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REGULATORY TOXICOLOGY

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Use of the dog as non-rodent test species in the safety testing schedule associated with the registration of crop and plant protection products (pesticides): present status

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Abstract The results from a survey of the expert information that is publicly accessible on the use of the dog as test species during the regulatory evaluation of agricultural chemicals and pesticides are reported. Methods that are being used or considered in order to reduce the number of dogs used for this purpose are described. Regulatory evaluation aims at establishing threshold values for safe human exposure; it is based on no-observed-adverse-effect levels (NOELs) determined in animal studies. Current regulations require testing in two species, a rodent species (usually rat or mouse), and a non-rodent species (usually the dog). Subchronic (90-day) and chronic (12-month) repeated-dose feeding studies must be routinely conducted in dogs. This report first focuses on the results from a retrospective study analysing data on 216 pesticides kept on record by the Bundesinstitut für Risikobewertung, BfR (German Federal Institute for Risk Assessment), the competent regulatory authority in Germany. The study was sponsored and coordinated by SET, the German Foundation for the Promotion of Research on Replacement and Complementary Methods to Reduce Animal Testing (Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen, Mainz) and conducted by the BfR. Since the data submitted for registration of a product is the property of the manufacturer, the study could only proceed with the collaboration of the German Association of Manufacturers of Agricultural Chemicals (Industrieverband Agrar, IVA). To ensure confidentiality, designated codes were used instead of the compounds' proper names when the study was published. The results support two major conclusions. The use of the dog for the testing of pesticides is indeed necessary because the dog has proved to be the most sensitive species for about 15% of the compounds examined. However, chronic studies are only of limited value since they only provide essential information that cannot be obtained in sub-chronic studies in about 5% of cases. These conclusions are supported by several retrospective analyses using data on pharmaceutical drugs carried out in the context of the International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH). Over 90% of drugs elicited no toxic symptoms in 12-month studies in dogs in addition to those that had been recorded previously in studies conducted for 90 or 180 days in dogs and rats. Another approach comparing the results from pre-clinical animal studies with clinical studies noted that animal studies predicted about 70% of the effects observed in volunteers, and in about 94% of cases the effects occurred in animal studies lasting not more than one month. Furthermore, the report summarises the current methods under consideration that could refine or reduce the use of dogs in toxicity testing: industrial data sharing and harmonisation of guidelines, in vitro methods, human studies, computational prediction models, and integrated testing approaches. The integrated Agricultural Chemicals Safety Assessment (ACSA) testing scheme, which is currently being developed in an international project initiated by the International Life Sciences Institute (ILSI, USA), is of particular relevance, since an ambitious attempt is being made to design a new comprehensive test framework incorporating modern scientific approaches and covering most aspects of current regulatory testing requirements. The ACSA project has access to the pesticide database of the US EPA's Office of Pesticide Programs (OPP). Preliminary results have

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confirmed the two major conclusions from the joint SET/BfR study conducted in Germany. Taking these results into account, it is recommended that the regulatory requirement for 12-month studies to be routinely carried out in dogs should be abandoned. While 90-day studies should be conducted in both rats and dogs, chronic studies should only take place in rats. If the dog is more sensitive than the rat in the 90-day study, an additional safety factor to the NOEL value obtained in the chronic rat study should be applied in order to set the threshold for safe human exposure, instead of conducting a 12-month study in dogs. This safety factor may be calculated from chronic NOEL data available in several pesticide databases. Chronic tests using dogs would then only be required if the test compound belongs to a new class of chemicals that has never been tested before. Thus, the report concludes that, according to current scientific knowledge, the routine 12-month studies in dogs are no longer required for agricultural chemicals and pesticides, and international regulations should be changed accordingly. Active international support of such measures is welcomed, from both an economical and an animal welfare perspective.

Keywords Pesticides · Regulatory testing · Chronic toxicity studies · Study duration · NOEL · Dog

Introduction and objective

Plant protection products have been developed, manufactured and used on a large scale since the 1940s. Correspondingly, legislation has evolved and been amended at times to make sure that human health and the environment are sufficiently protected. The basic toxicological test data requirements for the registration of pesticides have, however, remained essentially unchanged during the past 25 years, although requirements for certain neurotoxicity and immunotoxicity studies have been added. At the same time, technical and scientific knowledge has advanced tremendously. With the advent of molecular biology, efficient new tools have become available to study physiological processes. Computer modelling and statistical methods have also become more sophisticated. Concurrently, it was realised that previously neglected issues had to be addressed (Ross et al 2001; Ferrier et al 2002). The concern about cumulative risks posed by chemicals in food and the environment has led to new legislation (US Food Quality Protection Act: US EPA 1996) and to the development of the concept of ''reduced risk'' pesticides in the USA (Felsot 2001).

Public awareness of animal welfare issues has also grown since 1980 and resulted in the adoption of harmonised legislation in Europe in 1986 (EEC Publishing Office 1986). Ethical concern was fostered about usage of the dog, perhaps mainly because the dog is arguably the companion species with the closest rapport to man (Morris 1996). A scientific investigation initiated jointly by the BfR, the German Federal Institute of Risk Assessment, and the SET Foundation has conclusively shown that there is sufficient justification to reconsider the need to use dogs as test animals (Gerbracht and Spielmann 1998; Spielmann and Gerbracht 2001). The study presented herein focuses, therefore, on the issue of the necessity of using dogs for toxicological safety assessment, explores the options for reducing the extent of dog usage, and summarises suggestions put forward in the expert literature to improve on the current risk assessment test schedule and incorporate new scientific insights. Such a study appears timely, especially since a revision of European Council Directive 91/414/EEC (EEC Publishing Office 1991), concerning the placing of plant protection products on the market, is imminent.

The role of the dog as a non-rodent species in toxicity assessment

The evaluation of an active substance—be it a pesticide or a pharmaceutical drug—includes ''core'' studies of acute toxicity (such as LD50), repeated oral dosing (28 days to 2 years in separate studies), developmental toxicity, reproductive toxicity, genotoxicity, neurotoxicity and toxicokinetics. Each type of study comprises a large number of single observations and recording of numerous parameters. In the case of repeated-dose toxicity, body weight gain, liver weight, kidney weight, and liver and kidney pathology study are determined to delineate the lowest dosage level producing an effect (Weil and McCollister 1963; Heywood 1981; OECD 2000a). The studies listed in Tables 1 and 2 are commonly conducted for most pesticides.

In addition to the ''core'' studies, there may be mechanistic studies of primary effects, pharmacological activity screening, human volunteer studies or short-term range-finding studies, and studies of the impact on wildlife and the environment are also required. At least 9000 animals are needed to meet the regulatory data requirement for one single pesticide. More than 75% of the test animals are used to assess the effects on reproduction and development. A number of the toxicological endpoints determined in the different separate ''core'' studies are closely related (US EPA 2002). This redundancy has already been recognised by regulatory authorities. European Commission Directive 94/79/EC (EEC Publishing Office 1994) amending European Council Directive 91/414/EEC (EEC Publishing Office 1991) requires a sub-chronic study in dogs, but an additional chronic study in dogs only if the sub-chronic study shows that the dog is more sensitive than the rodent species tested in parallel and that the toxic effects may be relevant to humans. However, as compounds are usually marketed worldwide, 12-month dog studies are a standard part of the toxicity testing performed by industry to meet international requirements.

Subchronic and chronic repeated-dose toxicity testing makes use of mice, rats and dogs. In principle, the test animal species employed should be the one which bears

Table 1 Official figures for numbers of animals used for toxicological safety evaluation

Year	1 Total	2 Total (Pharm)	3 Total (Agro)	4 Total-dog	5 Dog (Pharm)	6 Dog (Agro)
EU						
1999	800788	433678	60997	8898	8373	357
Germany						
2000	219390	76847	29386	2210	1961	216
2001	189996	71607	36529	2039	1797	130

Quoted from the report of the Commission of the European Communities (2003) and the Animal Welfare Report for 2003 published by the German Government (German Federal Ministry of Consumer Protection, Nutrition and Agriculture 2003).
Column 1 shows the total number of animals used in toxicological and other safety evaluations for 1999, 2000 and 2001 as indicated. Column 2 shows the total number of animals used to test products, substances or devices for human medicine, dentistry and for veterinary medicine. Column 3 shows the number of animals used or intended for use mainly in agriculture. Column 4 shows the total number of dogs used in toxicological and other safety evaluations, in order to allow comparison between the usage of dogs for human medicine, dentistry and veterinary medicine (column 5) with the usage of dogs for agriculture (column 6).

Table 2 Types of toxicological studies performed to assess impact on human health, and species tested on

End point	Typical species
Acute testing	
Oral mortality	Rat
Dermal mortality	Rabbit, rat
Inhalation mortality	Rat
Eye irritation	Rabbit
Dermal irritation	Rabbit
Dermal sensitisation	Guinea pig
Acute delayed neurotoxicity	Domestic hen
Delayed neurotoxicity	Rat
Subchronic testing	
Subchronic oral	Rat, mouse, dog
Subchronic dermal	Rabbit, rat
Subchronic inhalation	Rat
Subchronic neurotoxicity	Rat
Chronic testing	
Chronic toxicity	Rat, dog
Oncogenicity	Rat, mouse
Reproductive testing	
Developmental toxicity	Rat, rabbit
Reproduction/fertility	Rat
Developmental neurotoxicity	Rat
Genetic testing (mutagenicity)	
Chromosome damage	Cells, organisms, mouse (micronucleus test)
Gene mutation	Cells, organisms
Pharmacokinetics/toxicokinetics	
Absorption	Rat, mouse, dog, monkey
Distribution	Rat, mouse, dog, monkey
Excretion	Rat, mouse, dog, monkey
Metabolism	Rat, mouse, dog, monkey

Adapted from Whitford et al (2003)

the closest resemblance to man in all relevant aspects. It is, however, not feasible to individually identify the most suitable species for each of the many chemical substances to be tested; hence the requirement to use several test species. The concept that a rodent species (''first'' species) should be routinely used in parallel with a non-rodent species (''second'' species) reportedly dates back to the early 1960s (Zbinden 1993). The non-rodent species is used to take into account the possibility that the rodent species may be insensitive to the effects of an active substance to which humans are sensitive. The use of a second mammalian species, which is to some degree distinct from mice and rat in phylogenetic terms, increases the likelihood that one or other of the test species will be at least as sensitive as humans. The dog became established as the non-rodent species largely because it was already widely used in the USA during the 1950s and was available as laboratory breed in sufficient numbers (Parkinson and Grasso 1993). There are also other reasons for using the dog. Dogs have a larger volume of blood than mice and rats, so more samples can be taken. The size of dogs also allows a number of separate physiological and clinical observations, which are more difficult to perform in rodents. These days, the preferred use of mice, rat and dog in toxicology can be explained by the existence of large databases on these species.

The joint SET/BfR study: comparison between the sensitivities of test species

In 1996, the German Animal Welfare Foundation SET sponsored and coordinated a large retrospective analysis of data on plant protection products kept on file by the German Federal Institute for Risk Assessment, BfR. Two BfR departments were involved in conducting the study: the Agency for the Regulation of Pesticides, and ZEBET, the German Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments. Consent for the study was obtained from the German Association of Manufacturers of Agricultural Chemicals, IVA. The study consisted of two parts.

The first objective was to compare the sensitivities of the test species. The analysis comprised toxicity data from 216 chemicals submitted for registration between 1953 and 1995. The data were obtained in studies conducted for four weeks (sub-acute), 13 weeks (subchronic) and/or 52 or 104 weeks (chronic) in mice, rats and dogs. To ensure data protection, an identification code was allocated to each substance (Gerbracht and

Spielmann 1998). For each coded compound, the intended use, year of submission, manner of administration, duration of study, no-observed-effect level (NOEL), lowest-observed-effect level (LOEL), organs targetted by the toxicity, and the toxic effects at LOEL were given.

The NOEL values obtained in four-week, 13-week and 52/104-week studies were used to compare the sensitivities of the three species in sub-acute toxicity testing (20 substances), sub-chronic toxicity testing (156 substances) and chronic toxicity testing (171 substances), respectively. The dog had the lowest NOEL of the three species for about 50% of the substances tested in sub-acute studies, for 52% of the substances tested in sub-chronic studies and for 41% of the substances tested in chronic studies. To evaluate the sensitivities across species, the LOEL values were included in the comparison as these were deemed to give a more relevant indication. Thus, the dog was considered more sensitive than the rat if the LOEL in the dog was lower than the NOEL in the rat, and it was considered the most sensitive of the three species if the LOEL in the dog was lower than the NOELs in both rat and mouse. When all of the toxicity data were included in the analysis, the dog was shown to be the most sensitive species for 28 out of a total of 157 substances in sub-chronic studies and for 25 of 172 substances in chronic studies. In summary, the dog was the most sensitive species in about 15% of the studies. This demonstrates conclusively that the use of dogs in toxicity testing is justifiable.

Only three other (much smaller) studies have been published comparing species sensitivity between dog, rat and mice (Appelman and Feron 1986; Dourson et al 1992; Storm et al 2000). There is not much overlap between the four studies; the SET/BFR study had only six substances in common with any of the three studies. Appelman and Feron (1986) obtained toxicity data from the general literature for 66 substances (pharmaceutical drugs, food additives and pesticides), with five substances having been administered to rat and dog for four weeks, 22 substances for 13 weeks and 39 substances for six months or longer. Of the 27 pesticides included, 20 had been used in chronic studies and seven in 13-week studies. The NOEL values of 13 pesticides showed the dog to be more sensitive than the rat, for 12 pesticides the rat and dog NOELs were about equivalent, and in two cases the rat was more sensitive than the dog. The authors noted tha, of the 66 compounds listed, the NOEL in the dog was more than ten times lower than the NOEL in the rat in only five cases. In order to reduce the use of dogs in toxicity studies, they suggested introducing an additional safety factor applicable to the NOEL in the rat: an Acceptable Daily Intake or ADI value (Lu 1988; WHO 1990, 1999). Dourson et al (1992) compiled NOEL values for 66 pesticides taken from US EPA's Integrated Risk Information System (IRIS, as at 1991). The data were obtained from 1-year to 2-year rat and 1-year to 2-year dog studies. The dog was more sensitive than the rat to 26 pesticides by a factor of 2–12. The NOELs for 23 pesticides were about equivalent (within a factor of 2). The rat was more sensitive than the dog to 13 compounds by a factor of 2–5 and to four more pesticides by a factor of 9.5–50. Storm et al (2000) collated data on organophosphorus pesticides that was available in the peer-reviewed literature, from pesticide manufacturers or from the US EPA Office of Prevention, Pesticides and Toxic Substances (OPPTS). The NOELs for red blood cell acetylcholinesterase inhibition were tabulated for 30 organophosphorus pesticides with data sets for both rat and dog for 27 substances. In two cases the dog was more sensitive than the rat, for 20 substances the NOELs in rat and dog were about equivalent, and for five pesticides the rat was more sensitive than the dog.

The SET/BfR study: observations concerning the duration of studies

The second objective of the joint SET/BfR study was to investigate whether chronic studies in the dog provide additional essential toxicological information, which is not obtained when sub-chronic or sub-acute studies are conducted in the dog in conjunction with studies using rodents (Spielmann and Gerbracht 2001). Data from chronic 52-week or 104-week toxicity studies on 172 pesticides carried out in mice, rats and dogs were analysed and compared with data from parallel studies of shorter treatment duration (four, 13 and/or 26 weeks). All toxic effects and affected organs were recorded. For 72 out of 141 compounds, comparative analyses yielded new findings in chronic studies, which were absent in sub-chronic 13-week studies. In 19 studies the effects observed did not correlate with functional or histopathological alterations and, therefore, were considered not relevant. In 12 chronic studies, the doses used were higher than in the corresponding sub-chronic studies and thus not comparable. For 27 pesticides, similar effects were seen in rats and/or mice. For eight pesticides, the additional effects noted were not considered to be of toxicological relevance by the company toxicologist. Thus, in only seven out of 141 studies was additional relevant information on the toxicological profiles of the pesticides obtained in chronic 52-week or 104-week studies, which was not evident from sub-chronic studies in dogs or from chronic studies in rats or mice. When data from four-week studies were compared with 52/104-week studies, two further cases of new toxic effects were found, giving a total of only nine cases out of 172 in which chronic studies identified new toxicologically-relevant effects not noted before in shorter-term studies. Moreover, the analyses showed that most of the organ-related toxicity of pesticides can be identified in 13-week studies in the dog, and that all relevant toxic effects were observed during 26-week exposure studies. Spielmann and Gerbracht (2001) concluded that toxicological testing of pesticides in dogs can be restricted to 13-week studies.

In 2004, a literature search was carried out by us using the publicly-available literature databases provided by the German database host DIMDI (http://www.dimdi.de), which gives access to over 70 databases (including MEDLINE) covering expert toxicological literature. The search was for publications dating from 1974 or later comparing data from subacute and/or sub-chronic with data from chronic studies in the dog. About 25 relevant publications were found, of which nine reported results from sub-chronic as well as chronic studies for single pesticides. None of the studies described new findings in chronic studies that were not seen in sub-chronic studies. This outcome shows that, for agricultural chemicals, the SET/BfR study is, at present, the only comprehensive and generally available publication addressing the relevance of the duration of repeated-dose toxicity testing in the dog.

Retrospective analyses for pharmaceutical drugs concerning the duration of studies

A larger number of comparative studies have been published on pharmaceutical drugs. Since 1985, the Centre for Medicines Research (CMR) in England has compiled comprehensive repeated-dose toxicology data from studies in various animal species, which were provided voluntarily by several British pharmaceutical companies (Lumley and Walker 1985; Lumley et al 1992). By 1995, the database permitted analyses of data on 117 pharmaceutical compounds tested in the dog (Parkinson et al 1995). For more than half of the compounds, all salient effects in the dog were seen for the first time within three months. Only 13 compounds showed new and possibly relevant effects in studies of six months' (four compounds) or 12 months' duration (nine compounds). Importantly, no particular therapeutic- or structurally-defined class of compound was implicated in these 13 cases. A similar result was obtained in a retrospective analysis of the database established by the Japanese Pharmaceutical Manufacturers' Association, JPMA (Igarashi 1993).

In 1990, the International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) was initiated as a joint regulatory-industry project to harmonise the registration process for new drugs in Europe, Japan and the USA. One of the Expert Working Groups established by ICH addressed the duration of chronic toxicity testing in animals. Taking into consideration the CMR and JPMA analyses, harmonisation was reached on a six-month duration for rodent chronic toxicity studies and a nine-month duration for non-rodent toxicity studies, which would be applicable for most categories of pharmaceutical drugs (DeGeorge et al 1999). After adoption of the guidance recommended by ICH, the lengths of repeated-dose studies legally required for product registration will differ between pesticides and pharmaceutical drugs as follows. For pesticides, a 28-day, a 90-day and a 12-month study are usually required, except that in the EU 28-day and 90-day studies suffice unless the dog proves to be the most sensitive species; if this is the case, a 12-month study must be done. For drugs, 14-day and/or 28-day and 90-day studies are usually carried out along with a six-month and/or a nine-month study in the EU, the USA and Japan; 12-month studies only need to be conducted for certain types of compounds in the USA (Dotzel 1999).

A second project involving retrospective analysis of data on pharmaceutical drugs was initiated by the International Life Sciences Institute (ILSI, USA). The aim was to examine the usefulness of animal studies in predicting and identifying different types of organ toxicity in humans (Olson et al 1998, 2000). The results showed a significant positive concordance of 71% between toxic symptoms observed in animals (rodents and non-rodents) in pre-clinical studies and in humans during clinical trials. In cases where the animal study was successful in predicting human symptoms, 94% of the effects on animal target organs were observed in studies less than or equal to one month in duration (over 50% were detected during the first day).

Two British animal welfare organisations have continued to pursue the subject. The Fund for the Replacement of Animals in Medical Experiments (FRAME) and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) prepared a position paper considering ways in which dog use could be reduced (Broadhead et al 1999) and held a meeting with representatives from the UK pharmaceutical industry, research institutions, the UK Home Office and the CMR (Broadhead et al 2000). A working group was set up which identified promising approaches to limit dog use and made proposals for further analysis (Smith et al 2002) presented in the following section.

Options available to replace, reduce or refine studies using dogs

Animal experiments need to be done in safety assessment since they are the only alternative to human testing. One should, however, consider that "if a study must be done with animals, then we are obligated to do it right the first time. This is the best refinement one can do to decrease the use of animals." (Rao and Huff 1990). Accepting this moral responsibility places emphasis on planning and designing toxicity studies in such a way that unnecessary use and suffering of animals is avoided. When conducting an animal study every observation should be meticulously recorded in order to collect as much information as possible, and the standards of Good Laboratory Practice (GLP) should be followed. In 2001, an international symposium on "Regulatory Testing and Animal Welfare" was held in Québec City, Canada, at which options to minimise dog use for pre-clinical safety testing were discussed (Smith et al 2002) and recommendations were made to implement "Best Scientific Practices" in

acute systemic toxicity testing (Stitzel et al 2002), sub-chronic/chronic toxicity and carcinogenicity testing (Combes et al 2002) and safety evaluation using non-rodent species (Weekley et al 2002).

Strategic planning

Strategic planning should be utilised to establish whether an experiment is really necessary or whether it can be partially or completely undertaken without animals. All information available should be used to identify realistic goals as well as to make provision for the use of methodologies that avoid unnecessary animal suffering (Combes et al 2002). Accelerated product development plans may reduce the number of studies. Single-dose toxicity studies, pilot dose escalation studies and dose range-finding studies may permit reduction of animal numbers in later studies or point the direction in which an investigation should proceed (Smith et al 2002).

Study design

Species selection

Industry often uses the dog in regulatory toxicology studies in addition to rodents because it has always done so, whereas other non-rodent species are not used as much because they are not readily available and less accepted by the authorities. However, the species used for testing should be the one that shows most resemblance to man in its responses to the substances tested (Smith and Trennery 2002). Although both a rodent species and a non-rodent species are used in safety assessment, it is conceivable that the use of only one species is sufficient. Appelman and Feron (1986) suggested the introduction of an additional safety factor for deriving ADI values to account for using only one animal species (the rat). Zbinden (1993) argued that the study of toxicity in one properly validated species in combination with appropriate monitoring in human studies is an adequate safety testing strategy. Alternatively, it has been suggested that a rodent as well as a non-rodent species should be used in short-term studies, whereas in longer-term studies only one species should be used, namely that which is deemed to be the most appropriate (Broadhead et al 2000). This more flexible approach would mean an overall reduction in the number of test animals, with the dog only being used in chronic studies when it is shown to be more appropriate on a case-by-case basis than the rodent.

Animal gender

Animals of both sexes are used in most toxicity studies due to metabolic differences. However, the differences in gender response may not always be very pronounced; for example Igarashi et al (1996) showed that there was no statistically significant gender differences in 78% of dog studies with pharmaceutical drugs. Using both sexes may thus not be necessary for all classes of compounds. Smith et al (2002) rated the use of single sex studies worthy of further analysis.

Group size

On average, three non-rodents are used for one-month and three-month studies, four non-rodents are used for each six-month, nine-month or 12-month study, and two animals are used to monitor recovery. Although the majority of companies are using group sizes consistent with regulatory guidelines, there may be opportunities to harmonise (Weekley et al 2002). The optimum number per dose group may be obtained by statistical analysis of in-house data (Igarashi et al 1996). Best practice in study design may also reduce the use of recovery animals or the number of control groups (Smith et al 2002).

Dose levels

Careful selection of dosages at the beginning of a toxicity study dictates the quality of the data generated. Toxicokinetic and pharmacodynamic endpoints should be used in dose selection to improve the evaluation of toxicity with repeated dosing. The initial dose should be based on rat acute toxicity data or estimated therapeutic dose. Pain and discomfort may be minimised by conducting pilot studies using dose escalation (Combes et al 2002; Weekley et al 2002).

Frequency of repeated measurements and time of terminal measurements

In 1996, a joint international committee convened to provide harmonised expert recommendations for clinical pathology testing of laboratory animal species used in repeated-dose studies (Weingand et al 1996; OECD Guidance Notes on repeated-dose studies: OECD 2000a). The frequency and timing of clinical pathology testing is dependent on study duration, study objectives, biological activity of the test substance, and the species tested. For repeated-dose studies in non-rodent species, clinical pathology testing is recommended at study termination and at least once at an earlier interval. It is important to take care that test substances are administered and blood samples taken for analysis without causing more distress than is unavoidable (Diehl et al 2001). Throughout the study animals should be observed closely to allow early detection of toxic effects or signs of discomfort. Extreme endpoints, such as signs of severe distress and moribund condition, should be avoided whenever possible (OECD Guidance Document on the use of clinical signs as humane endpoints: OECD 2000b; Smith et al 2002).

Improvement in communication and harmonisation

Improvement in communication and harmonisation is widely recognised as playing a pivotal role in furthering animal welfare as well as creating opportunities for economising in product development and registration. Industrial cooperation in data sharing may reduce the number of exploratory studies required for dose range finding or to investigate substance class effects (Smith et al 2002; Smith and Trennery 2002). International workshops on the topic of sharing information about industrial chemicals assessment have already been held under the auspices of the OECD and the European Centre for the Validation of Alternative Methods, ECVAM (OECD 1997; Todd et al 1998). Both have produced recommendations about the conditions under which proprietary data could be shared with regulatory authorities, research institutions and/or animal welfare organisations. Communication between the competent authorities of different countries could likewise facilitate reductions in animal testing required for registration and re-registration of pesticides. Sharing the work of reviewing agricultural pesticides is possible because the same pesticides are often used in many countries (OECD 2002).

In vitro studies

In vitro studies alone are generally not regarded as providing quantitative data of sufficient confidence to substitute in vivo toxicity studies or reliably derive ADI values (Walton et al 1999). The report of an international workshop held by ECVAM (Pfaller et al 2001) points out that, despite limitations, in vitro systems can make a substantial contribution to toxicological risk assessment (Eisenbrand et al 2002; Snodin 2002; Wakefield et al 2002; Worth and Balls 2002). In vitro systems provide good models for characterising the mode of action for critical effects, although the findings need to be validated in vivo. In vitro systems may aid in the extrapolation from high to low dose and from test animals to humans, since potential qualitative and quantitative species differences in toxicity can be studied (Holme and Dybing 2002). They thus lend themselves to use in dose-range finding studies and in reducing the number of animals needed (CSTEE 2004). Moreover, in vitro studies will contribute to the identification of the most sensitive species. This may be achieved by the use of a battery of cell lines that have been genetically modified to express particular molecules, which are relevant for metabolic pathways and derive from different species, including man (Krebsfaenger et al 2003).

Human data

Human data is essential for risk assessment in order to validate existing animal models and facilitate appropriate extrapolation from animal data. The data can also serve as a basis to design more relevant new models. Human data stems from either clinical cases of intoxication or volunteer studies. The concept of toxicovigilance has been introduced for the evaluation of clinical cases, which is based on detailed medical evaluation of case reports of acute or chronic intoxication and allows for the identification of the causal relationship between toxic exposures and pathological conditions (Descotes 2003). In contrast, volunteer studies can be designed to address specific questions, such as derivation of threshold doses for pesticides, without using animals (Gemert et al 2001). Ethical considerations play a key role in the conduct of volunteer studies, especially if the effects of potentially harmful substances such as pesticides are investigated (Charnley and Patterson 2003). Studies have to comply with the principles set down in the Declaration of Helsinki (World Medical Association 2002) or the Good Clinical Practice guidelines developed for drug testing (CPMP/ICH 1997). Test persons must be fully informed about the nature and purpose of the tests and any health consequences that are reasonably foreseeable, and participation must be voluntary (''informed consent'').

Computational methods

Quantitative structure–activity relationships play an increasing role in predicting the effects of newly-discovered or synthesised substances, for example, in order to select chemicals for further product development (Combes et al 2002). One important aspect is the deduction of potential toxicity. During the last 20 years, our understanding of the mechanisms underlying molecular interactions as well as insights into basic physiological and biochemical processes have grown substantially. There have also been great advances in computer technology and mathematical modelling of biological reactions, such as dose–effect relationships, permitting predictions of the biokinetic and biodynamic behaviours of compounds within the body (Boobis et al 2002; Blaauboer 2003). Computational techniques combine data on the physicochemical properties of chemical compounds (such as type of functional groups and lipophilicity) with knowledge of physiology and metabolism. Thus, the uptake of a compound by different routes, its distribution into different tissue compartments and its metabolic fate can be estimated with increasing accuracy for physiologically-based pharmacokinetic/toxicokinetic modelling (Ridings et al 1996). This is an area that is progressing rapidly, and software is being developed and improved all of the time. At present, several pharmacokinetic parameters such as absorption and distribution can already be modelled quite satisfactorily (Boobis et al 2002). Computational methods are also being increasingly applied to examine more realistic scenarios of assessing exposure to pesticides, or the simultaneous exposure to several compounds and the cumulative effects this might have on human health (see,

for example, the US Food Quality Protection Act: US EPA 1996). This is a relatively new line of investigation in risk assessment, emphasising the need to develop strategies for evaluating health risks, which include new computational approaches (Teuschler et al 2002). On the whole, computational methods could provide a promising route toward significantly reducing the use of animals for risk assessment in the future (Ferrier et al 2002).

Integrated testing approaches

Aside from the application of individual methods to risk assessment presented above, there are strategies incorporating a variety of methods into one single, comprehensive or integrated testing scheme. The evaluation of a compound's toxicity is based on the combined use of physicochemical properties, in vitro data, human data, animal data, computational methods and software permitting modelling of toxicodynamic and biokinetic parameters. Typically, the methods and data are assessed in parallel or in sequence (Blaauboer et al 1999). An important example of an integrated strategy is the scheme developed in the ECITTS project (ERGATT-CFN Integrated Toxicity Testing Scheme), which was set up in 1991. Special emphasis is placed on employing other methods before conducting any animal tests (Blaauboer 2001). Although the results are encouraging, the ECITTS scheme cannot yet be applied to determining NOEL values without using animal data (Eisenbrand et al 2002; Blaauboer 2003).

Another integrated approach is currently being developed especially for the assessment of plant protection products. The Agricultural Chemical Safety Assessment (ACSA) project was initiated in 2001 at a workshop organised by the ILSI Health and Environmental Sciences Institute (ILSI-HESI). The aim is to develop new strategies for safety evaluation. Three task forces have been set up to address different areas. The Systemic Toxicity Task Force is undertaking to design a tiered testing framework for diverse endpoints such as neurotoxicity, immunotoxicity, carcinogenicity, and chronic toxicity. Data on pesticide compounds from the database at US EPA's Office of Pesticide Programs (OPP) are taken to evaluate the usefulness of dog studies. Preliminary analyses have shown that the dog needs to be included in the testing scheme because it is more sensitive than other test species in a significant number of cases (Moretto 2004). It was also concluded that 1-year to 2-year dog studies do not add significant information to that obtained in a 90-day study in dogs evaluated in conjunction with other studies (Dellarco 2004). Both results are a confirmation of the findings of the SET/BfR study. According to a work-in-progress report (Moretto 2004), the proposed testing scheme envisages a reduction in the number of dogs from the 70 required in current guidelines (for preliminary, 90-day and one-year studies, with three doses and one control) to 40 dogs, by omitting the one-year study. This figure is, however, preliminary, as the scheme has not yet been endorsed by the ACSA Technical Committee.

Discussion

This survey set out to give an overview of the specialist literature and other generally-available information relating to the current status of the use of the dog as test species in the toxicological safety evaluation of plant protection products, and to give a summary of the efforts directed at reducing the extent of such usage. It centres on the findings and conclusions from the study funded by the SET Foundation and conducted by the BfR, the competent regulatory authority in Germany. The SET Foundation made possible the retrospective analysis of the pesticide data kept in the files of the BfR with the consent of the IVA, the German Association of Manufacturers of Agricultural Chemicals. This data is kept secret in order to prevent infringement of proprietary rights. By allocating codes to the names of the substances, confidentiality was preserved. Data on a total of 216 substances were examined; this makes the joint SET/BfR study the largest of its kind conducted on pesticides to date. The results were published in two parts and two main conclusions were drawn.

First, the SET/BfR study showed conclusively that studies on dogs provide essential information and are necessary for the toxicological safety evaluation of pesticides, not only for practical but also for scientific reasons (Gerbracht and Spielmann 1998). Thus, the dog was shown to be the most sensitive species in comparison with mouse and rat for about 15% of the substances studied. The need for studies in dogs during the assessment of pesticides was confirmed in the ILSI-HESI-ACSA project (Moretto 2004). The second conclusion from the results of the SET/BfR study relates to the length of the studies required to identify and establish toxic effects elicited by pesticides and their relevance in humans. Only nine out of 172 substances fed to dogs in chronic studies identified new toxicologically-relevant effects that had not been noted before in shorter-term studies in either dogs or rats. The analyses showed that most of the organ-related toxicity of pesticides can be identified in 13-week studies in the dog and that all relevant toxic effects were observed during 26-week studies (Spielmann and Gerbracht 2001). Toxicological safety testing of pesticides in dogs can be restricted to sub-chronic studies of 13 weeks, since studies of longer duration do not provide additional essential information. Again, this conclusion has been confirmed in the ILSI-HESI-ACSA project: preliminary analysis of data taken from the database of the US EPA's OPP showed that conducting one-year to two-year dog studies provides no advantage over conducting 90-day studies (Dellarco 2004). Studies with dogs of 12 months in length should not, therefore, be routinely required.

The SET/BfR study has thus proved that collaboration between authorities, industry and animal welfare organisations can produce fruitful results to the benefit of all. If the conclusions drawn from the study are translated into regulatory practice, the advantages will include savings both in terms of product development costs as well as animal lives and suffering, while, at the same time, maintaining the present high standards of safety for human health.

Two proposals deriving from the SET/BfR study, and a survey of the literature

In 1963, Weil and McCollister concluded from their observations made over 15 years "that the feeding of a chemical to dogs for 90 days should be an ample period to show whether this species would be more sensitive than the rat. If this proved to be the case, then long-term tests should be performed using dogs. If not, this step should be eliminated." This corresponds to the data requirements laid down in the currently effective European Council Directive 91/414/EEC concerning the placing of plant protection products on the market (EEC Publishing Office 1991; amended by Commission Directive 94/79/EC: EEC Publishing Office 1994) for 90-day studies in both rat and dog with a trigger for 12-month studies if the dog is more sensitive. However, provision for such a trigger is not implemented in the requirements stipulated for agricultural chemicals outside the European Union. Elsewhere, 12-month feeding studies are mandatory.

Support for the way the duration of dietary studies for pesticide assessment is currently regulated in Europe is found in the recommendations issued by the ICH of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH). The ICH considers studies of up to nine months in duration as sufficient. The US FDA modified its requirements in response to these recommendations and stipulates six- or nine-month studies for most drugs, and 12-month studies only for certain drugs destined for particular uses. The ICH based its recommendations on retrospective studies of pharmaceutical drugs, which had reached conclusions similar to those regarding pesticides of the SET/BfR study (Lumley et al 1992, 1993; Igarashi 1993; Parkinson et al 1995). These studies had shown, moreover, that the small number of drugs that were found to elicit new findings after administration for three or six months did not belong to any particular therapeutic class and did not share structural similarities. Appelman and Feron (1986), studying 66 substances, which included pharmaceutical drugs, food additives and pesticides, did not report either that the dog was exclusively susceptible to substances of any particular chemical structure. The same is true for the results from the SET/BfR study. However, pharmaceutical drugs as well as pesticides comprise diverse classes of substances with a wide range of different chemical structures and distinguished modes of action. Thus, it seems that, in general, the structure of a chemical substance, be it pesticide or drug, gives no specific indication per se of whether the dog or the rat will be more sensitive to the substance, or whether any additional toxic effects will be seen after three months' oral feeding or not.

For the above reasons the following proposal is made for further consideration. In view of the recommendations of the ICH for studies to be conducted with pharmaceutical drugs, one might consider adapting pesticide legislation accordingly. This would mean a requirement for 90-day studies in both rat and dog with a trigger for either a six-, nine- or 12-month study in dogs; usually a study duration of six or nine months, but a 12-month study in cases of particular toxicity elicited by certain classes of pesticides, as is prescribed for drugs destined for particular therapeutic uses, such as in the treatment of AIDS [see provisions made by US FDA (see Dotzel 1999)]. Although this proposed adaptation of the requirements regarding study duration would mean a substantial refinement in animal testing by reducing the term of potential suffering in dogs, the proposal does not yet take into account that the SET/BfR study concluded that studies of a duration of more than 13 weeks need not be conducted routinely.

Many authors of toxicological studies recommend evaluation of the toxic potential of a compound on a case-by-case basis and suggest a more flexible approach in risk assessment (Parkinson and Grasso 1993; Broadhead et al 2000). Meeting the regulatory requirements on a case-by-case basis has the beneficial effect of furthering communication between manufacturers and regulatory authorities and, moreover, could result in a reduction of the number of studies requiring dogs.

Review of the literature suggests the following proposal for limiting the requirement for dog studies conducted routinely for the evaluation of pesticides to studies with a duration of 90 days (13 weeks). Appelman and Feron (1986) calculated the ratios between NOEL-dog and NOEL-rat for 66 compounds and found the ratio to differ, in general, by a factor of ten at most. They suggested setting an additional safety factor when deriving ADI values from chronic rat data in order to account for the dog being more sensitive than the rat in a significant number of cases. It is therefore proposed that a 90-day study in rat and dog should be performed first to determine which species is the more sensitive as is required in the EU. However, if the dog proves more sensitive than the rat in the 90-day study, it is further proposed that the safety factor be applied to the NOEL value obtained in a subsequent chronic rat study and that no longer-term studies in dogs be carried out at all. The value of the safety factor should be determined from the ratio between chronic NOEL values in rat and dog for compounds to which the dog was more sensitive than the rat. These chronic NOEL values should be obtained from retrospective analyses of previous data. The safety factor could be better defined if the database of Appelman and Feron was expanded with the NOEL data of

Gerbracht and Spielmann (1998) and the data on organophosphates published by Storm et al (2000); this would provide NOEL ratios for more than 250 compounds. Since no proprietary information beyond the compound name and the ratio between NOEL-dog and NOEL-rat is needed in chronic studies, even more chronic NOEL ratios could be obtained by approaching the database administrators at CMR International, US EPA and WHO. This should provide sufficient data to give the proposed safety factor a sound basis. Data on pharmaceutical drugs can also be included because, as mentioned above, new toxic findings were seen with drugs with diverse chemical structures (Lumley et al. 1992, 1993; Igarashi 1993; Parkinson et al 1995). Following this proposal, long-term studies in dogs would only be required if the compound belongs to a novel class of chemical substances for which no previous data exists.

Outlook

There is a strong case for proposing that dog studies conducted routinely in the safety evaluation of pesticides should be limited to studies of duration no longer than 90 days (or 13 weeks), and to do so would not compromise human safety. This follows clearly from the results of the SET/BfR study and the ILSI-HESI-ACSA project using the US EPA's OPP database. This data should also be considered together with the supportive evidence from studies with pharmaceutical compounds in the ICH process and the ILSI Human Toxicity project (Olson et al 1998, 2000) as well as with the above consideration of introducing an additional safety factor. Taken together, this is regarded as providing sufficient weight in favour of the argument that routine testing in dogs should last no longer than 13 weeks, and therefore to renounce 12-month studies on a routine basis. The regulatory directives should therefore be adapted accordingly. Active international support for introducing such change into the currently effective regulations is welcome from both an economical and an animal welfare point of view.

In comparison, it is difficult to judge how an ambitious and complex framework such as the multifaceted ILSI-HESI-ACSA testing strategy can be implemented once it has been finalised. As representatives from the US EPA and its OPP pesticide database have been involved in developing the scheme, an obvious option is that the scheme will be reviewed first within the US EPA and other US Federal Agencies (Dellarco 2004). Alternatively, the proposals for the scheme might be incorporated into the deliberations on the impending revision of the EU Directive on plant protection products.

The approaches pursuing non-integrated strategies as alternatives to animal testing, such as optimisation of study design, human studies, in vitro systems or computational methods, should each be evaluated on their merits, to be incorporated into legislation once they are validated well enough. In order to make this possible, the competent decision-making bodies should make provisions in the legal framework for allowing new knowledge conforming with the 3*R* concept of Russell and Burch to be adopted and introduced into the regulations, whenever sufficient advances are made in the future, in order to modify and improve on existing procedures and rapidly substitute practices no longer ethically tenable, especially when failing to meet current scientific standards.

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