an important means of protecting the consumer's health, of ensuring quality and of reducing trade barriers, particularly in the rapidly integrating market of Europe and aimed at coordinating the growing number of food standards programmes undertaken by many organizations. In November 1961 the eleventh session of the Conference of FAO passed the resolution by which the *Codex Alimentarius Commission* was established, was joined by the WHO in June 1962, and held the first session in Rome in October 1963. Some 120 participants from 30 countries and 16 international organizations attended. Among the first documents, that on the General Principles of Food Hygiene was issued in 1969. In 2009 the Maximum Residue Limits (MRLs) for Pesticides was issued and revised in 2011.

37.5 General Conclusions

It is a long time that the necessity to supply agricultural products to feed the ever-increasing world population and to produce the commodities necessary to sustain increasingly higher standards of goods availability makes pest control a priority in the global agenda.

To overcome the continuous emerging of pest resistance there is a need to discover and use new products and to adapt the use of existing ones to changing needs, avoiding excessive use and unnecessary contamination of valuable planetary resources.

Since plants are naturally ever since engaged in fighting their natural predators, the panoply of plant allelo-chemicals can be exploited as a source of chemical diversity to imitate and improve plant strategies against crop-spoiling organisms.

Availability of natural products as plant protection products may show important differences with respect to production of man-made molecules, since the latter can be produced at any economically sound rate by up-sizing chemical plants while the former must rely on the natural growth of producer organisms, be plants, or micro-organisms.

Both in the field of crop protection and in food production '*Natural is not necessarily harmless*'. Allelo-chemicals employed as plant protection products can be as powerfully toxic to humans, domestic animals and cattle, to useful insects and pollinators and to non-target organisms as are to pests. 'Natural' products used as substitutes of synthetic pesticides therefore need the same level of Risk Assessment, of Risk Management and of Risk Communication as man-made molecules. Food with a long tradition of consumption can in fact contain natural substances the toxicity of which can result at least worth a health risk assessment.

Globalization calls for agreement on the properties of foods in order that they can be marketed world-wide without health restrictions. Also their nutritional value should be agreed upon by sharing of methods for production and for the determination of their nutritional properties and of the unavoidable residues of plant protection products, of veterinary drugs, of environmental contaminants and of manufacturing by-products, so that the latter are kept at levels for which no health concern is raised even for the most sensitive sub-groups of the human population.

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Chapter 38
Initiatives and Actions on the Elimination of Obsolete Pesticides Risks/Hazards in the Republic of Moldova

Mariana Grama, Iurie Pinzaru, Corneliu Mirza, and Veronica Tertea

Abstract In spite of the fact that Moldova has never produced pesticides, including persistent organic pollutants (POPs), over 50 years of overuse of pesticides left a damaging legacy. At present, the Republic of Moldova has an estimated 7,245 ton of obsolete pesticides and dangerous chemicals, which remain scattered in 22 central warehouses and 4,000 ton that are buried in the dump pesticides site in the southern part of the country. Among the pesticides that have been used in Moldova in the past are organo-chlorinated pesticides listed in the Persistent Organic Pollutants Convention of Stockholm; these are thought to pose the highest health and environmental risks due to their toxicity, persistence and bioaccumulation potential. The Government of the Republic of Moldova acknowledged that elimination of obsolete pesticides (included POPs) would serve the long-term interests of public health, environment, and economic development of the country. Since 2001, after signing the Stockholm Convention, it was defined as a separate field of actions, which became a priority one among the main environmental issues of the country. In dealing with POPs, the country followed a process that includes developing a clear understanding of the situation, setting priorities and establishing realistic objectives for actions. Preparation of the Moldova National Implementation Plan for the Stockholm Convention followed such a process utilizing a participatory approach whereby all interested partners in society (national and local government, economy, science,

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energy, agriculture, education, NGOs) had an active role in the decision-making and assumed their full share of responsibility for the National Implementing Plan.

Keywords Obsolete pesticides • NATO • OSCE • GEF • WB • Ministry of Defense of the Republic of Moldova • Environmental security • Wine's export certification • Database of stockpile's nature • Public awareness

38.1 Background

The presence of obsolete pesticides on the territory of the Republic of Moldova is one of the most significant risk factors for the national security as environmental, public health, food security, social, etc.

During the 1970–1980 Soviet dominated period the agricultural sector was used on an experimental basis for research on the use of pesticides. Moldova accumulated at least 7,245 ton of pesticide waste of more than 110 different types of pesticides by the end of 1980. Among the pesticides that have been used in Moldova in the past are more than 49 compounds which have been prohibited or restricted for use in agriculture as organochlorine pesticides listed in the Persistent Organic Pollutants Convention; these are thought to pose the highest health and environmental risks due to their toxicity, persistence and bioaccumulation potential.

The use of pesticides and other chemicals, toxic to both human health and the environment grew dramatically during the last 40 years in Moldova. In what follows we will use the term "pesticide" for all chemical products that serve as herbicide, insecticide, fungicide… Due to poor management practice and the lack of imposition of bans in the use of particular chemicals, Moldova has accumulated over the years large amounts of obsolete pesticides, in particular persistent organic pollutants (POPs). These substances possess toxic characteristics, are persistent, accumulate in the tissues of most living organisms and are likely to cause adverse human health or environmental effects near to and distant from their sources.

Therefore, over the period 1977–1987, more than 4,000 ton of pesticides waste, collected from various locations in the country, was buried there, including 654.1 ton of DDT. The pesticides landfill at the Cismichioi site is considered as one of the national priority sites and requires urgent attention in order to eliminate acute risks. The 2.3 ha site contains 12 distinct burial mounds, most of which are visible from the surface. In only four of these sites the wastes were buried in protected conditions, in the others the chemicals are only kept isolated from the surrounding soil with a layer of plastic foil. The site is only a few km away from the Ukrainian and Romanian borders and close to watersheds discharging in the Prut River and the Lower Danube near to its estuary.

According with statistical data, in the period 1950–1990 an estimated total amount of 560,000 ton of pesticides were used in Moldova including 22,000 ton of organochlorinated POPs (OCPs). The pesticides use registered a peak during the period 1975–1985, but reduced significantly over the last 10–12 years (from 38,300 ton in 1984 to 2,800 ton in 2000) [8]. However, according to the data from the Ministry of Agriculture and Food Industry, the State Enterprise "Moldselhozhimia" and the State Ecological Inspectorate, information is not always accurate and the quantities and types of the chemicals used differ a lot. An approximate estimation of the available data shows that the average amount of POPs out of the total stock of pesticides is about 20–30 %.

It is well known that pesticides are able, by virtue of their various physical and chemical properties to enter all environmental compartments and affect human, animal and plant organisms through various pathways. The health of local people and the quality of their environment are still severely compromised by the consequences of presence of obsolete pesticides, including POPs and unidentified stocks, and by conditions of their storage in more than 340 warehouses over the country, which are still located closely to residential areas (mainly rural ones) or/and nearby pastures, arable land and orchards/vineyards. Some of the stores are located within areas, which are regularly passed or visited by unprotected people. The result is that children, women, men and livestock are exposed to pesticide vapors, dust and contaminated soil, water and other materials on a virtually constant basis. It is also important to note that over 1,500 pesticide warehouses and a similar number of grounds for preparation of pesticide solutions had been in use in Moldova in the past. It can be estimated that a few thousand areas, which are potentially polluted and pose health and environmental risks, have been found in Moldova.

It is also important to recognize that contamination of agro-products has potential impacts on the urban population's health, as many products are sold in local markets which have no rigorous pesticides residuals control. The economic losses, driven by contamination of agriculture and food products, can significantly limit Moldova opportunities on the external markets.

38.2 Actual Situation

Currently, the total amount of obsolete pesticides in Moldova is estimated at more than 6,000 ton of obsolete pesticides and other hazardous chemicals waste: 1,949 ton which remains scattered in 23 regional central storages of 26 districts and 4,000 ton buried in the pesticide landfill in the southern part of the country (Territorial Autonomy of Gaguz-Yeri), and cca 150 ton of pesticides waste in the uncontrolled region called "Transnistrian region" [1]. Until the year 2008, the quantity of harmful pesticides was more than 7,400 ton of pesticides. 3,245 tons were scattered or buried in 424 poorly equipped or unfitted facilities which lack proper monitoring and security. 1,296 tons were disposed in the Global Ecological Fund/World Bank Project "Management and disposal of the POPs stockpiles" by the French Company TREDI during the years 2007 and 2008.

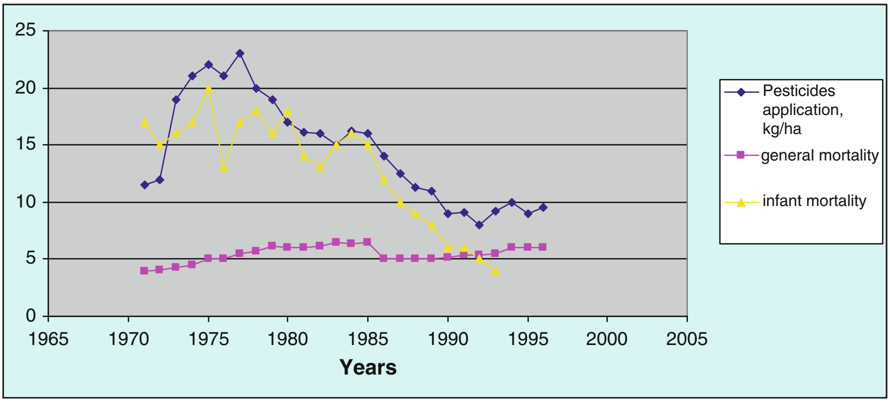

Fig. 38.1 Total pesticide application in agriculture, general and infant (3) mortality

During the time, the urban and rural area were extended and the pesticides warehouses are situated close/or into the residential area, aquatic basins, state protected areas, agricultural land, etc. and become a potential contamination sources of the soil and the potable water, which gravely affects people‘s and animal‘s health, and biodiversity, especially in rural area.

Because of aggression of the liquid pesticides during 2004–2011, the steel drums start to be rusty, cracked and linking and present new high risks for the environment and public health.

The Government of the Republic of Moldova has a difficult to guarantee a constitutional rights of healthy life style for the people which live close/or in the area of pesticides waste warehouses. A lot of complain letters from Local Authority, people couldn’t be solved.

Life expectancy at birth in Moldova shows patterns similar to the one observed in neighboring countries and in some Eastern Europe countries. Although the decrease of this indicator has been reversed and presently it constitutes 68.4 year, it continues to register values much lower than the average for the EU states [10]. The maternal mortality rate is now 16.0 maternal deaths per 100,000 live births as compared to 43.9 in 2001. However, these indicators are still higher than average European figures. The mortality of the economically active population is higher than in the EU countries. The most important death causes in Moldova include diseases of the circulatory system, poisoning, malignant neoplasm, cancer and diseases of the liver, gall bladder and pancreas. More frequently illnesses for economically active population are registered such as allergy diseases, digestive tract diseases, inflammatory diseases of skin and subcutaneous tissue (dermatitis, eczema, etc.) and others. Figure 38.1 presents an overview of the general pesticides application in agriculture in connection with general and infant mortality of the population from Moldova.

The share of persistent OCPs also decreased in favor of other pesticide groups. During the period 1976–1990, soil samples showed pesticide contamination levels

Fig. 38.2 Conditions at typical storage site in the Moldova

exceeding the maximum allowable concentration (MAC) from five times in the southern zone of the country to 50 times in the Central zone. Research undertaken by the Institute for Experimental Meteorology of the State Committee for Meteorology showed that during the period 1979–1985 about 60 % of soil samples were polluted with DDT at concentrations exceeding the MAC, in spite of the fact that DDT had been prohibited since 1970. Beginning in 1989, due to reduction in pesticide application, investigations showed a decrease of pesticides-related pressure both in annual and perennial crops. During the period 1990–1995, the regional Centers of Preventive Medicine of the Ministry of Health analyzed the contents of 28 pesticides' residuals in ten agricultural crops and foodstuffs. Pesticide residuals were found in 56 % of the tomato samples and in 40 % of the grape samples analyzed.

By the early 1990s, over 1,000 warehouses for pesticide use have been built in collective farms. During 1991–2003 about 60 % of these were destroyed or dismantled with only 20 % of the remaining ones were maintained in a satisfactory condition. These were recently transformed into regional storage facilities for pesticides and fertilizers. Significant amounts of obsolete pesticides were stored there in the open. Deteriorated packaging enhanced the risk of harmful effect on people's health and environment. Some warehouses are situated close to residential areas. In addition, the pesticides should have been packaged and stored in these warehouses according to the following instruction and rules that were developed by the Ministry of Agriculture of the Soviet Union in 1985: "Rules on Pesticides Receiving, Keeping and Delivery at Agricultural Chemistry Storages"; and "Instruction on Collecting, Preparing and Transporting of Old and Banned Pesticides and Their Packages". However, as shown in the examples in the photographs in Fig. 38.2, the warehouses have deteriorated to the stage that there was considerable mixing of different categories of pesticides, both in the solid and the liquids sections. Occasionally, there was even mixing of solids and liquids in the same section. In many cases bags of pesticides had broken and drums of liquids had corroded and were leaking.

It became clear that the segregation system envisioned in the 1985 was not followed in the 1990s and the stored pesticides could no longer be classified according to this system. Hence in the recently organized repackaging process the majority of the pesticide packages can only be categorized as "Unknown" and can only be classified as "hazardous waste with the potential of POPs contamination". The photographs in Fig. 38.2 show examples of the typical state of these warehouses prior to the repackaging program.

Also, it is important to notice that after the collapse of USSR, the Republic of Moldova lost a large part of its manufacturing sector, including the fact that the country's industrial hub was located in the breakaway region of the Transnistrian Region. Actually, the wine industry plays a significant role in the Moldovan economy. Moldova is highly dependent on wine production; it is considered the backbone of the agricultural sector. In global terms, Moldova ranked 7th in the list of world wine exporters in 2005, exporting 2.3 million hectoliters of bottled wines. In 2005, the Moldovan wine collection "Mileştii Mici", with 1.5 million bottles, was included in the Guinness Book, as the largest wine collection in Europe.

In March 2006 Russia imposed a total ban on Moldovan wine based on claims of contamination by pesticides and heavy metals which pose health risk. This financially devastating ban led to losses officially reaching 180 million USD in a country that was already Europe's poorest [3, 4].

Besides, annually, the Republic of Moldova spent 260.000 EUR for security of the pesticides regional storages, etc.

38.3 National Framework

Memorandum of Understanding between Government of the Republic of Moldova and the NATO Organization for Maintenance and Supply (*NAMSO*) *on logistic co-operation*, signed at 28 June 2001, ratified by Law No.541-XV of 12 October 2001, promulgated by Presidential Decree No.292 of 31 October 2001, contributed to the institutionalization of the NATO/PfP Trust Fund for demilitarization of various small arms, light weapons and munitions in 2002, financed by the NATO member countries designed to destroy an allotment of anti-person mines and expired-term ammunition from the subsidy of the National Army, as well as an important quantity of missile fuel the melanj type. Due to this project were eliminated an ecological threat and foresights on interdiction of anti-person mines of the Ottawa Convention were accomplished. Hereby, Republic of Moldova was the first country to interdict this type of mines from its arsenals.

Another project implemented with the NATO financial support regards the annihilation of the reserves of interdicted and unusable pesticides from Republic of Moldova, into Implementing Agreement 12 May 2006, Bruxelles.

The Individual Partnership Action Plan Republic of Moldova – NATO, adopted by the North Atlantic Council on 19 May and, respectively, by the Government of Moldova on 24 May 2006, sets a number of important goals, such as deepening of Moldova's cooperation with the European and Euro-Atlantic structures and institutions, promoting democratic reforms in various fields, reform and modernization of the defense and security areas, strengthening democratic control over armed forces, etc.

The National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants, approved by the Government Decision in October 2004, aims to provide a framework and management options in order to meet the obligations taken by Moldova by joining the Stockholm Convention on Persistent Organic Pollutants and to reach the national objectives and priorities regarding the POPs [8].

The Economic Growth and Poverty Reduction Strategy Paper (*2004–2006*) was the overarching policy framework for the sustainable development of the Republic of Moldova in the medium term. It served as the basis for developing and implementing new assistance strategies by international financial organizations and donor countries, as well as for the preparation of the annual state budgets for 2005 and 2006. The environmental component of the Paper made direct reference to the improvement of unusable pesticides waste management and the reduction of pesticides waste, actions which directly would influence on reduction of soil degradation, growth of ecological agriculture products, etc.

The Midterm Strategy for Socio-economic Development of the Republic of Moldova to 2005, approved by the Government Decision in 2001, stipulated that ecological economic goals should include: regulating impacts from economic activities on the environment; preventing environmental pollution and ensuring environmental quality and rehabilitation; improving methods for utilization of natural resources and ensuring their continuous and safe exploitation; improving environmental education; enhancing environmental research and implementation of environmentally clean technologies. Also it was mentioned that international environmental standards would be introduced in all sector of national economy, particularly the ISO 14000 standards.

The Concept of the Environmental Policy of the Republic of Moldova, approved by Parliament Decision No.605 of 02 November 2001, reflects two key objectives on prevention and reduction of negative impacts of economic activity on the environment, natural resources and health of the population within a framework of national sustainable development, and on insurance of ecological security of the country. Both directions have to be conducted by principles of "economy through ecology" and "cost-benefit".

The *National Environmental Health Action Plan* (NEHAP) provides directions for the next 10 years for protecting human health and assigns responsibilities among Government agencies, with specific focus on aligning the country with EU by ensuring harmonization with its policies, procedures and practices. NEHAP has a few references to pesticides waste, including in: § 4.1-Water stipulating elaboration of a pesticide decontamination system for waste water discharges; § 4.2-Air stipulates development of pesticides air emissions monitoring systems and of a program for "neutralization" of the referred emissions; § 4.3-Soil seeks to encourage improving existing legislation and elaborating new maximum allowed concentration for various pesticide as well as provides for establishing a national pesticides waste inventory; § 4.5-Food – stipulates improved control of pesticides contamination of food; § 4.10-Natural Catastrophes and Industrial Accident provides for creation of a national register of potentially toxic chemicals; § 5.4-Agriculture stipulates developing regulations regarding importing, storage and use of pesticides.

The main objectives of the national framework consist of:

- To guarantee the fundamental human rights in according with Charter of the fundamental rights and ensure the rights to a healthy environment (EU 2010/C 83/02, the Universal Declaration of Human rights, the Constitution of the Republic of Moldova, national policy and strategies on human rights) [12], etc.
- To prevent illegal trafficking of hazardous waste, in special pesticides waste (strong links need to be maintained with the Basel Convention and Stockholm Convention).
- To prevent using of pesticides waste as chemical weapons in the terrorist actions (Geneva Protocol, 1925).
- To protect human health and the environment from pesticides waste as persistent organic pollutants not only from Moldova but entire Southern European Region (Stockholm Convention on Persistent Organic Pollutants).
- To address international security issues linked to elimination of acute risks of hazardous waste. Solution range from regularizing exchange of information across the border to a full application of the Espoo Convention on environmental impact assessment in transboundary context.
- From dialogue to sustainable settlement through engagement of Moldova's Transnistrian region into environmental cooperation for destroying hazardous waste in the region.
- To increase environmental security with impact on pesticides migration into water distribution in the Lower Danube Delta and Black Sea, recovery ecological balance of the Danube River and Black Sea To assist implementation of the Healthcare System Development Strategy for the period 2008–2017 [9, 10], National Strategy on implementation of Stockholm Convention on POPs in the RoM [8], The National Strategy for Sustainable Development of the Agricultural Infrastructure for 2008–2015 [11].

38.4 Actions and Initiatives

38.4.1 At the National Level

During the last decade the problem of obsolete pesticides was placed on the environmental agenda of the Republic of Moldova as part of toxic substances and waste management programmes.

In dealing with obsolete pesticides and other dangerous chemicals, the country followed a process that includes developing a clear understanding of the situation, setting priorities and establishing realistic objectives for actions.

The Moldovan authorities recognized that the long term storage of obsolete pesticides is not sustainable option since a series of deficiencies revealed in the process of repackaging and storage raised concerns about the integrity and security of the storage facilities.

In 2001, the Government adopted a decision [5] stipulating the measures to be undertaken, timeframe and responsibilities of involved ministries, departments and local public authorities with regard to centralized storage and disposal of obsolete pesticides. In particular, it asked the Ministry of Agriculture, the Ministry of Ecology and the local authorities to select three to four storehouses in each judet (former administrative unit in Moldova) to be used for centralized storage; the Ministry of Agriculture, the Ministry of Healthcare, the Institute of Chemistry of the Academy of Sciences and the Department for Standardization and Metrology to participate in laboratory investigation of pesticides to determine their chemical composition and amount of active substance; the Ministry of Agriculture and the local authorities to rehabilitate the storehouses; the Institute of Chemistry to investigate the possibilities of further incineration of pesticides; the Ministry of Agriculture, the Ministry of Healthcare and the Department for Standardization and Metrology to make provisions for transportation of pesticides to cement factory for further elimination while the Ministry of Agriculture was given the task to apply to the Council of Europe for a grant to support the elimination of pesticides.

In 2002 the Government adopted the decision No. 1543 “On Additional Measures for Central Storage and Disposal of Out-of-Use and Banned Pesticides” which stated that the Ministry of Defense and the Department of Emergency Situations is to ensure the transportation of pesticides as well as other relevant technical actions [6].

In 2003 the Government adopted the Decision No. 1389 “On approval of modifications and agenda in the Governmental Decision 1543 as of 29 November, 2002” [7]. The latest Decision stated that heads of rayons, and mayors of settlements must select one storehouse on the administrated territory, and in coordination with the Ministry of Defense and Department of Emergency Situations should make provisions for the repackaging, transportation and guarding of pesticides until a destruction option is found. Besides, the Ministry of Defense of the Republic of Moldova has been nominated as the national authority for implementation of the programs for the destruction of the obsolete pesticides stockpiles and/or for evacuation from the Republic of Moldova territory in according with Government Decision No.1543 of 29 November 2002 “On additional measures for central storages and neutralization of the forbidden and unusable pesticides” and Parliament Decision No.90 of 12 May 2011 “On approval of the National Action Plan for human rights for period 2011–2014”, and other national actions plans or strategies [12]. For the efficient implementation of the programs, the Ministry of Defense cooperate with all state structures at the Central level (Ministry of Agriculture and Food Industry and its territorial Inspectorate for Fitosanitation; Ministry of Health and its territorial Public Health Services; Ministry of Environment and its territorial State Ecology Inspectorate, etc.) and Local level as Local Public Administration through Territorial Emergency Situation Commission created at the district level, which include responsible authorities of Mayors, Civil Protection, Ecological Inspectorate, Public Health Services, etc. The implementation of this Decision is under way.

In the same time, since 2001, after signing the Stockholm Convention, it was defined as a separate field of actions, which became a priority one among the main environmental issues of the country. In dealing with obsolete pesticides like persistent organic pollutants, the country followed a process that includes developing a clear understanding of the situation, setting priorities and establishing realistic objectives for actions. Preparation of the Moldova National Implementation Plan (NIP) for the Stockholm Convention followed such a process utilizing a participatory approach whereby all interested partners in society (national and local government, economy, science, energy, agriculture, education, NGOs) had an active role in the decision-making and assumed their full share of responsibility for the National Implementation Plan.

Later, in October 2004, the Persistent Organic Pollutants National Implementation Plan was approved which aimed to provide a framework, management options and measures in order to meet the obligations taken by Moldova through joining the Stockholm Convention on Persistent Organic Pollutants. National objectives and priorities were defined.

Despite the existing difficult economic situation, the Republic of Moldova is committed to fulfilling its obligation regarding obsolete pesticides under international treaties. This required increased internal institutional and financial resources and the mobilization of international assistance.

38.4.2 Cooperation with International Organizations Into Environmental Security Initiative

To address and prevent increasingly high risks to population and the environment, NATO, in collaboration with other international organizations embedded in the Environment and Security (ENVSEC) initiative for Eastern Europe (the United Nations Environment Programme, the United Nations Development Programme and the Organization for Security and Co-operation in Europe), accepted supporting Moldova to achieve its objectives in the management and safe destruction of its chemical hazards and the fulfillment of its obligations under international Conventions, Agreements and protocols, especially the Stockholm Convention.

On 12 May 2006 the Implementing Agreement between the Government of the Republic of Moldova and the NATO Maintenance and Supply Organization (NAMSO) for destruction of pesticides and dangerous chemicals, was signed at the NATO Headquarters, in Brussels. This Agreement aims to improve the environment and health situation of the population in Moldova through the reduction of negative impacts of old environmental burdens – remnants of obsolete pesticides scattered on whole territory of the country through implementation of four distinct phases:

- Repackaging, relocation and safe storage of 3,245 ton of obsolete pesticides from 424 to 37 regional central warehouses;
- Identification of the nature and composition of the pesticides stockpiles;
- Disposal of the pesticides and dangerous chemicals;
- Remediation of contaminated facilitation.

Table 38.1 General distribution of project funding by sources

	UN/ADR package	Non UN/ADR package	Total
NATO/PfP-OSCE/ENVSEC Trust Fund	1,269	309	**1,578**
Other sources	27	344	**371**
Total	**1,296**	**653**	**1,949**

These four phases have been implemented in four projects with the intention to address Moldovan environmental issues, which are perceived to threaten security, social stability and peace, human health and sustainable development at the local level, as well as in the entire South East European Region.

Repackaging and safe storage of obsolete pesticides was managed by the NATO Maintenance and Supply Agency (NAMSA) on behalf of the Lead Nations (Belgium and Romania).

The first phase of the project, rendering the chemicals safe repackaging and storage, was funded through a NATO Partnership for Peace (PfP) Trust Fund project (EUR 804,000). Contributing nations were Bulgaria, Czech Republic, Ireland, Lithuania, Luxembourg, the Netherlands, Norway, Romania, Sweden and Turkey, Belgium, Finland, and Germany (Table 38.1).

The process of *repacking of 3,245 ton of pesticides and dangerous chemicals* from 424 poorly equipped/demolished places/warehouses and safe storage in the 37 centralized facilities was completed in June 2008, with collaborative efforts by the Moldavian State Budget and the National Ecological Fund (430,000 EURO), NATO/Partnership for Peace Trust Fund – OSCE/ENVSEC (658,000 EURO, including the Dutch NGO MILIEUKONTAKT (150,000 EURO)).

Actually status showed, that from totally amount of 1,949 ton of pesticides, 1,296 ton of pesticides are repacked in the UN/ADR package, and 653 ton – in Non UN/ADR package.

Since 2003 year, in the project's activities were involved more than 720 military personnel and 35 military techniques from NBC units of the National Army of the Republic of Moldova (Fig. 38.3).

The benefits of this project are:

- Overall reduction of the environmental, health, and socio-economic risks associated with obsolete pesticides dissipation in the environment:
  - Eliminating the health and environmental risks of further releases of obsolete pesticides from temporary unsafe storage sites;
  - Raising opportunities for organic agriculture thus increasing the income levels of small farmers and the rural poor by improving the export potential of agriculture products and increasing the land value in the proximity of emptied warehouses;
- Assisting Moldova in meeting its obligations under international Conventions, Agreements and protocols.

Fig. 38.3 Cleaning of an abandoned obsolete pesticide storage site

Besides the repackaging and safe storage of the obsolete pesticides, ***the POPs Information Management and Reporting System and Monitoring Network*** in the Republic of Moldova have been developed in order to improve POPs (including new POPs) environmental management and reduce their potential negative impacts.

In the framework of the *NATO Science for Peace and Security Project* "*Clean-up chemicals – Moldova*" (*298,000 EURO*), were established the analytical laboratory with sophisticated equipment for systematic characterization and analysis of the pesticides stockpiles, based on gas chromatography-mass spectrometry (GC-MS), high pressure liquid chromatography (HPLC), thin layer chromatography (TLC) and ion selective electrode (ISE). A NATO-funded laboratory is located at the State Centre for Certification and Registration of Phyto-Sanitary Means and Fertilizers in Chisinau. The project employs specialized personnel from Ministry of Defense of the Republic of Moldova for sampling and general project management and specialist scientists in the "phytosanitary products and fertilizers" of the Ministry of Agriculture for the laboratory activities. In addition, the Moldavian State University participates with specialists in analytical chemistry including a number of young scientists with general scope on training a new generation of scientists, that have been involved in the future in the Republic of Moldova for the pesticides issues. All personnel became operationally in standard sampling, analysis and data management techniques.

By the end of July 2009, an overview was obtained of the nature and composition of 3,245 ton of harmful pesticides stored in two municipalities, 32 districts of Republic of Moldova and the Territorial Autonomy Gagauzia. A total of 5,000 samples were tested by GC-MS, HPLC, TLC and ISE methods of analysis, which provided the identification in cca 87 % of samples.

More than 70 active ingredients and their metabolites were detected. Among the identified compounds 49 of them have been prohibited or restricted for use in agriculture, in Moldova since 1970, while the use of 24 substances was prohibited or restricted in the UE countries.

The NATO-funded laboratory has been played an important role in solving the problems related with obsolete pesticides, as follows:

- To make the incineration process safer and more efficient, TREDI used a database of the chemicals composition and toxicological evaluation, created by NATO-funded laboratory for the destruction of 1,296 ton of obsolete pesticides;
- To enhance food security in the country, with sophisticated equipment test agricultural products, in October 2007, Russia, Belarus and Ukraine recommenced importation of Moldavian wine after more than 650 samples of wine from 35 companies were certified by the lab to be pesticide-free. Since December 2008, the laboratory has been started certification of the pesticides-free in the apples for export, too;
- During January 2009–January 2010, the project activities were focused on environmental issue on the systematic study of the waste dump from s. Cismichioi, Teritorial Autonomy Gagauzia – the national strategic object, where more than 4,000 ton of pesticides have been buried in the early 1980s. The site is located at few km away from the Ukrainian and Romanian borders and close to watersheds discharging in the Prut River and the Dniester River.

In the same time, the NATO-laboratory played a great role in the obsolete pesticides analytical study for the TAUW Consortium (Tauw bv, Milieukontakt International, International HCH and Pesticides Association, Witteveen + Bos Environmental Consultants and Green Cross Switzerland) activities into the World Bank Project entitled “The obsolete pesticides technical study in the Kyrgyz Republic, the Republic of Tajikistan and the Republic of Uzbekistan” [3].

Regarding the analysis of POPs in environment components were done by laboratory of the Monitoring Centre of the State Hidrometeorological Services, funded by GEF/WB Project “Management and destruction of POPs stockpiles” in order to develop a map of polluted areas. More than 1,580 potentially polluted sites were investigated. For 252 sites (about 16 % of the total number) the data (expressed as a sum of all POPs detected on-site in composite soil samples) showed concentrations exceeding 50 mg/kg. At this level of pollution the soil can be classified as hazardous waste (Fig. 38.4).

Regarding ***the disposal of obsolete pesticides stockpiles*, *including persistent organic pollutants***, in March 2006 the Government of the Republic of Moldova received through World Bank and Global Environmental Facilities a grant for the Persistent Organic Pollutants Stockpiles Management and Destruction Project. Within funding out of this project and co-financing from state budget (3,6 mln $ SUA (including Moldovan Contribution of 1,2 mln $ SUA)) an amount of 1,296 ton of obsolete pesticides were shipped abroad, in France, during 2007–2008, and disposed of.

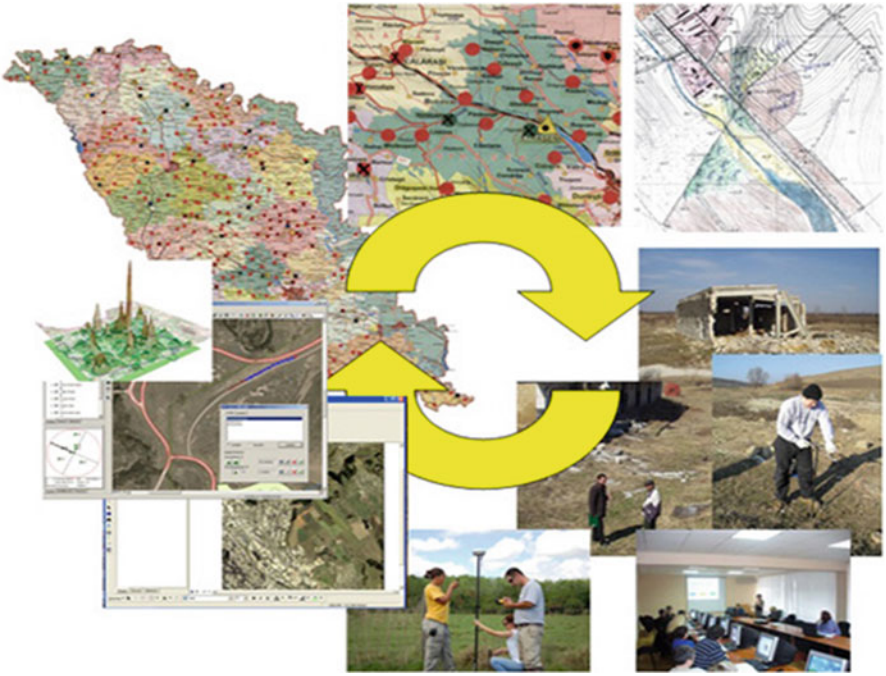

Fig. 38.4 A publicity poster of the GEF/WB Project "Management and destruction of POPs stockpiles"

Currently, the on-going Project "Remediation of environmental burdens by pesticides waste in Moldova" funded by Czech Development Agency, aims to destroy in Germany 200 ton of pesticides waste with EUR 500,000.

There are in place a new sub-project under NATO Trust Fund on disposal of the 1,269 ton of obsolete pesticides stored at 15 regional central storages. The Ministry of Defense on behalf of the Government of the Republic of Moldova signed 3rd Implementing Agreement for the destruction of pesticides and dangerous chemicals with NATO/NAMSO at 22 June 2011, in Bruxelles.

The Project will be managed by NAMSA on behalf of Romania as Lead Nation. Romania, Bulgaria, Estonia, Ireland, and Czech Republic have already specifically contributed or pledged for this Phase.

The Republic of Moldova will have essential contribution as:

- will assure necessary security at all stages of the project in the country;
- will provide cash contribution of 325,000 EUR and in-kind contribution of 557,000 EUR through manpower for any repacking, palletisation, labeling and loading activities, transport of pesticides waste between regional central storages, medical support, assistance of the territorial experts for monitoring and carrying out pesticides control, inspections and surveillance activities, and security at regional central storages (circa 260,000 EUR annually).

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Concerning ***the remediation of the contaminated sites*** with obsolete pesticides, including persistent organic pollutants, into GEF/CIDA Project "Remediation of POPs pesticides polluted areas and decontamination of PCB polluted oils in electric equipment" (2007–2009) were selected and tested the obsolete pesticides contaminated areas remediation technologies and techniques adjusted to Moldovan conditions (isolation of contaminated materials in cofferdams covered by protection layers (site Congaz, Teritorial Autonomy Gagauzia and site Step-Soci, Orhei district) and biological remediation with Daramend (site Bujor, Hincesti district)). But more than 1,500 places/warehouses or contaminated areas are still waiting to be remediate (*cca* 1,500 ha of land), because of lack of financial resources.

The ***awareness and educational activities*** related to obsolete pesticides issues is one of the pillars of Moldovan authorities based on the Communication Strategies, therefore is to increase citizens' awareness on environmental issues and foster public participation through strong cooperation with NATO, OSCE, ENVSEC, Moldovan state actors, NATO Information and Documentation Center, Association INQUA-Moldova, Regional Environmental Centre for Moldova, Moldovan Ecological Movement, Garamond Studio SRL and Casa Imago SRL [2].

In the conclusion, a lot of work has been done already, but still there are many urgent things need to be solved: including another 5,000 ton of obsolete pesticides waiting for being disposed of, more than 1,500 obsolete pesticides polluted areas to be remediated. Nowadays, Moldovan Government is strongly looking for additional funding from international organisations which will help to solve obsolete pesticides problems in the country in order to promote a safe environment and protect the public health as part of the sustainable development of Europe.

Acknowledgements Thanks go to the NATO/PfP countries, FAO, EU, OSCE, GEF, WB, Green Cross, Dutch NGO Milieukontakt, IHPA, Development Agency of the Czech Republic, Canadian Government, etc. that have permanently contributed and supported Moldovan initiatives in the clean-up and disposal of obsolete pesticides and other hazardous chemicals.

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Chapter 39
General Approaches and Procedures for Pesticide Legislation

Stefan Mandic-Rajcevic, Federico M. Rubino, and Claudio Colosio

Abstract The use of synthetic chemicals such as plant protection products (PPPs or 'pesticides'), especially in the last 60–70 years, greatly contributed to an impressive progress in the availability of food to an increasingly numerous mankind. However, the long-term consequences of the deliberate and unavoidable dispersion of these chemicals into the environment were long overlooked and generated health consequences to professional applicators, to bystander general population and to food consumers. An increased awareness of the potential threats of uncontrolled use of substances of poorly known toxicity led to a substantial change in the approach, gradually resulting into an improved legislation in Western Europe and in other developed countries, to the point that today licensed PPPs are among the substances of which the chemical and toxicological properties are best known, much before their introduction into the market and even better than requested for human pharmaceutical drugs. We will trace the pathway which leads to the birth of new PPPs and to their authorization according to the legislation of the European Union. In particular, while active substances are licensed for use in a 'positive list' at EU level, the different formulations suitable for use on different cultivations are authorized for the different geographical areas of EU with a 'mutual recognition' procedure between member States. Protection of agricultural workers, of consumers, of the environment are embedded into the authorization procedure by requesting that targeted studies run under normalized conditions are conducted prior to marketing. Several chemical and toxicological parameters which are

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pivotal to risk assessments towards humans, non-target plants and animals are measured and health-based safety levels are established for agricultural workers, for bystanders, for the general population, for the residual presence of the active substance and of its decomposition products in food and in natural drinkable water. For enhanced safety, authorization is released in 10-year periods, in order that unexpected harmful consequences for man and environment can be timely examined and, in case, tackled. The financial cost of this procedure is, of course, not without consequences. Since authorizations are issued in 10-year periods and are subject to voluntary rejuvenation by the licensees, there is a pressure to invest in newer, more profitable active substances rather than to keep into market older ones, which may be as efficient and cheaper, but for which the faintest evidence of health or environmental hazard may prematurely terminate corporate interest. The genesis of EU legislation will be discussed and examples will be brought to highlight key issues.

Keywords Plant protection products • European Union • Authorization • Risk assessment

39.1 Characteristics of PPP

Plant protection products, or pesticides (insecticides, fungicides, herbicides) are chemical formulations which consist of one or more active ingredients (or active substances) and other ingredients. Their role is the protection of plants and crops in agriculture, horticulture, forestry and gardening. While Plant Protection Products (PPPs) differ in many ways from other chemical substances produced by humans, especially for manufacturing and industrial uses, they share several similarities with pharmaceuticals.

First, they are produced to control living species and therefore they are necessarily toxic; second, they are deliberately spread into the environment to reach their targets, therefore can be source of environmental pollution and human exposure (workers and consumers); third, they are produced to fight against pests, but the specificity of their toxicity for their targets is limited, therefore their use can endanger non target species, from useful insects such as bees to humans.

Moreover, it is accepted that without the use of pesticides a significant proportion of the agricultural production goes lost to spoilage in the fields and to rotting and deterioration throughout the production and distribution process, in particular in tropical countries, their use is unavoidable. In this perspective, the environmental and health risks related with their use need to be balanced by the benefit they yield to agricultural production and, in the fight to disease-bearing parasites, to the benefit to public health.

The role of regulation processes is therefore to keep under constant control the consequences of the use of pesticides, with reference to a risk-benefit evaluation, and to prevent serious consequences to human health and to environment self-sustainability related to the use of these compounds.

Since the Council Directive of 1991, the European Union recognizes that plant production is very important for agriculture and plant protection products are one of the most important ways of protecting plants and plant products against harmful organisms including weeds, and of improving agricultural production [8]. Well alike pharmaceuticals, and different from most other commodities and consumer products, PPPs are long subject to pre-marketing authorization process which calls for knowledge of key information (physical, chemical, environmental and toxicological) characteristics to perform scenario-specific risk assessment. The introduction of the REACH (Regulation on Evaluation and Authorization of Chemicals) for nearly all industrial and consumer products takes most of its principles from the experience gained in the risk assessment of these high value-added products.

39.2 Regulation of Chemical Substances: A Glance to Its History

Although its principles were implicitly well-known since at least two centuries (the assessment of risk in long-distance trading of goods, which is the basis for setting insurance premium, is as old as Mesopotamic ages) Risk Assessment (RA) was formalized as a discipline in the USA towards the end of the 1970s. The necessity to frame the process arose as a consequence of two episodes which greatly stressed the public opinion and brought considerable debate into the scientific community: the consequences of the use of thalidomide as a drug and of vinyl chloride in the synthesis of PVC.

In the case of thalidomide, a sedative drug against morning sickness of pregnant women was deemed as fully safe after the reassuring outcome of what was reputed at the time to be sufficiently extensive safety tests on animal models and was marketed all over the world. It was prescribed to and taken by millions of pregnant women and, as a consequence, an epidemics of teratogenic effects soon developed, which stroke millions of children throughout the world and their families, before the use of the drug was banned [4]. In the case of vinyl chloride, a chemical commodity was supposed to be reasonably safe in normal conditions of use and was widely used both as a monomer in the production of polymers for manufacturing uses, as a propellant for spraying in consumer products and it was evaluated even for the use as anesthetic gas. As manufacturing and use increased over a few decades, evidence of its carcinogenic properties accumulated, finally leading to a ban in its use as a consumer product and to an increased level of care in its production and use as a chemical commodity [19].

These two crucial episodes highlighted the need of performing a full toxicological assessment *before* any chemical substance could be authorized for industrial, pharmaceutical or other uses, and in particular if their use was, as in the case of pesticides, intrinsically dissipative.

Pesticides are one of the best examples to follow the thread of the development of risk assessment of chemical substances. Since its first introduction into the market, in 1939, DDT showed very good insecticidal properties and its production and use grew, widening its application from public health (control of human external parasites, such as lice and scabies), to environmental application in the eradication of Anopheles mosquito, the vector of malaria, from several endemic areas in temperate and tropical countries, to an efficient control of agricultural pests and even. Starting from the early 1960s, with the publication of Rachel Carson's *Silent Spring*, concern on the environmental consequences of its widespread use reached the public opinion and the scientific community over the world started to investigate the bioaccumulation and bio-magnification properties of DDT through the food chain and its possible link to possible effects on human health. As awareness strengthened in the 1970s, limitations and bans were raised in the most developed Countries, leading to a significant reduction of DDT production and use and finally to a generalized international ban (see [22]). The same fate was followed by a few other pesticides, which are all characterized by common chemical characteristics leading to very long persistence in the environment, to transmission through the human food chain and by potential long-term toxicity.

Public perception of failures of pesticide regulation leading to strong public health concern and severe and persistent environment contamination has been a strong driving force towards improvement and harmonization of the requirements for authorization of plant protection products. The approach which is currently adopted for regulation of pesticides is "reactive/preventive", since it responds ('reactive') to damaging impacts for which there is convincing evidence of cause-effect relationship and takes regulatory action to ensure that similar impacts do not arise with new generation chemicals ('preventive'). It is also "risk-based" since it relies on cost-benefit analysis as a basis for scientifically rational decision making [27].

This approach is however not fully satisfactory, since existing knowledge of the complex interactions at the planetary scale is not sufficient to ensure that severe, irreversible consequences to the environment would not occur as the unexpected consequence of actions deemed devoid of risk according to existing knowledge. To cope with such cases, more or less compelling versions of the (still undefined; [32]) Precautionary Principle were appended. The 1992 Conference on the Environment and Development [29], stated that: "*Where there are threats of serious and irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost effective measures to prevent environmental degradation*".

In the 1970s, to substitute for the fairly efficient but now unacceptably dangerous poly-chlorinated insecticides, new classes of products, devoid of accumulating potential and as quickly effective were developed, mainly the organo-phosphorous compounds (OPs), which derived from already well experimented although banned chemical warfare agents (the parent pesticide, parathion, was discovered at the German trust IG Farben in 1940). Due to their high acute toxicity and low specificity, at the beginning of their use several cases of acute and even fatal poisoning occurred to the point that workers often complained, requesting to authorize again the use of a compound perceived as safer during application, as is DDT.

The regulatory system for *new* chemical substances was therefore conceived to avoid to the widest possible extent the hazardous consequences of new products before they reach the market, rather than ex-post, on a case-by-case basis. As examples, when organophosphate pesticides replaced organochlorine pesticides, the higher acute toxicity of the former focused more attention on the safety of spray operators while, later, the introduction of pyrethroid insecticides, which have a much lower toxicity on humans and on mammalian animals, highlighted aquatic environment toxicity as the highest concern [27].

This approach grew in time to require the evaluation of toxic effects of new designer chemical entities to be used as PPPs towards several tens of different living organisms throughout the evolutionary cloud. As a consequence, knowledge of the biological effect of pesticides is even wider than that on pharmaceutical drugs, since it also covers toxicity towards non-target species and environmental fate. The necessity to obtain such data mandated the standardization of toxicological tests to be performed on sufficient numbers of animals to ensure statistically sound results, which in turn led to a widespread use of animal testing on bureaucratic, or 'defensive' rather than information-seeking basis. The heightened, albeit at the start and still minority, public perception of the existence of 'animal rights' (however this was declined in individual opinions) led to the necessity to arbitrate the opposite and little reconcilable positions by improving the quality of animal care and by better exploiting the advances in biochemical technologies (genetic engineering of animal strains, cell cultures, computational simulations) to achieve the '*3R*' aims of reduction (of the number of employed animals), of replacement (of animal tests by alternative techniques) and of refinement (of animal tests to achieve crucial information unavailable by alternative techniques).

39.3 Development of New Pesticides

That of pesticides-against-pests is, like that of antibiotics-against-microbes, a direct consequence of natural evolution on Earth. As pesticides are increasingly used to protect crops from their natural hosts, which compete with mankind for feeding on the same soil products or warm-blood animals from insects feeding on them, evolutionary pressure weeds out sensitive individuals and strains in favor of resistant ones. As an example, DDT-resistant mosquitoes were first detected in India in 1959, and they have increased so rapidly that when a local spray program is begun now, most mosquitoes become resistant in a matter of months rather than years. Mechanisms of acquired resistance include [14, 21, 25]: (**a**) an improved metabolic detoxification through a point mutation of the gene coding for a P450 enzyme and by an increased activity of glutathione-S-transferase, (**b**) knockdown resistance (or kdr), caused by a reduction in the sensitivity of the insect nervous system to the insecticide, mostly through *point mutations* in sodium channel genes, which also allows resistance to pyrethroids [20]; (**c**) behavioural resistance or a shift in the insect's dwelling from the interior of treated houses to untreated outdoor areas [3, 31]. Analogous examples exist for the resistance of weeds to herbicides [24].

Developing new pesticides thus means finding chemical entities with multiple requirements:

- *activity* towards the targeted living species (weed, insect, warm-blood animal) through interference with an appropriately vulnerable biological pathway, which can develop at practically attainable doses;
- *resistance* to the environmental conditions of field application (sunlight, rain, high temperatures), in order that biologically active doses can reach the targeted living species;
- sufficient *selectivity* towards the targeted living species, *i.e.*, at environmental levels the product does not display toxicity towards non-target species, including humans, innocuous plants and insects, wildlife animals and fish, crops (including the plants to be treated), pollinating insects;
- farmer, consumer and environment *safety*, *i.e.*, can be employed in such conditions that agricultural workers are exposed at levels below which there is concern for their health. the residues of the product do not enter into the human food chain and persist into food and water at levels above those of concern for health of the general population; the applied product does not persist in the environment after its efficacy is no longer required at levels of no concern for non-target species;
- *marketability*, *i.e.*, can be protected by patents, manufactured, authorized, delivered worldwide and applied safely in a range of climatic conditions, at a sustainable cost for the farmer.

While in 1972 'only' *approx.* 10,000 different chemical substances had to be screened for this characteristics in order to find one active substance deemed suitable for the market, in 2001, this number rose by 20 fold, meaning that 200,000 chemicals need to be screened to weed out one with all the required characteristics.

The scheme of Fig. 39.1 outlines the general pathway followed for the selection of new candidate active substances and in their development as PPPs in industrial research. This pathway is closely similar to that followed to develop pharmaceutical drugs. Both activities are currently run in a global, highly competitive and highly regulated environment where fundamental research (or, better, fundamental research methodology) is aimed at investigating the molecular mechanisms of the physiology of target organisms, to seek unique metabolic pathways the disruption of which leads to death (Mode-of-Action; MoA). Chemical compounds able to interfere with target organism viability or with specific sensitive pathways are now selected with high-throughput screening platforms based on complex robotics, which enable them to test over 100,000 potential active molecules per year against whole living organisms or cultivated tissues. Potentially active molecules in agrochemical research are mainly of synthetic origin rather than natural substances as now increasingly common in pharmaceutical research, although there are several exceptions.

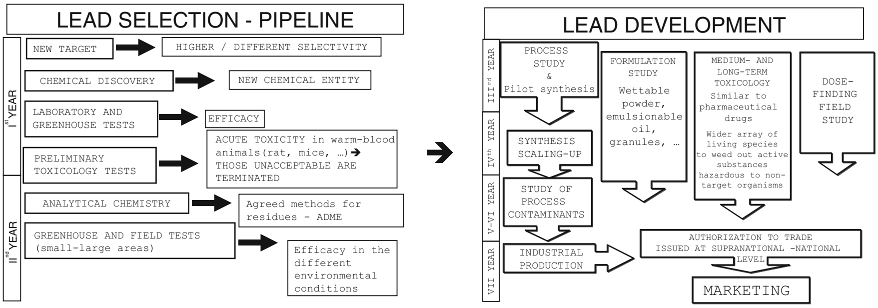

Fig. 39.1 General pathway followed for the selection of new candidate active substances and in their development as PPPs in industrial research

Useful compounds (‘*hits*’) are developed into families of structural analogues (‘*leads*’), among which one or more with the most promising characteristics are selected for further study. Molecules with unfavorable characteristics (those less active, less stable, with unacceptably high acute toxicity) need to be sieved out as soon as possible, to ensure that intellectual and financial resources are focused on those most likely to end up into commercially viable products.

Thus it is only after several years of in-house research that a Company may be ready to apply for authorization of a new plant protection product. A substantial body of information, even greater than that required for a human drug, is supplied to the Authorizing bodies (in the EU, a Commission headquartered in Brussels) in a standardized and legally binding form and subject to non-disclosure agreement (‘Dossier’). This is a large technical-legal document, now distributed in digital form, in which the outcome of experimental tests requested by the authorization process and performed under relevant Good Practice codes are summarized in tables and refer to original data collected in files. Even after the authorization process has positively ended into an authorization or the product has been superseded or commercially abandoned the information is not disclosed for public use.

39.4 The Main Contents of Current Legislation on Pesticides in the European Union

The regulation for the approval of active substances, of herbicide safeners and of synergists, enforces the precautionary principle as cited by Article 191.2 of the Consolidated Version of the Treaty on the Functioning of the European Union, which reads [13]:

> Union policy on the environment shall aim at a high level of protection taking into account the diversity of situations in the various regions of the Union. It shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should as a priority be rectified at source and that the polluter should pay

The main legislation to regulate the registration procedure of pesticides in the EU was the Council Directive 91/414/EEC of July 1991 concerning the placing of plant protection products on the market [8]. In 2009, in the light of experience gained from the application of the 1991 Directive and the "recent scientific and technical developments" that Directive was replaced by the Regulation No 1107/2009 of the European Parliament and of the Council [11], which is fully applicable as of 14 June 2011. This Directive has repealed, by way of simplification, the Council Directive 79/117/EEC [7], which prohibits the placing on the market and use of plant protection products containing certain active substances.

According to current legislation, in the EU only Plant Protection Products with a current authorization ('*positive list*'; www) can be legally used, to the exception of any other. According to the principle of subsidiarity stated in Article 3b of the Treaty of the EU [13], which reads:

> *The Community shall act within the limits of the powers conferred upon it by this Treaty and of the objectives assigned to it therein.//In areas which do not fall within its exclusive competence, the Community shall take action, in accordance with the principle of subsidiarity, only if and in so far as the objectives of the proposed action cannot be sufficiently achieved by the Member States and can therefore, by reason of the scale or effects of the proposed action, be better achieved by the Community.//Any action by the Community shall not go beyond what is necessary to achieve the objectives of this Treaty.* (Art. 3b of the Consolidated Version of the Treaty on the Functioning of the European Union, Official Journal of the European Union)

The authorization of active substances is issued at EU level, while that of formulated products is issued by Member States' national authorities (usually the Ministry of Health) by adopting the procedure of Mutual Recognition.

The general principle of Mutual Recognition of EC ensures the free movement of goods within the EU by avoiding the unnecessary duplication of controls. In the field of PPPs the standard procedure under the old legislation [8] was that every product had to be authorized in each Member State separately, while the current one fully applies this fundamental EU principle (*see* Sect. 39.4.3).

39.4.1 Authorization of Active Substances by the European Union

In the European Union (EU), no plant protection product can be used unless it has first been scientifically established that: (***a***) they have no harmful effects on consumers, farmers, local residents and passers-by; (***b***) they do not cause unacceptable effects on the environment; (***c***) they are sufficiently effective against target pests. As a direct consequence, the components of plant protection products placed on the market must not adversely affect human or animal health or the environment. The current regulation also allows the States members of the European Union to apply the precautionary principle where there is scientific uncertainty as to the risk with regard to human or animal health or the environment posed by the plant protection products.

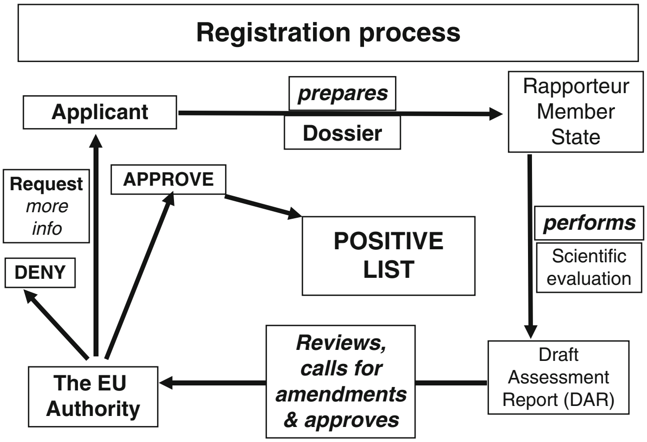

Fig. 39.2 Procedure leading to authorization of a new active substance as PPP in the EU

The complex procedure leading to authorization of a new active substance as PPP is outlined in the scheme of Fig. 39.2.

The Notifier or Applicant is (usually) the Company willing to have the active substance authorized for PPP use in the EU. The review of the information supplied in the Dossier is assigned to one Member State (Rapporteur), which is usually selected on the basis of the putative or requested use of the active substance and the expertise gained in the Country in reviewing products for that specific use (*e.g.*, grape in Southern European countries like France, Italy or Spain, potato in Central European countries like Germany or Ireland).

To apply for authorization of an active substance as PPP, the Applicant submits a dossier to the Member State which has been designated as the Rapporteur Member State (RMS). The dossier comprises all the study reports, data and information which are required by the Council Directive. The dossier is evaluated by the RMS and the results are summarized in a Draft Assessment Report (DAR). The DAR is then discussed by the Member States in a peer review process. The Member States, the EFSA and interested parties can comment on the DAR. Open matters to do with evaluation can be discussed in expert meetings (PRAPeR Expert Meetings) organized by the European Food Safety Agency (EFSA) for this purpose. Following the peer review, the EFSA sends a summary report, which includes its conclusions, to the European Commission. At the end of this process, the European Commission and the 27 Member States decide in a meeting with the Standing Committee on the Food Chain and Animal Health on the inclusion or non-inclusion of the active substance in Annex I of the Council Directive. The complete list of active substances submitted for EU approval is publicly accessible at the website of the Directorate General for Health and Consumers (SANCO, in the EU administrative jargon) [6]. For each active substance it is indicated the status and outcome of the authorization procedure. Sofar, nearly 500 active substances have been authorized.

This regulation, which is publicly available at the SANCO website [6], applies to active substances that are intended for one or more of following uses:

- Protecting plants or plant products against all harmful organisms [. . .];
- Influencing the life processes of plants (e.g. growth, other than as a nutrient);
- Preserving plant products (excluding products subject to EU provisions on preservatives);
- Destroying undesired plants or parts of plants;
- Checking or preventing undesired growth of plants.

The EU regulation applies also to other categories of substances which are usually mixed with the active substance in the production process, or prior to application:

- *Safeners*: substances or preparations which are added to a plant protection product to eliminate or reduce phototoxic effects of the plant protection product on certain plants;
- *Synergists*: substances or preparations that can give enhanced activity to the active substance(s) in a plant protection product;
- *Co-formulants*: substances or preparations which are used in a plant protection product, but are neither active substances nor safeners or synergists;
- *Adjuvants*: substances or preparations consisting of one or more co-formulants, to be mixed by the user with a plant protection product to enhance its effectiveness.

Therefore the European Union will authorize only active substances that are sufficiently effective under reasonable conditions of use; that do not have immediate or delayed harmful effect on human health, including that of vulnerable groups and on animal health, directly or through drinking water, food, feed or air, or consequences in the workplace or through other indirect effects; that do not have any unacceptable effects on plants or plant products; that do not cause any unnecessary suffering and pain to vertebrates to be controlled; and finally that do not have any unacceptable effects on the environment.

39.4.2 Authorization of Formulations

In accordance to the Regulation 1107/2009 of the European Commission, Plant Protection Products (PPPs) can be authorized in a Member State and can be placed on the market only if it complies with the requirement that:

- All substances in it; active substances, safeners and synergists have been approved and, if any of them is produced by a different source, their properties cannot deviate significantly from those included in the Regulation approving the substance;

- It does not contain co-formulants which are included in Annex III of the Regulation 1107/2009 (List of co-formulants which are not accepted for inclusion in plant protection products)
- It is formulated so that user exposure or other risks are limited as much as possible without compromising the efficacy of the product;
- It complies with all the safety requirements for active substances and PPPs
- Its physical and chemical properties have been determined and deemed acceptable for the use and storage of the product;
- The nature and quantity of all components and its residues in the environment and in crops and food can be determined by appropriate methods.

39.4.3 National Authorization and Mutual Recognition

The regulation 1107/2009 has laid down harmonized rules for the approval of active substances and the placing on the market of plant protection products, including the rules on the mutual recognition of authorizations and on parallel trade. The goal is to increase free movement of such products and availability of these products in the Member states.

Under the new legislation, authorizations granted by one Member State should be accepted by other Member States where agricultural, plant health and environmental (including climatic) conditions are comparable. To facilitate such mutual recognition, the 27 Member States of the EU are assigned each to one of three zones with such comparable conditions. The zones of mutual recognition have been established as follows:

Zone A – North: Denmark, Estonia, Latvia, Lithuania, Finland, Sweden
Zone B – Centre: Belgium, Czech Republic, Germany, Ireland, Luxemburg, Hungary, Netherlands, Austria, Poland, Romania, Slovenia, Slovakia, United Kingdom
Zone C – South: Bulgaria, Greece, Spain, France, Italy, Cyprus, Malta, Portugal

The Member State may amend an authorization issued by another Member State, or refuse to authorize the plant protection product in their territory, where there are agricultural or environmental circumstances that require so, or where high level of protection of human and animal health, and the environment cannot be achieved.

39.4.4 Renewal and Review of Active Substances

To have the same level of protection for all Member States, the decision to approve a PPP, to deny or to withdraw the approval is taken at Community (EU) level and authorization is subject to renewal to account for new information that may be emerging from field use. This procedure is analogous to farmaco-vigilance carried by EMEA and by the National authorities for pharmaceutical drugs.

A comprehensive renewal procedure was first laid down in 1991 [8], and in 1993 the European Commission launched the work program on the Community-wide review for all active substances used in the European Union. By that time, there were about 1,000 active substances and 10,000s of PPPs on the market. It was requested that each substance was re-evaluated to understand whether it could be still used safely with respect to human and environment health. To harmonize technical requirements and acceptance criteria, Directives have laid out comprehensive risk assessment and authorization procedures for active substances and products containing these substances. It is the responsibility of industry to provide the data showing that a substance can be used safely with respect to human health and the environment.

The decisions only started to be taken in 2001, since and in March 2009 last decisions were taken. From around 1,000 active substances on the market in at least on Member State before 1993, only 250 (26 %) passed the harmonized EU safety assessment. For the majority of eliminated substances (67 %) dossiers were either not submitted, were incomplete or the industry spontaneously withdrew them from the market. This results show that most of the substances in use were fairly safe, as demonstrated by the fact that only about 70 substances failed the review and have been removed from the market, because the evaluation carried out did not show safe use with respect to human health and the environment.

By the new Regulation, 1107/2009, first approval of a product can be for a period not exceeding 10 years, after which all active substances approved have to be reviewed to be renewed. Renewal cannot be granted for a period longer than 15 years, and for some active substances (those covered by Article 4(7): substances that do not comply to all of the conditions required by this Regulation, but are necessary to combat a pest in an urgent matter) authorization cannot be renewed for a period longer than 5 years.

This effort now provides assurance that the substances currently on the EU market are acceptable for human health and for the environment.

39.5 Setting the Limits: AOEL, ADI, MRL, ARfD

The use of PPPs necessarily entails the spread of active substances in the environment and the possibility that they contaminate workers, subjects of the general population and, through the general environment, also food and water sources. To protect humans from the possibly unavoidable contact with active substances in unnecessary and excessive amounts, limits need to be established during the regulatory process for the presence of PPP active substances at workplaces (mainly during pesticide application by farmers), as residues or contaminants in food and water and in the general environment. For each of these scenarios a different limit value has been developed.

39.5.1 Acceptable Operator Exposure Level (AOEL)

Protection of agricultural workers' health when using Plant Protection Products features several fundamental differences and further difficulty with respect to the much simpler case of workers in the manufacturing industry, mainly outdoor rather than indoor work, continuously changing job, time and exposure patterns rather than Tayloristic schemes, prevalent skin than respiratory absorption route of employed chemicals. These differences point at whole-body dosimetry rather than environmental monitoring as the most convenient strategy to allow quantitative risk assessment. To this purpose, an Acceptable Operator Exposure Level (AOEL) has to be established. The AOEL is a systemic dose, normalized as milligrams of active pesticide substance per kilogram of body weight (mg * $kg_{bw}{}^{-1}]_{day}$) which an agricultural worker can absorb through professional exposure in any one working day so that there will be no negative health consequences. The AOEL is determined through an "health based approach", that is on the basis of animal toxicology experiments which take into consideration as endpoint the biological effect (relevant to the human) which occurs at the lowest exposure level (the Lowes Observed (Adverse) Effect Level, LO(A)EL), then look for the (often extrapolated) exposure dose at which the effect is no longer observed in the most sensitive animal species (No Observed (Adverse) Effect Level, NO(A)EL). In turn, NO(A)EL values are extrapolated from animal studies (typically oral short-term toxicity studies; 90-day study, or occasionally 1-year dog study), performed in the pre-marketing development of a candidate active substance [5].

NO(A)EL is translated into AOEL by further dividing the 'safe' dose assessed in the suitable animal model by empirical reduction factors which account for the uncertainties existing in the extrapolation from animal toxicity data to safeguard levels for the human population. The current hazard assessment for toxic endpoints for which the existence of a no-effect threshold dose is assumed employs a (default) 100-fold uncertainty factor to extrapolate a 'safe' dose level for the general population based on animal toxicity data. The global 100-fold uncertainty factor is based on the assumption of a conservative ten-fold higher sensitivity of the human with respect to the most sensitive (or the default) animal species, and of a ten-fold difference of inter-individual variability in sensitivity between human subjects of the general population of different age, gender and general health status. In some cases, due to the low levels of intra-specie variability, the uncertainty factor applied for workers can be lower (2.5), but not (or seldom) lower values of the uncertainty factors can be applied when it is deemed that current unavoidable uncertainties recommend a more conservative approach to ensure that even the most sensitive human subject will go unharmed.

While it is commonplace that the use of agrochemicals by farmers is limited in time to the relatively short periods of application, whereas in the scenario of manufacturing industry exposure is considered to be continuous and appreciably constant throughout the working life of the person in the specific task, however it is also well-known that farmers' working life spans a much wider period of their lives, even from late childhood to late post-retirement age. As a consequence, adverse health effects occurring late in age as the consequence of chronic exposure should also be taken into account.

To improve risk assessment, any information on human exposure derived from scientifically sound and ethically sustainable observations and studies can be used to confirm the validity of regulatory limit values derived from animal studies, but at the moment it is not allowed to perform *ad-hoc* studies in human subjects to derive information for regulatory purposes and, in particular, no data collected on humans can be used to lower the safety margins resulting from tests or studies on animals [10, 11]. Of course, during the re-authorization process, specific attention is addressed at the collection of epidemiological data coming from the use of the compound under evaluation.

39.5.1.1 The Use of the AOEL for Pre-marketing Risk Assessment

Pre-marketing risk assessment for occupational exposure to Plant Protection Products is a procedure aimed at demonstrating that the active substance, formulated as the commercial product(s) intended for marketing, is able to perform its task (*i.e.*, to suppress the target organism under field conditions) without causing inacceptable harm to the farm worker.

As in any risk assessment, the risk is calculated as the ratio of actual internal dose to the regulatory limit: acceptable risk is exceeded if the ratio is >1, *i.e.*, if the internal dose is higher than that allowed by the regulatory limit. This task is accomplished by (***a***) evaluating the dose of active substance which reaches the farmer during agricultural activities, (***b***) estimating the resulting internal dose and (***c***) comparing with the maximum dose allowed by the toxicity characteristics of the active substance and established as part of the authorization process.

As anticipated, all calculations use the main parameters which are obligatorily part of the information collected in the evaluation Dossier or in the studies supplied with the active substance for which the application has been submitted: the Acceptable Operator Exposure Level (the health-based AOEL), the skin absorption factor (which is either experimentally measured or defaulted to 100 % if its direct determination is not feasible) and the concentration of active substance in the product.

The internal dose is calculated from the dose reaching the skin and the skin absorption coefficient, when this is available (otherwise defaulting to 100 % skin absorption). In turn, the dose reaching the skin needs to be estimated from the amount of pesticide employed in a typical working day.

This quantity is often measured by performing studies in experimental farms, under standardized conditions, with workers doing spraying activities in different working scenarios, using different kinds of machineries, and different levels of protection. The resulting exposure is measured according to standardized methods, following the Guidelines of European Union [23].

The measured levels of exposure for each working scenario and level of protection are then generalized as milligrams of active substance deposited on farmers' clothes per kilogram of active substance used (exposure), and the levels of

Table 39.1 Main determinants of exposure considered in the models for pre-marketing risk evaluation of Plant Protection Products in the EU

Formulation type:	Liquid
	Wettable powder
	Wettable granules
Characteristics of the product:	Concentration of the active substance in the product
	Quantity of product used per hectare of field surface
Personal Protection Devices: worn in different phases	Respiratory protection (none, mask with filters)
	Hands protection (none, gloves)
	Head protection (none, hat)
	Body protection (none, coverall and footwear)
Container type (UK-POEM):	Capacity
	Closure (narrow or wide)
Work scenarios:	Target
	Tractor
	Hand-held
	Home-garden

protection afforded by different types of Personal Protection Devices are expressed as the percent fraction of exposure that reaches the worker's skin.

The various conditions which are able to influence the overall value of the fraction of used active substance which actually reaches the operator's skin are referred to as 'determinants of exposure'. The determinants covered by most models are collected in Table 39.1.

Accepted models used to perform calculations in the regulatory setting are often available as computer spreadsheets [28] or as technical documents reporting the equations and from which appropriate calculation codes can be developed by interested professionals. Among those most commonly used are: the German Model [18], the EURO-POEM [30], the UK-POEM [15]. Publications in the refereed scientific literature often refer to these products as *de facto* standards for which informal agreement between practitioners exists, rather than formal independent validation.

One major criterion to consider a formulated product as safe for use is that the dose absorbed by the worker even in the absence of, or with the adoption of, minimal protection equipment is lower than the AOEL in all working scenarios which are relevant to product application.

39.5.2 Acceptable Daily Intake (ADI)

This concept was first introduced in 1961 by the Council of Europe and later the Joint FAO/WHO Expert Committee on Food Additives (JECFA), a committee maintained the Food and Agriculture Organization FAO and the WHO World Health Organisation of the UNO. The ADI takes into account the unavoidable presence of residues of PPPs in food and in drinking water which derive from the legitimate use of the formulated active substance, applied on crop cultures to protect them from pests, on crop products to prevent deterioration in their transport to food processing, to markets and to consumers, incorporated into meat and dairy from pasture and from silage, leaked into water reservoirs from use in the field.

An ADI value is established in the authorization process, based on the results of long-term studies on animals, by applying the same general criteria described above for the AOEL. Also the ADI is usually given in milligrams per kilogram of body weight (mg * $\mathrm{kg}_{\mathrm{bw}}{}^{-1}]_{\mathrm{day}}$).

The ADI is considered a safe intake level for a healthy adult of normal weight when intake is appreciably constant in time. This may raise concern for substances with a tendency to show bio-accumulation in the organism or bio-magnification in the human food chain, as was the case with organo-chlorine pesticides, but currently the requirement for new active substances is that they are *per se* chemically labile in the environment, so that potential for build-up of levels of concern in the environment is now mostly negligible.

Increased safety factors for infants have been discussed, but are not needed, because elimination of chemicals is in fact often more rapid in children than in adults. The ADI does not take into account allergic reactions that are individual responses rather than dose-dependent phenomena.

39.5.3 Maximum Residue Levels (MRLS)

Conceptually closely related to the ADIs are the limit values referring to the maximum tolerable presence of residues in the several types of food which are produced and marketed downstream to the crops and in drinking water. Under the EU regulations, the Maximum Residue Levels (MRLs) are the upper legal levels of a concentration for pesticide residues in or on food or feed based on good agricultural practices and to ensure the lowest possible consumer exposure. The European Food Safety Authority (EFSA) is the administrative body of EU responsible for setting those limits. Regulation (EC) No 396/2005 [9] establishes the MRLs of pesticides permitted in products of plant or animal origin intended for human or animal consumption.

MRLs are derived after a comprehensive assessment of the toxicological properties of the active substance (on the basis of which the ADI is established) and on the residue levels measured on or in crops treated according to the good agricultural practices defined for the product. Since consumer safety is the final aim for setting MRLs, values of the MRL are set at levels such that consumer intake of the active substance even in unbalanced diets based on food with the highest presence of residues does not exceed the ADI.

The maximum pesticide residue level in foodstuffs is 0.01 mg/kg for each active substance identified. This general limit is based on the expected sensitivity of available analytical methods and is applicable 'by default', *i.e.* in all cases where an MRL has not been specifically set for a product or product type. Some of the specific MRLs listed in Annex II are higher than the default limit, since there is evidence that the active substance is harmless to consumers' health.

In some cases, provisional MRLs may be set and should then be listed in Annex III. Provisional MRLs should in particular be set in the some cases, among which are the occurrence of exceptional circumstances (*e.g.*, emerging local phenomena of food contamination), and in the course of harmonization procedures.

The Member States have to carry out official controls on pesticide residues in order to enforce compliance with Maximum Residue Levels. The results of the controls have to be reported to the Commission, to the other Member States and to EFSA, which publishes an Annual Report on Pesticide Residues in the EU based on the monitoring information.

Products which do not comply with the fixed limits cannot be marketed to consumers and may not be diluted with products with a lower level of residues in order to lower the mean level to below the limit. except in the case of certain processed and/or composite products listed by the Commission (Annex VI). In exceptional cases, products which do not comply with the limits set in Annexes I and II may be authorized by a Member State if the products do not represent an unacceptable risk. It should in fact be considered that agricultural products are produced at a substantial environmental, labour and economic cost and that unnecessary discard and destruction or diversion from their food use is ethically unjustified unless higher-rank interests, such as that to health protection need to be enforced.

39.5.4 Acute Reference Dose (ARFD)

The need to consider acute effects of pesticide residue intake has been acknowledged for many years, and the concept of the Acute Reference Dose (ARfD) was developed by the Joint FAO/WHO Meeting on Pesticide Residues [16] in 1994. Since then, there has been a progressive increase in the establishment of ARfDs for particular pesticides to address potential exposure to residues in food and drinking water at relatively higher doses for short-term periods, due to accidental or incidental events. JMPR has continuously updated its procedure on the setting of ARfDs.

The ARfD is defined as "an estimate of the amount a substance in food or drinking water, normally expressed on a body weight basis, that can be ingested in a period of 24 h or less without appreciable health risks to the consumer on the basis of all known facts at the time of the evaluation" [17].

39.6 Risk Assessment in Post Marketing Phase

Even as an active substance is authorized in European Union, and products containing this active substance are authorized and marketed, there is still a need for risk assessment to communicate and to manage risk with regard to the different groups of stakeholders and to the general population as a whole [10, 11].

There may be concern within the general population for the alleged high level of residues in food staples, which may harm the long-term health of parents of unborn children, of babies, of otherwise weakened and of strongly health-seeking individuals. As a consequence, increased numbers of the population may move from the conventional food market to more demanding alternatives, such as less abundant products of biological agriculture, thus posing an often unnecessary pressure on their production and, due to their higher cost, to the economic system as a whole.

Leakage of herbicides and pesticides from treated soils and contamination of water tables, perturbation of insect populations of economic value, accidental if rare localized dispersion of bulk amounts of pesticides from production and packaging plants or during transportation and distribution are events through which the public opinion may feel higher-than-real threats and on regard of which fair and authoritative risk assessment, communication and management is both necessary and dutiful. Farmers may be concerned for their long-term health although occupational exposure to pesticides in the EU is traditionally considered to occur at low level and the consequent health risk is far from being a real source of concern. These different needs, although common in their necessity to be addressed by the public health authority, call for different systems to yield the required answers.

Continuous monitoring of pesticide residues in foodstuff is explicitly included in Regulation (EC) No 396/2005, which also requires publication of the results as a measure of risk communication to the EU population. The Annual Report on pesticide residues in food issued on a yearly basis by the EFSA [12] is based on data received from 27 EU Member States and from two EFTA countries (Iceland and Norway). The EU MRL food monitoring programmes are one of the most comprehensive worldwide, covering more than 60.000 food samples every year which are analysed for up to approx. 800 different pesticides. Member States report more than 15 million determinations of pesticide residues on a yearly basis. The report also assesses the exposure of European consumers to pesticide residues through their diets.

To fill this task, analytical methods have been harmonized and standardized and method-compliant instruments, such as gas chromatograph and liquid chromatograph systems with tandem mass spectrometry identification and detection are marketed by most international suppliers of analytical chemistry instruments [1].

Risk assessment of agricultural occupational exposure (as well as for other exposures) performed in the pre-marketing phase is aimed at ensuring that a formulated active substance, when applied in the field under the conditions established as Good Agricultural Practices, is safe for use and does not pose harm to farmers' health. In real-life working conditions, however, risk assessment is seldom, if any, performed since the task has many difficulties, mainly linked to economic cost, to the limited availability of trained personnel and logistics necessary to reach small, family based enterprises, which are often poorly covered by occupational health services, to the variability of working patterns, of climatic conditions and of the frequent use of mixtures of pesticides. The existence of epidemiological studies [2] and of case reports which suggest that chronic low-level pesticide exposure can have long-term effects on the health of agricultural workers also suggest the necessity to perform risk assessment also in 'real-life', region specific field conditions.

In the field, exposure to pesticides comes from three main routes: dermal, inhalation and oral. During open-field farming (and pesticide spraying), the contribution of the oral route is considered negligible and inhalation has been demonstrated to contribute very little to the overall exposure, while exposure by absorption from the contaminated skin (the dermal route) accounts as that quantitatively most relevant. From the point of view of risk assessment, work with pesticides can be classified into three phases, each corresponding to specific modalities of farmer exposure: preparation of the product for application (mixing and loading), spraying (application) and finally maintenance of the agricultural equipment. In each of these phases, the worker can be exposed to the pesticides to a different extent, partly by direct contact with the mixture, and partly from contact with contaminated items.

This difficulty leaves field (environmental) and biological monitoring as the most informative strategies for risk assessment. In particular, field monitoring allows to investigate how contamination builds up on operator's clothes and skin in the different phases of pesticide work in the farm including worker-to-worker variability in different ambient and organization conditions. Biological monitoring measures the dose of pesticide actually absorbed by a worker through all routes of exposure, mainly as the excretion into urine of a representative metabolite of the active substance.

What hampers a more frequent use of biological monitoring for individual risk assessment of farmers during use of pesticides is lack of reference or limit values for the interpretation of results. A pioneering approach to establish such reference has been recently demonstrated in proof-of-principle coordinated studies of farmers who sprayed herbicides (propanil) for weed control in maize cultivation [26] and of fungicides (mancozeb) on vineyards. Briefly: (***a***) health risk was calculated from comparison of internal dose to the AOEL of the active substance; (***b***) internal dose was calculated from skin dose and the skin absorption coefficient; (***c***) skin dose was assessed in a field study, along with doses on farmer's hands and clothes and with recording of employed amounts and application conditions. A (double-logarithmic) least-squares linear regression of excreted metabolite dose in urine *vs.* (the reciprocal of) health risk affords as the intercept of the equation the value of dose corresponding to absorption of a systemic dose corresponding to one AOEL, *i.e.*, the maximum allowable daily dose. Once validated, this approach can be developed into a simpler tool for risk assessment of workers exposed to pesticides and lead to the necessity to establish the Biological Exposure Index as an additional parameter requested for the regulatory authorization of Plant Protection Products.

39.7 Conclusions

Considering that the use of Plant Protection Products is not deemed to be dispensed from if crop production will continue to be the staple source of humankind, strict regulation is necessary to avoid mistakes of the past, such as the use of chemically robust, bio-accumulating organics, as the frontline of pest control. Current EU regulation on the authorization of pesticides is one of the tiers of a global action aimed at improving the quality of agricultural production in the EU by protecting crops from pests with the safest active substances, used in the most rational ways to minimize unnecessary contamination of food, of the environment and health risk for the farmers.

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