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REGULATORY TOXICOLOGY

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The use of dogs as second species in regulatory testing of pesticides

Part II: subacute, subchronic and chronic studies in the dog

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Abstract Data on 172 pesticides (fungicides, herbicides, insecticides and other pesticides) submitted for regulatory purposes during the past 40 years to the German Federal Institute for the Health Protection of Consumers and Veterinary Medicine (BgVV) were analysed to determine whether chronic studies in dogs (52/104 weeks) provide essential additional specific toxicological compared with subchronic (13 weeks) or subacute (4 weeks) studies in the same species. Comparison of the lowest observed effect levels (LOELs) in dogs revealed no significant differences between subchronic and chronic studies but a significant difference between subacute studies and subchronic or chronic studies. Moreover, there was a significant correlation between the LOELs determined in subchronic studies and those determined in chronic studies in dogs ($r = 0.78$–0.84). The distribution of target organ toxicity determined in chronic studies in dogs was not significantly different from the distribution determined in subchronic studies, except for effects on the spleen in studies on herbicides which were only observed in chronic studies and in combined subchronic/chronic studies, but never in subchronic studies. Organ-specific effects that were observed in chronic studies but not in subchronic studies were found in 30 of 55 studies on fungicides, in 25 of 44 on herbicides, in 17 of 38 on insecticides and in 10 of 16 on other pesticides. Compared with 26-week studies, additional organ-specific toxic effects were found in three of five, in three of four, in one of three and in one of one 52/104-week studies on fungicides, of herbicides, of insecticides and other pesticides, respectively. The organ-specific effects that were seen only in the chronic dog studies were evaluated according to their severity, e.g. significant damage to organs versus changes in enzyme activities that do not affect organ function or histology. Such effects were not considered to be specific for dogs in chronic studies if similar effects were also found in chronic studies in rodents (rat or mouse). In 15 of 141 studies in dogs serious side effects were observed in chronic studies that were not observed in subchronic studies. Furthermore, for 9 of 172 pesticides significant new effects were seen in 52/104-week studies when compared with 4- or 13-week studies and in 7 of 141 52/104-week studies when compared with 13-week studies. Analysis of the severity of organ-specific toxic effects of pesticides revealed that chronic long-term studies (52/104 weeks) in dogs do not provide specific additional information to 26-week studies in the same species.

Key words Pesticides · Regulatory testing · Chronic toxicity studies · Dog · Interspecies comparison

Abbreviations *ALAT* alanine aminotransferase · *a.ph.* alkaline phosphatase · *BUN* blood urea nitrogen · *ChE* cholinesterase · *CNS* central nervous system · *LOEL* lowest-observed-effect level (mg/kg) · *MCH* mean corpuscular haemoglobin · *MCV* mean corpuscular volume · *NOEL* no-observed-effect level (mg/kg) · *RBC* red blood cells · *WBC* white blood cells

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Introduction

In developing new pesticides, sensible regulatory requirements have to be met to protect public and environmental health. In the EU, toxicity testing has to be performed in two animal species, a rodent (usually the rat) and a non-rodent (usually the Beagle dog) in order to

identify differences in the susceptibility of species (European Commission 1988, 1994). In the dog, a long-term chronic study is required if the subchronic study provides evidence that the dog is the most sensitive species and if the toxic effects may be of importance to humans.

Toxicity testing in dogs has increasingly been criticized by the general public in several OECD member countries (Appelman and Feron 1986; Parkinson and Grasso 1993; Parkinson et al. 1995; Zbinden 1993). It is therefore important to analyse the scientific impact of studies in this species. In recent years, several databases have been established using data from drug testing which have enabled the relationship between the length of repeat-dose toxicity studies and the relevance of the toxicological information generated to be analysed. This evaluation has shown that for pharmaceuticals 12-month studies in dogs provide no essential benefit compared with 3-month studies and that no important additional information is obtained when extending 3-month studies to 6 months (Igahashi 1993; Parkinson and Grasso 1993; Parkinson et al. 1995). In the majority of cases, the use of the dog does not provide additional relevant information on target organ toxicity of the drugs (Broadhead et al. 1999).

Since the majority of toxicity studies on pesticides are conducted for regulatory purposes, the results are confidential and stored in the files of either industrial companies or regulatory agencies. Therefore, these data cannot be used for analysing the impact of studies in the dog on regulatory decisions in comparison with studies in other species, e.g. rats and mice. As outlined in the first part of this study (Gerbracht and Spielmann 1998), to overcome the problem of confidentiality, 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*, SET) decided to fund a study on confidential data kept in the files of the competent regulatory authority for the Regulation of Pesticides in Germany, the Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV), in Berlin. This study was carried out jointly by two departments of the BgVV: the Agency for the Regulation of Pesticides, and the German Center of Evaluation and Validation of Alternatives to Animal Testing (ZEBET). The study was scientifically monitored by toxicologists from the German Pesticides Manufacturer's Association (*Industrieverband Agrar*, IVA). In that study, the confidential data from 216 pesticide toxicity studies kept in the files of the BgVV were evaluated. The IVA agreed that these data could be used in the present study providing confidentiality was maintained by coding the chemical agents.

In part I of the study the NOELs from 4-, 13- and 52/104-week toxicity studies on pesticides in dogs were compared with the values determined in studies of the same length in rats and mice in order to determine the relevance of the NOELs determined in dog studies for the safety assessment of pesticides in regulatory testing (Gerbracht and Spielmann 1998). This study revealed that studies in the dog are indeed essential for hazard identification and risk assessment of pesticides. However, the information required may be obtained by focusing on only a few of the currently established subacute, subchronic and chronic studies in dogs. Thus, in the second part of our investigation we sought to determine from the same set of data whether all the long-term studies currently performed in dogs are required to allow regulatory decisions for pesticides.

In the present investigation we therefore evaluated the toxicity data from long-term studies in dogs for 172 pesticides from the files of the BgVV to determine whether specific information is provided by chronic studies which is not obtained in subchronic studies in dogs or in chronic studies in other species, e.g. the rat.

Materials and methods

Study design

The toxicity data used in the present study were obtained from confidential reports submitted by pesticide manufacturers to the regulatory agency for this group of chemicals in Germany, the BgVV. The toxicity data on pesticides were generated between 1953 and 1995. The 172 chemical entities which had been tested in shorter subacute (4 weeks) and subchronic (13 or 26 weeks) studies as well as in the long-term chronic studies (52 or 104 weeks) were divided into fungicides, herbicides, insecticides and other pesticides (acaricides, molluscicides, nematicides, rodenticides, synergists for insecticides and growth regulators or hormones) and coded to ensure confidentiality (H herbicides, F fungicides, I insecticides and O other pesticides). In Appendices 1 and 2 the complete toxicity profiles of the 172 chemicals, the duration of the studies, the dose ranges, the affected organs and the target organs for toxicity identified by the regulatory agency are shown.

Ranking of studies according to lowest LOELs

From the LOELs determined in subacute, subchronic and chronic studies in dogs three ranking categories were established. In this evaluation the lowest ranking of 1 was given to the study with the lowest LOEL, a ranking of 2 to the study with the intermediate LOEL and a ranking of 3 to the study with the highest LOEL. The same ranking was given to studies of different length but the same LOEL. If only two studies of different length were available (i.e. subchronic and chronic, or subacute and chronic) only rankings of 1 and 2 were given. Using this system the ratios of the rankings for the chronic and subchronic studies in dogs were calculated for the different groups of pesticides to identify the most sensitive long-term study.

Organ-specific toxicity

To assess organ-specific toxicity, the following toxicological methods were used: clinical observations, laboratory investigations, functional observations, necropsy, organ weight and histopathology. In addition to gravimetric and morphological/histopathological organ toxicity, alterations in thyroid hormone levels in blood and plasma were also regarded as thyroid effects. Deviations in phenol red retention, BUN, serum creatinine as well as changes in urinary status were regarded as organotypic effects indicating kidney damage. Changes in Bromsulphalein retention, liver γ-glutamyltransferase and cytochrome P-450 as well as serum

Table 1 Rank values of the LOELs from studies of insecticides in dogs. The ratio of LOELs in dogs from 52/104-week studies to those from 13/26-week studies was 0.87 ($n = 30$). The correlation coefficient between 13/26-week studies and 52/104-week studies was 0.802 ($n = 30$; $y = 0.092 + 0.815x$)

	Study duration (weeks)		
	4	13 or 26	52 or 104
Total rank value	18	45	49
Number of studies	9	31	35
Mean rank value	2.00	1.45	1.40

Table 2 Rank values of the LOELs from studies of fungicides in dogs. The ratio of LOELs in dogs from 52/104-week studies to those from 13/26-week studies was 0.86 ($n = 51$). The correlation coefficient between 13/26-week studies and 52/104-week studies was 0.843 ($n = 51$; $y = 0.3 + 0.840x$)

	Study duration (weeks)		
	4	13 or 26	52 or 104
Total rank value	33	80	75
Number of studies	14	51	52
Mean rank value	2.36	1.57	1.44

Table 3 Rank values of the LOELs from studies of herbicides in dogs. The ratio of LOELs in dogs from 52/104-week studies to those from 13/26-week studies was 0.72 ($n = 39$). The correlation coefficient between 13/26-week studies and 52/104-week studies was 0.783 ($n = 39$; $y = 0.632 + 0.677x$)

	Study duration (weeks)		
	4	13 or 26	52 or 104
Total rank value	43	71	54
Number of studies	21	42	40
Mean rank value	2.05	1.69	1.35

aspartate aminotransferase and glutamate dehydrogenase were considered to indicate hepatic side effects. In addition, alterations in multiple hepatic enzyme activities or metabolites in serum (i.e. increased ALAT together with increased alkaline phosphate and/or cholesterol) were also regarded as indicating liver damage. Symptoms such as convulsions, hypersensitivity, emesis and salivation were taken as indicating effects on the CNS. Changes in consistency of the feces (diarrhoea, soft feces) were taken as indicating effects on the digestive tract. Haematological symptoms of bleeding and histopathological abnormalities (i.e. haemosiderin deposits in various organs) were regarded as specific effects on the haematopoietic system. An inhibitory effect on ChE was only considered when a decrease in ChE activity was found in RBC, plasma and brain.

Evaluation of new toxicological information (affected organs and corresponding symptoms) from chronic studies (52 or 104 weeks) or in 26-week studies and comparison with symptoms in rats and mice

The new affected organs and the corresponding findings were categorized according to their toxicological relevance, whether they were possibly important or whether they were questionable in nature due to lack of nonspecificity or adverse effect. The category of nontoxicologically significant was assigned to symptoms that were part of the same syndrome in rats or mice (affected organs also identified in rats or mice), that consisted of separate nonspecific alterations without a histopathological or morphological correlate, i.e. only alteration in organ weight, or isolated nonspecific alterations in, for example, enzymatic activities, and that were seen only at higher doses in long-term as opposed to short-term toxicity studies

The studies with significant findings were examined again and the company's toxicological expertise was taken into consideration. When the toxicologist's opinion was that the new finding of a study was not toxicologically significant the study was eliminated from further evaluation.

Table 4 Rank values of the LOELs from studies of "other pesticides" in dogs. The ratio of LOELs in dogs from 52/104-week studies to those from 13/26-week studies was 1.23 ($n = 12$). The correlation coefficient between 13/26-week studies and 52/104-week studies was 0.812 ($n = 12$)

Ranking	4 weeks	13 or 26 weeks	52 or 104 weeks
Total value	9	16	18
Number of studies	4	12	15
Mean rank value	2.25	1.33	1.20

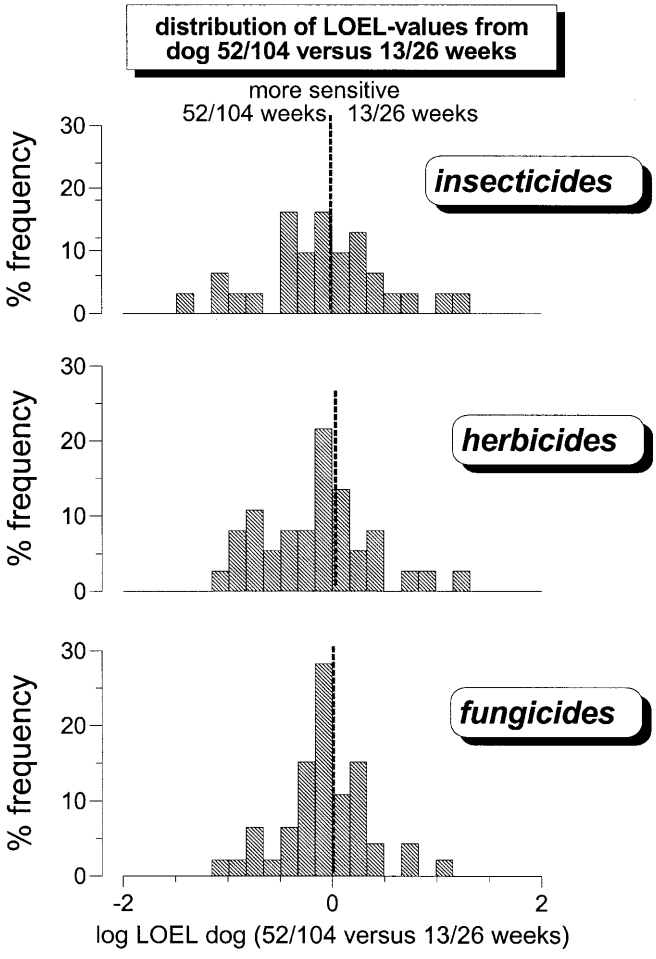

Fig. 1 Distribution of LOEL values for insecticides, herbicides and fungicides in 52/104-week studies compared with 13/26-week studies in dogs. The comparison was made by calculating the ratio of the LOEL values from the chronic versus those from the subchronic studies. The vertical lines indicate studies in which the ratio of the LOELs of the two studies was 1 (log 0) indicating the same sensitivity. Available for the evaluation were 31 studies on insecticides, 40 on herbicides and 53 on other pesticides

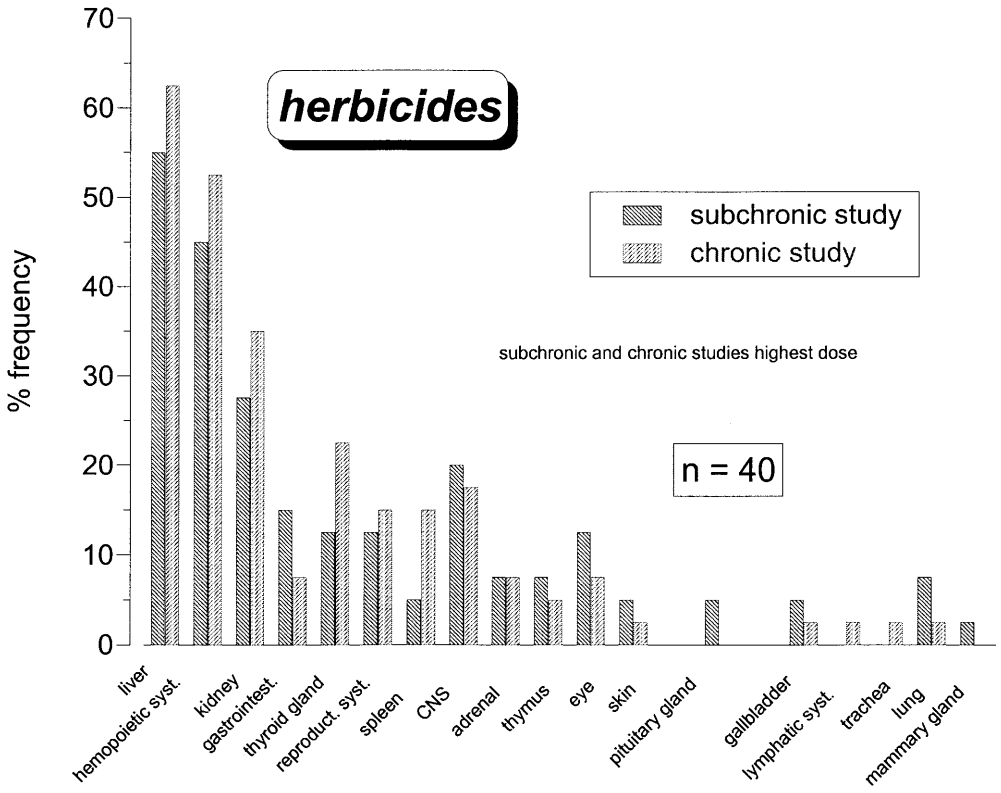

Fig. 2 Target organ toxicity of herbicides at the LOEL in subacute, subchronic and chronic studies in dogs. Target organs are given on the *x*-axis and the percentage of 40 studies showing organ-specific toxicity are given on the *y*-axis

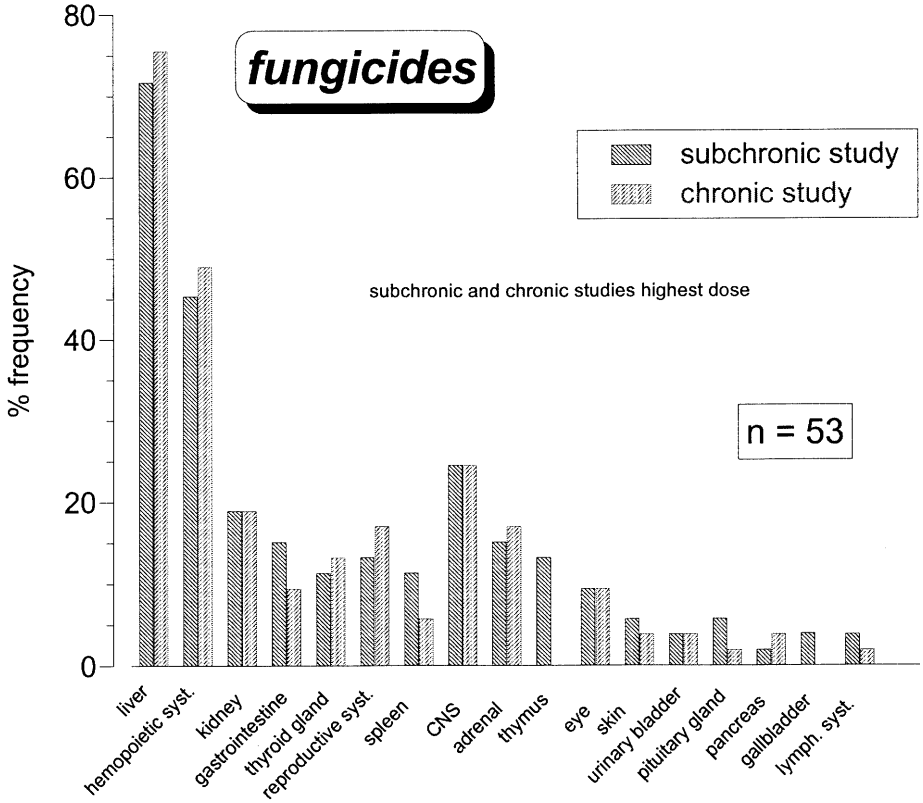

Fig. 3 Target organ toxicity of fungicides at the LOEL in subacute, subchronic and chronic studies in dogs. Target organs are given on the *x*-axis and the percentage of 53 studies showing organ-specific toxicity

Results

The rank values calculated from the LOELs from the subacute, subchronic and chronic studies are given in Tables 1, 2, 3 and 4 for insecticides, fungicides, herbicides and other pesticides, respectively. The following results were obtained: the most insensitive study schedule was the subacute schedule which yielded

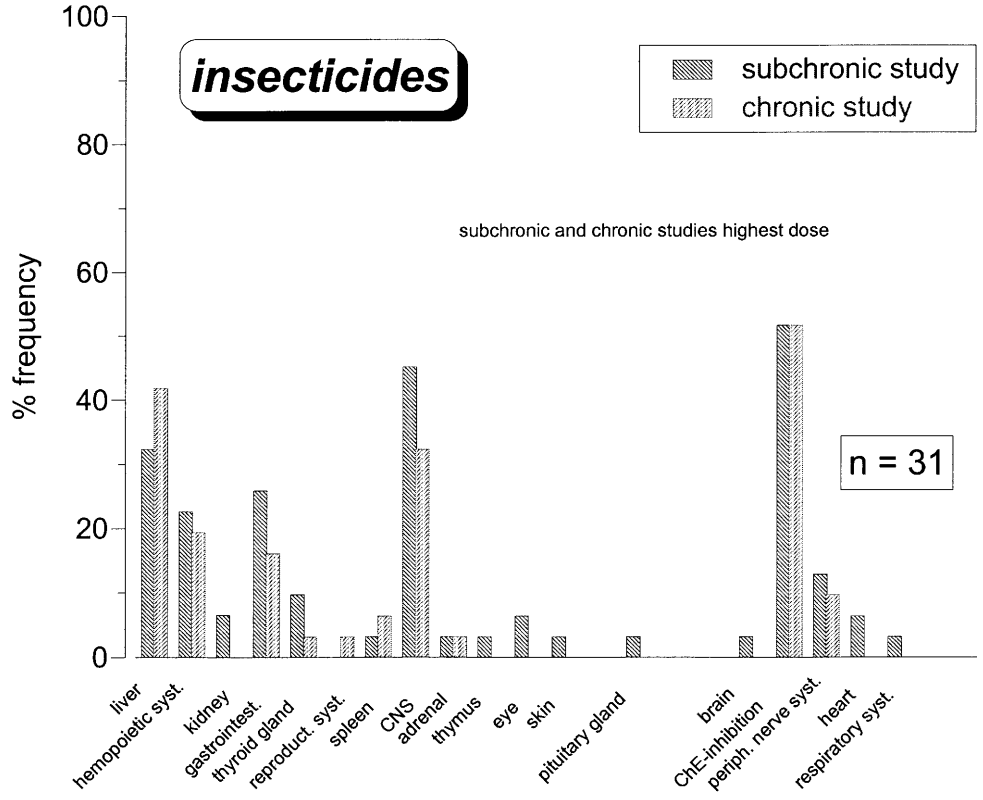

Fig. 4 Target organ toxicity of insecticides at LOEL in subchronic and chronic studies in dogs. Target organs are given on the *x*-axis and the percentage of 31 studies on insecticides showing organ-specific toxicity

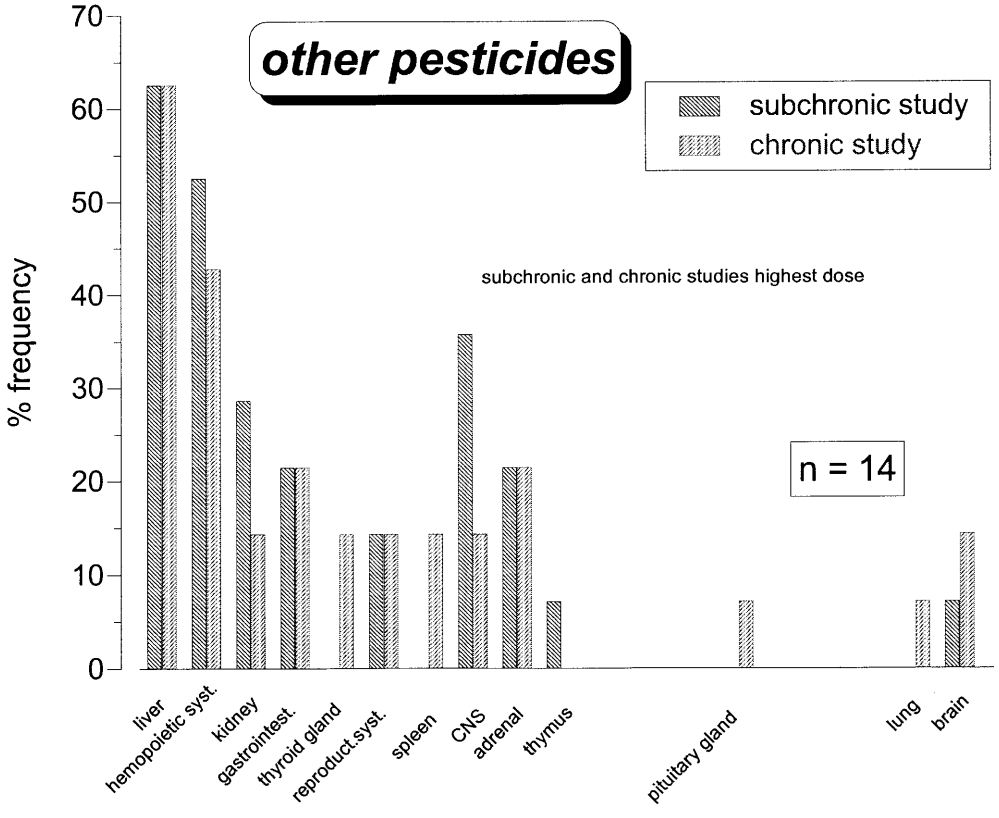

Fig. 5 Target organ toxicity of other pesticides at LOEL in subchronic and chronic studies in dogs. Target organs are given on the *x*-axis and the percentage of 14 studies on other pesticides showing organ specific toxicity are given on the *y*-axis

rank values from 2.00 (insecticides) to 2.36 (fungicides). No clear difference in sensitivity was observed between the subchronic (rank values from 1.33 to 1.69) and chronic studies (rank values from 1.20 to 1.44). The following ranking ratios between the chronic and subchronic studies were obtained: insecticides 0.87, fungicides 0.86, herbicides 0.72, other pesticides 1.23.

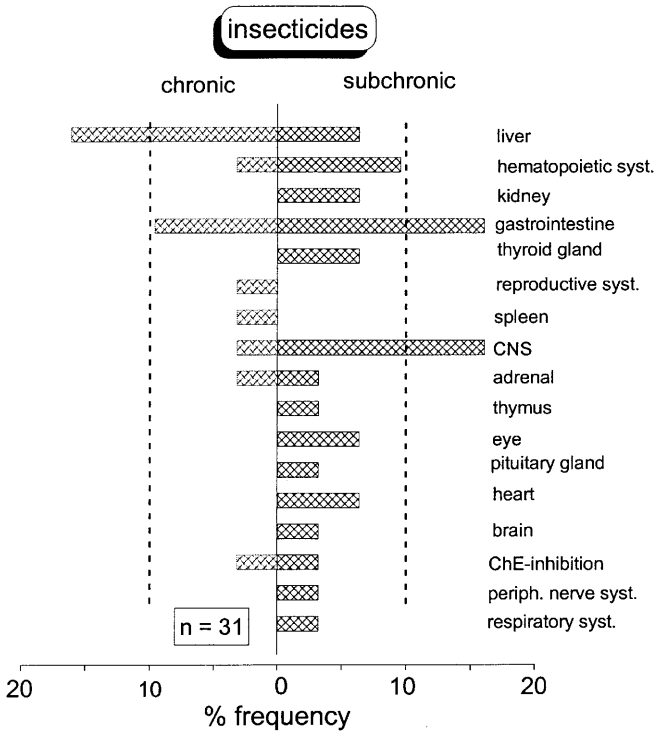

Fig. 6 Frequency distribution pattern of organs affected only in subchronic or chronic studies on insecticides in dogs. A total of 31 studies were compared in the evaluation

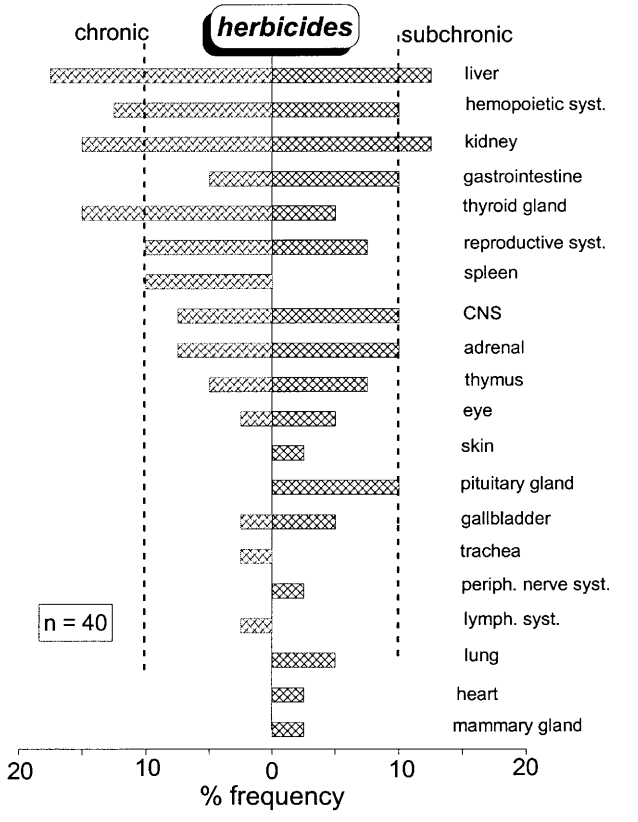

Fig. 7 Frequency distribution pattern of organs affected only in subchronic or chronic studies on herbicides in dogs. A total of 40 studies were compared in the evaluation

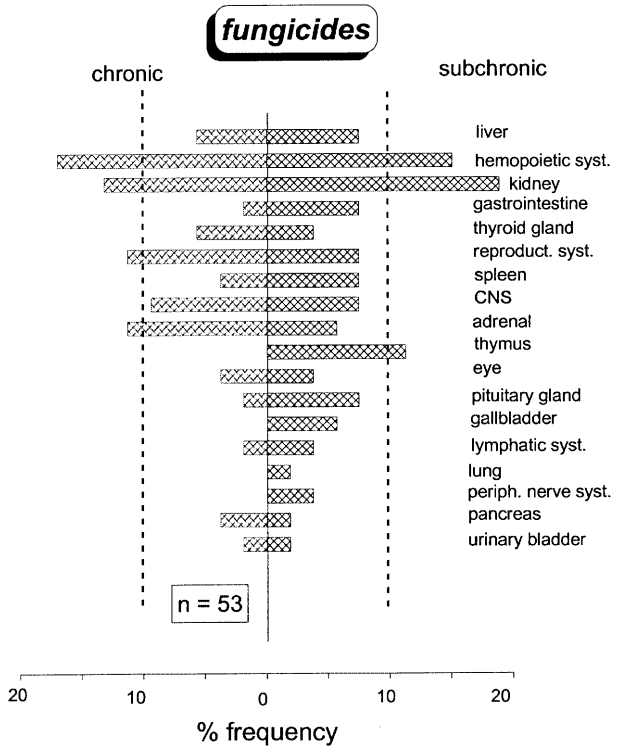

Fig. 8 Frequency distribution pattern of organs affected only in subchronic or chronic studies on fungicides in dogs. A total of 53 studies were compared in the evaluation

The ratios of the LOELs determined in subchronic studies to the LOELs determined in chronic studies in the dog were calculated for each chemical, and the results are plotted in Fig. 1. Figure 1 shows that the difference in the distribution pattern of the LOELs was not significant between the two treatment regimens.

Regression analysis of the LOELs from the 13- or 26-week studies versus the 52- or 104-weeks in the dog revealed a good correlation for all chemical classes (regression values, *r*: fungicides 0.843, herbicides 0.783, insecticides 0.802, other pesticides 0.814; see Table 1, 2, 3 and 4).

Target organ toxicity of pesticides in subchronic and chronic toxicity studies in the dog

The distribution of target organ toxicity in dogs exposed to herbicides, fungicides, insecticides and other pesticides is presented in Figs. 2, 3, 4 and 5. In this evaluation only data from 13-week versus 52- or 104-week studies were used. The target organs are given on the *x*-axes, and the relative frequency of animals with alterations in target organ on the *y*-axes.

In subchronic and chronic studies on herbicides most of the toxic effects were observed in the liver, haematopoietic system and kidneys (Fig. 2). Effects on the trachea and lymphatic system were seen only in the long-term studies after 52 or 104 weeks. However, such side effects were fairly rare and identified in only 1 of 40 studies.

Moreover, no significant difference in organ toxicity was seen between subchronic and chronic studies except for the spleen (5% versus 15%) and thyroid gland (12.5% versus 22.5%). The toxic symptoms in the spleen were not supported by histopathology. Serious effects on the thyroid gland that were found in a single study were not species-specific being also found in rat and mouse studies of the same chemical.

Effects on the liver, haematopoietic system and CNS were the most frequent toxic findings in animals exposed to fungicides (Fig. 3). No significant difference in organ toxicity was observed between chronic and subchronic studies. The most frequent toxic symptoms in dogs exposed to insecticides were inhibition of ChE activity in plasma, RBCs and brain, and effects on the CNS and liver (Fig. 4). In chronic studies more organ-specific toxic effects were seen in the liver (42% versus 32%) and the spleen (6% versus 3%).

After administration of "other pesticides" the liver and the haematopoietic system were most affected in the subchronic and chronic studies (Fig. 5). Some organ alterations were only observed after chronic exposure, i.e. in the thyroid gland, spleen, pituitary gland and lung. However, these effects were rare and observed only in 1 of 14 studies.

In Figs. 6, 7 and 8 the frequencies of organ-specific effects which were only found in subchronic and chronic studies are given for insecticides, herbicides and fungicides. It is important to note that due to higher dosing of test chemicals the number of target organs showing side effects was higher in subchronic than in chronic studies. However, only after exposure to herbicides (Fig. 7) and after chronic exposure to insecticides were toxic effects more frequently seen in the liver (Fig. 6) and after exposure to fungicides in the adrenal glands (Fig. 8). Generally, in the majority of studies the affected organs were identified in both subchronic and chronic studies.

New information (affected organs and toxic symptoms) on pesticides observed in chronic studies in dogs

The toxicological profile of each pesticide is given in Appendix 1. Next to the intended use of each pesticide are presented the duration of treatment in dogs, the dose tested, the affected organs, the target organs taken from the reports of the regulatory agency, the complete toxicological profile, additional findings (chronic versus subchronic or subacute study) and the rank value. New affected organs were observed in 30 of 55 studies with fungicides, 25 of 44 studies with herbicides, 17 of 38 studies with insecticides and 10 of 16 studies with other pesticides. Compared with 26-week studies, new affected organs were found in 52/104-week studies in three of five studies of fungicides, three of four studies of herbicides, one of three studies of insecticides and one of one study of other pesticides.

New information (affected organs and toxic symptoms) on pesticides observed only in chronic studies and comparison with symptoms in rats and mice

Since most data were available from subchronic 13-week studies and from chronic 52/104-week studies, these studies were evaluated intensively, and the 4-week studies were omitted from further analysis. The new toxicological information regarding pesticides which is marked in bold type in Appendix 1 was evaluated in Appendix 2. In Appendix 2 the expertise of the appropriate company toxicologist and other remarks as well as corresponding findings from rat and mouse studies are given together with the new information.

The following findings were considered not to be relevant:

Table 5 Analysis of toxicity studies in dogs on herbicides, insecticides, fungicides and other pesticides (52/104-week versus 13-week studies)

	Herbicides	Insecticides	Fungicides	Other pesticides
Total number	42	32	54	13
All salient effects in dogs identified within 13 weeks	18	20	25	5
New salient findings in dogs identified in chronic studies	24	12	29	8
New salient findings in chronic studies which were seen in studies in rats/mice	11	5	8	3
Higher doses in chronic studies than in 13-week studies	3	4	5	1
Toxicological effects without relevance[a]	6	2	8	2
Symptoms without toxicological effect in toxicologist's expert opinion[b]	2	0	4	2
Relevant toxicological effects	2	1	4	0

[a] Toxicological effects without relevance were: effects upon organ weights (without a histopathological and/or functional correlate); single (isolated) alterations in enzymatic activity (without organ weight change or histopathological correlate); clinical effects on the CNS (when the chemical was administered via capsule or stomach tube); or, if isolated symptoms were diagnosed (only emesis etc.), effects on haematopoietic system only if a single haematological parameter was affected without a histopathological correlate or if haemosiderosis was found without a haematological correlate; effects on gastrointestinal tract (diarrhoea or fluid feces without a histopathological correlate)

[b] These symptoms are indicated in Appendix 2 and were found neither in 13-week studies in dogs nor in studies in rats and mice. Following the expert opinion of the toxicologist, these symptoms were taken as having no toxicological relevance

Table 6 Insecticides

Compound	Dog studies compared	Affected organs	Target organs taken from the agency	Corresponding findings	Remarks	Corresponding effects in rats or mice
1	52 versus 4 weeks	CNS, *haemato - poietic system	ChE	Tremor, convulsions, salivation, *RBC parameters	↓Brain ChE after 4 weeks; *Higher doses used	Neurotoxic effects in rats
2	52 versus 13 weeks	**Adrenal**	Nervous system, liver, lung	**Focal degeneration, inflammatory changes, increased lipid deposits**		
3	26 versus 13 weeks	CNS, *Lymph nodes, *haematopoietic system	Nervous system	Emesis, ataxia, *Anaemia, *infiltration in lymph nodes	*Higher doses used in 26-week study	Same effects in rats and mice
4	26 versus 13 weeks	(Heart, brain)		↑Relative heart, brain weight	Body weight reduced, no histopathological correlate	
6	52 versus 2 weeks	Gastrointestinal tract	CNS	Diarrhoea	Not observed after 104 weeks at the same dose	
9	104 versus 13 weeks	Oesophagus	ChE	Erosion of mucosa	Higher doses used in 104-week study	
10	52 versus 13 weeks	Spleen	Blood, spleen, liver	↑Weight	Higher doses used in 52 week study	Also seen in rats and mice
11	107 versus 13 weeks	Thyroid	Liver, thyroid	Thyroid weight, ↓thyroxine	Thyroid weight after 13 weeks	Thyroid effects in rats (hyperplasia)
12	52 versus 12 weeks	Gastrointestinal tract, *liver	ChE, liver	Diarrhoea cytochrome P-450, N-demethylase, albumin	*Hepatic function not determined in 12-week study	
16	104 versus 13 weeks	Liver	ChE, liver	Hepatocellular oedema, liver weight	Hepatic function normal	Hepatic effects in rats (fat vacuoles, bile stasis)
17	52 versus 4 weeks	(Adrenal)	ChE, (liver, adrenal)	↑Weight	No functional or histopathological correlate	Adrenal hyperplasia in mice
19	52 versus 13 weeks	Gastrointestinal tract, CNS, mortality	Liver	Fluid feces, ataxia	Higher doses used in 52-week study	
20	52 versus 13 weeks	Haematopoietic system	ChE	Anaemia	Found after 13 weeks but not after 52 weeks	Anaemic effects in rats and mice
21	104 versus 4 weeks	CNS	ChE	Tremor, sedation, vomiting	Higher doses used in 104-week study	

27	52 versus 4 weeks	(Liver)	ChE	↓Liver weight	No functional or histopathological correlate	Hepatic effects in mice
28	104 versus 13 weeks	(Liver)	ChE	↑Relative liver weight	No functional or histopathological correlate	
37	104 versus 13 weeks	*Liver, CNS		*Hepatomegaly, emesis	Higher doses used in 104-week study	*Liver adenomas in mice, hepatic effects in rats also found in rat
38	52 versus 13 weeks	ChE	Nervous system	↓Plasma, RBC parameters and brain ChE	Higher doses used in 52-week study	
39	52 versus 13 weeks	Liver	Blood	Serum liver enzymes, histopathological lesions	Higher doses used in 52-week study	

- when only some isolated nonspecific parameters were altered (i.e. only organ weight or enzymatic activity in blood samples without a functional or histopathological correlate)
- when higher doses were used in chronic studies
- when the affected organs observed in chronic studies in dogs were also identified in studies in rats or mice
- if in the expert opinion of the author the findings were considered not relevant for toxicity in humans.

In 1 of 32 studies on insecticides, one new affected organ was identified: the adrenal gland (focal degeneration, inflammatory changes and increased lipid deposits). These findings or similar effects were found neither in subchronic studies in dogs nor in studies in rats and mice.

In 4 of 42 studies on herbicides new affected organs were identified after chronic administration of herbicides. In one case the gallbladder (cholelithiasis), spleen (atrophy) and the CNS (salivation and tremor) were affected. However, following the author's opinion, the effects on the gallbladder and spleen were considered not of toxicological relevance. In another study mucosal hyperplasia of the trachea was observed but was not seen in other studies, and was possibly important. Alterations in the thyroid gland (focal hyperplasia and increased organ weight), adrenal gland (corticular fatty degeneration) and thymus (involution) were considered not to be relevant substance-specific effects.

Most of the new findings in chronic studies of fungicides were of a degenerative nature. In 8 of 54 studies new additional organs were affected. Testicular degeneration (failure in spermatogenesis or reduction in testis weight) after administration of Dichlofluanid or Fosethyl, degenerative changes in the cells of the zona fasciculata of the adrenal gland (Dichlofluanid), alteration in the specific gravity of the urine with calcification of Bowman's capsule (Pyrazophos) and the occurrence of small granuloma and leucocyte infiltration in the urinary bladder (Iprodion) were classified as specific side effects by the appropriate company toxicologists. However, detailed analysis of studies on Dichlofluanid revealed that in contrast to chronic studies no histopathology and no gravimetric measurement of testes had been performed in subchronic studies, and alterations in urinalysis seen after chronic exposure to Pyrazophos were also identified in a study in rats. The other additional findings observed after administration of Bromuconazol (adrenal and kidney), Diniconazol (reproductive system) and Fuazinam (haematopoietic system) were considered not to be important.

In 2 of 13 studies new affected organs were identified after chronic treatment with other pesticides. In one case testicular degeneration and in the other hippocampal and lateral mid brain vacuolization were considered to be spontaneous in nature. When 52/104-week studies were compared with 26-week studies, none of the 8 studies with additional findings were regarded as important for toxicological evaluation.

Table 7 Herbicides

Compound	Dog studies compared	Affected organs	Target organs taken from the agency	Corresponding findings	Remarks	Corresponding effects in rats or mice
1	52 versus 13 weeks	Testes, spleen, **gallbladder, thymus, CNS**	Thyroid, blood, urinary bladder, testes	**Salivation, tremor, cholelithiasis in gallbladder, thymic atrophy,** inflammation and reduced spermatogenic activity in testes, pale spleen		Testes and spleen effect also in liver
2	52 versus 13 weeks	*Haematopoietic system, **lymphatic system	Liver, kidney	*Haemosiderin in spleen and liver, **lymphofollicular hyperplasia in gastric mucosa	*No haematological findings = no adverse effect, **Higher doses used	*Effects also in rats
4	52 versus 26 weeks	Haematopoietic system, liver	Not defined	RBC parameters, ↑liver weight	Higher doses used in 52-week study	
5	104 versus 13 weeks	(Thyroid)	Liver, kidney	↑Weight	No functional or histopathological correlate	
8	52 versus 13 weeks	CNS, mortality	Not defined	Salivation, emesis, tremor, ataxia	Higher doses used in 52-week study, 13 weeks administered orally via capsule	
9	52 versus 13 weeks	Liver	Blood, liver, uterus, testes	Liver weight, liver enzymes, no histopathological findings	Liver weight in 13-week study	Hepatic effects in rats and mice (adenoma)
11	26 versus 13 weeks	Haematopoietic system, **kidney**	Liver, blood	Anaemia, haemosiderosis, **fatty degeneration of kidney epithelium**	No functional or histopathological correlate	Haematopoietic effects in rat
12	52 versus 4 weeks	Pituitary, **CNS, gallbladder, skin**	Thyroid, pituitary	Hypertrophy of pale cells, **lethargy, hyperplasia of gallbladder, dermal acanthosis**		Pituitary effects in rats and mice
14	52 versus 13 weeks	Liver	Blood, liver, thyroid	Weight, hypertrophy		Neoplasia in mice
15	52 versus 13 weeks	(Liver, thyroid)	Not defined	↑Liver weight, ↑relative thyroid weight	No functional or histopathological correlate	
16	52 versus 13 weeks	*Haematopoietic system		Anaemia	Accumulation of brown pigments in bile canaliculi after 13 weeks	
17	52 versus 13 weeks	(Spleen)	Blood	↑Weight	No histopathological correlate	
19	52 versus 13 weeks	Liver	Liver, kidney	Hepatomegaly, a.ph., ALAT, cholesterol		Hepatic effects in rats and mice
23	55 versus 13 weeks	**Adrenal, thymus**, kidney	Liver, kidney, blood, eye	**Cortical fatty vacuolation in adrenal, thymic involution**, bile pigments in urine		Renal effects in rats
24	52 versus 13 weeks	Gastrointestinal tract	Kidney, liver, blood, eye	Diarrhoea		
29	13 versus 4 weeks	Haematopoietic system, *liver, *skin, *kidney	Liver, kidney	RBC parameters, *hepatic and renal function, *conjunctivitis (one dog)	*Higher doses used in 13-week study	Haematopoiesis in rats and mice, liver and kidney effects in mice

31	52 versus 13 weeks	Thyroid, liver	Blood	Hyperplasia of follicular epithelium, hepatomegaly	Thyroid and liver weight increased in 13-week study	Thyroid and hepatic effects in rats and mice
32	52 versus 4 weeks	Liver, thyroid, **CNS**	Blood	Liver enzymes without histological correlate, thyroid follicular hyperplasia, **tremor, ataxia, spasm**		Liver enzymes and thyroid follicular hyperplasia in rats
33	13 versus 4 weeks	Haematopoietic system, mortality	Liver, blood, kidney	Anaemia with histopathological correlate		Blood effects also in rats
35	52 versus 13 weeks	Haematopoietic system, liver, kidney	Liver, kidney, blood, eye	MCV, MCH (only after 13 weeks), ↑liver and kidney weight, renal pigment	Higher doses used in 52-week study	All effects seen in rats and mice
36	52 versus 13 weeks	Thyroid	Kidney	**Focal hyperplasia, ↑weight**		
38	26 versus 13 weeks	CNS, gastrointestinal tract	Blood, liver, mammary gland	Irregular nervous system response, intestinal insult (haemorrhage)	Administered orally via capsule	
39	104 versus 13 weeks	Liver, haematopoietic system	Liver, thyroid	Mild hepatotoxic effects, haemosiderosis		Hepatic and haematopoietic effects in rats
40	52 versus 13 weeks	Kidney, (spleen)	Lung	↑Specific gravity of urine, ↑spleen weight	No histopathological correlate	Renal lesions in mice
41	104 versus 13 weeks	Haematopoietic system, liver, kidney, thyroid, adrenal, testes, prostate, (spleen)	Not defined		Higher doses used	Haematopoietic and hepatic effects in mice
44	13 versus 4 weeks	Kidney, *gastrointestinal tract, *skin	Liver, kidney	Renal function, ulcers and haemorrhage in gastrointestinal tract, ulcerative change in skin	*Higher doses used	Functional and histopathological renal effects in rats and mice
50	52 versus 13 weeks	Testes, eye, **trachea**	Liver	Testicular degeneration, **mild tracheal mucosal hyperplasia**, spontaneous corneal lipidoses		Ocular lesions and testicular degeneration in mice
51	52 versus 13 weeks	(Prostate gland)	Liver	↓Prostate weight		Prostate adenomas in rats
52	52 versus 14 weeks	Kidney	Mammary gland	↑Creatinine	No histopathological correlate	
53	52 versus 13 weeks	Haematopoietic system, *kidney	Not defined	RBC parameters at 26 weeks only, hyaline droplets in females	*Higher doses used	Both effects seen in rats
54	104 versus 13 weeks	Liver	Liver	↑A.ph., ALAT, ↑liver weight	↑A.ph. in 13-week study	Hepatic effects in rats

Table 8 Fungicides

Compound	Dog studies compared	Affected organs	Target organs taken from the agency	Corresponding findings	Remarks	Corresponding effects in rats or mice
1	52 versus 13 weeks	Haematopoietic system	Liver, kidney, adrenal, ovary	RBC parameters	Males only	Also found in rats and mice
4	52 versus 13 weeks	**Adrenal, kidney, pancreas**	Liver, skin	**Fatty vacuolation in adrenals, hypertrophy of zona fasciculata (degenerative change) pigments in renal cells, ↑urine volume, exocrine hypersecretion in pancreas (male), toxicological significance unclear**	↑Adrenal and kidney weights in 13-week study	
6	52 versus 13 weeks	Prostate, spleen, thyroid, testes	Blood	Prostate atrophy, Leydig cell hyperplasia, ↑spleen, thyroid, testes weight	Spleen, thyroid and testes only organ weight without any histopathological correlate	Prostate (fibrosis), testes (Leydig cell hyperplasia)
9	103 and 52 versus 13 weeks	Kidney	Liver, kidney	**↓specific gravity of urine (52 weeks), calcification of Bowman's capsule (104 weeks)**		↑Specific gravity of urine in rats
11	52 versus 13 weeks	Spleen, CNS	Thyroid	Enlargement, emesis	13 weeks administered orally via capsule	↑Spleen weights in rats
12	104 versus 13 weeks	CNS	Liver	Vomiting	13 weeks administered orally via capsule	
13	52 versus 13 weeks	Nose, CNS, haematopoietic system	Liver	Nasal dryness (one female), salivation, **RBC parameters, myeloid/erythroid ratio**	No histopathological correlate	
14	52 versus 13 weeks	Haematopoietic system, *liver	Liver	↓Activated partial thromboplastin time, liver weight, bile stasis, ↑N-demethylase	Higher doses used in 52-week study	Single haematopoietic parameters in rats, hepatic neoplasia in rats
15	52 versus 13 weeks	Liver	Liver	↑Liver weight, a. ph, lipidoses	↑A.ph. in 13-week study	Hepatic effects in rats and mice (histopathological)
16	104 versus 13 weeks	Liver, kidney, gastrointestinal tract	Kidney, stomach, liver, blood	Fibrosis, hepatocyte atrophy, degenerative change in proximal tubules, glomerulocytosis, chronic gastritis	Higher doses used in 13-week study	Gastrointestinal effects (erosion, ulcer, neoplasia) and renal effects (nephrosis, hyperplasia) in rats and mice, hepatic effects not found in rodents
17	52 versus 13 weeks	Adrenal	Liver, eye, blood	Fatty metamorphosis of cortical adrenal cells, ↑adrenal weight		Degenerative adrenal effects in rats
20	52 versus 13 weeks	Kidney	Liver, kidney, thyroid, bone	↑BUN, creatinine, glucosuria, hypertrophy of cortical tubules	Higher doses in 52-week study	
23	52 versus 13 weeks	*Adrenal	Liver	*Fatty vacuolation of zona fasciculata	Not observed after 104 weeks	

24	104 versus 13 weeks	CNS, haematopoietic system, kidney	Liver	Spasm, salivation, RBC parameters, ↑kidney weight	13 weeks administered orally via capsule, higher doses used in 104-week study	
25	52 versus 13 weeks	Adrenal, *kidney	Liver, blood	Vacuolation of zona fasciculata, ↑adrenal weight, kidney weight	*Higher doses used in 52-week study	Same renal and adrenal effects in rats
28	52 versus 13 weeks	Haematopoietic system, (kidney, thyroid)	Kidney in rat and mouse	RBC parameters, pigments in kidney, ↑weight		RBC and urinary effects in rats
29	104 versus 17 weeks	**Testes, adrenal**	Liver, thyroid, bone, kidney	**Testis weight, failure in spermatogenesis (one male only), degenerative changes in cells of zona fasciculata of adrenal**	Studies were performed in 1966	
30	104 versus 13 weeks	Testes	Liver, testes	Degeneration, ↓weight	Due to bacterial infection	
32	52 versus 13 weeks	Liver	Liver, testes	Functional and histopathological alterations (liver effects in 4-week study, 13-week study from 1968)		Liver effects in rats
34	104 versus 13 weeks	Kidney, liver	Liver, blood, thyroid, kidney	Urinary albumin, ↑BUN, inflammatory liver change, cirrhosis, mortality	↑Relative liver weight in 13-week study	Mortality in mice, renal tubular degeneration in rats, hepatocellular hypertrophy in rats
36	26 versus 13 weeks	Gastrointestinal tract, kidney	Liver	Emesis, diarrhoea, ↑BUN	Higher doses used in 52-week study, administered orally via capsule	
41	52 versus 13 weeks	**Reproductive system**	Liver, blood, thyroid	**Onset and frequency of oestrus slightly delayed in females**		
44	52 versus 13 weeks	CNS	Thyroid, liver, kidney, blood	Tremor	Administered orally via capsule	
45	52 versus 13 weeks	Haematopoietic system	Liver, thyroid, urinary bladder	↑WBC, ↓clotting time		Haematopoietic effects in rats
47	104 versus 13 weeks	Eye	Eye (dog)	Loss of reflectability of tapetum ludicum	Higher doses used than in 13-week study, rodents do not have a tapetum ludicum	
48	52 versus 13 weeks	Haematopoietic system	Liver, kidney	↑Platelets	Males only	↓Lymphocytes in mice
49	52 versus 13 weeks	Haematopoietic, liver, **urinary bladder**, (adrenal)	Not defined	Anaemia; hepatic cord atrophy; **small granulomata in urinary bladder and leucocyte infiltration**, ↑adrenal weight	↑Liver weight in 13-week study	Haematopoiesis in rats, hepatic, and adrenal effects in rats and mice
50	104 versus 13 weeks	Testes	Urinary organs (rats)	**Testicular degeneration**		
51	52 versus 13 weeks	**Lymphatic system**	Liver, urinary bladder, stomach	**Lymphoid hyperplasia**		
56	52 versus 13 weeks	Haematopoietic system, *pancreas	Liver, (thyroid)	Anaemia (RBC parameters, brown pigments in hepatocytes) ↑relative weight	*Higher doses used in 52-week study	Haematopoietic effects in rats (RBC parameters)
58	104 versus 13 weeks	Pituitary		↑Weight without histopathological correlate		

Table 9 Other pesticides

Compound	Dog studies compared	Affected organs	Target organs taken from the agency	Corresponding findings	Remarks	Corresponding effects in rats or mice
1	52 versus 13 weeks	Spleen	Liver, kidney, spleen, blood	Weight	No histopathological effects	↑Spleen weight in rats and mice
2	52 versus 13 weeks	Liver	Kidney, urinary bladder, kidney, blood	Hepatocellular degeneration, fibrosis, bile duct proliferation, ↑BSP retention	Histopathological effects in liver after 4 weeks	Histopathological effects in rats and mice (neoplasia etc.)
3	52 versus 4 weeks	Gastrointestinal tract	CNS	Diarrhoea		
4	104 versus 13 weeks	Kidney, adrenal, thyroid, spleen, lung	Liver, ChE	Organ weights, pigmentation of kidneys	No functional or histopathological correlate	
5	52 versus 13 weeks	Liver	Liver, blood	Histopathological changes	Some functional changes in 13-week study	Histopathological changes in rats and mice
6	52 versus 13 weeks	**Testes**, thyroid gland	Liver, kidney, thyroid gland	**Reduction in spermatozoa, seminiferous tubular atrophy**, thyroid weight	Thyroid weight without functional or histopathological correlate, ↓testis weight after 13 weeks	
10	52 versus 13 weeks	gastrointestinal tract, **brain**	kidney, liver, **brain**	Fecal changes, **hippocampus and lateral mid brain effects**		
13	52 versus 14 weeks	*Liver	Liver, blood, bone, testes	Liver function and weight	Higher doses used in 52-week study	Same and more hepatic effects in rats and mice
14	52 versus 4 weeks	Adrenal, liver, haematopoietic system, kidney	Liver, adrenals	Adrenocortical hypertrophy, ↓RBC, haemoglobin, haematocrit (male) only after 3 months; hepatocellular hypertrophy, ALAT, a.ph., kidney weight	No haematology or clinicochemistry available at 13 weeks	Adrenal, liver and blood effects also seen in rats and mice
17	52 versus 13 weeks	Pituitary gland	Liver	Relative weight	No histopathological correlate	

It must be taken into account, however, that all but one of the additionally affected organs had not been evaluated as target organs by the regulatory agency. The only exception was the hippocampus and lateral mid brain vacuolization after chronic exposure to Trinexapac-ethyl (''other pesticides''). This specific effect must be viewed with some caution, since the biological significance is unknown and it was not associated with any pathological changes or any overt neurological signs.

Table 5 summarizes the conclusion from the analysis of the toxicological information obtained in the studies on herbicides, fungicides, insecticides and other pesticides in 52/104- versus 13-week studies. The data show that in 72 of 141 studies new findings were seen in chronic studies which were not observed in subchronic studies. However, in 27 studies the affected organs were also identified in studies in rats and/or mice, and in 12 chronic studies the new findings appeared at higher doses which were not tested in subchronic studies. In 19 studies there was no substantial evidence for toxic side effects since the histopathological findings did not correlate with the clinical symptoms reported in the studies. In 8 of the remaining 15 studies, according to the expert opinion of the company toxicologist, the clinical symptoms had no toxicological relevance.

Discussion

It was the aim of the second part of our study on the use of dogs as second species in the regulatory testing of pesticides to analyse the relevance of the NOELs obtained from studies in the dog for the safety testing of pesticides. Toxicity studies on pesticides in animals are

Table 10 Insecticides

Compound	Comparison	Affected organs	Target organs taken from the agency	Corresponding findings	Remarks	Corresponding effects in rats or mice
2	52 versus 13 weeks	Adrenal	Nervous system, liver, lung	**Focal degeneration, inflammatory changes, increased lipid deposits**	In zona fasciculata and zona reticularis	
3	26 versus 13 weeks	CNS, *lymph nodes, *haematopoietic system	Nervous system	Emesis, ataxia, *anaemia, *infiltration in lymph nodes	*Higher doses used in 26-week study	Same effects in rats and mice
4	26 versus 13 weeks	(Heart, brain)		↑Relative heart, brain weights	Body weight reduced, no histopathological correlate	
9	104 versus 13 weeks	Oesophagus	ChE	Erosion of mucosa	Higher doses used in 104-week study	
10	52 versus 13 weeks	Spleen	Blood, spleen, liver	↑Weight	Higher doses used in 52-week study	Also seen in rats and mice
11	107 versus 13 weeks	Thyroid	Liver, thyroid	Thyroid weight, ↓thyroxine	Thyroid weight after 13 weeks	Thyroid effects in rats (hyperplasia)
12	52 versus 12 weeks	Gastrointestinal tract, *liver	ChE, liver	Diarrhoea cytochrome P-450, N-demethylase, albumin	*Hepatic function not determined in 12-week study	
16	104 versus 13 weeks	Liver	ChE, liver	Hepatocellular oedema, liver weight	Hepatic function normal	Hepatic effects in rats (fat vacuoles, bile stasis)
19	52 versus 13 weeks	Gastrointestinal tract, CNS, mortality	Liver	Fluid feces, ataxia	Higher doses used in 52-week study	
20	52 versus 13 weeks	Haematopoietic system	ChE	Anaemia	Found after 13 weeks and not after 52 weeks	Anaemic effects in rats and mice
28	104 versus 13 weeks	(Liver)	ChE	Relative liver weight	No functional or histopathological correlate	
37	104 versus 13 weeks	*Liver, CNS		*Hepatomegaly, emesis	Higher doses used in 104-week study	*Liver adenomas in mice, hepatic effects in rats
38	52 versus 13 weeks	ChE	Nervous system	↓Plasma, RBC and brain ChE	Higher doses used in 52-week study	Also found in rats
39	52 versus 13 weeks	Liver	Blood	Serum liver enzymes, histopathological lesions	Higher doses used in 52-week study	

carried out for hazard identification and safety assessment to protect the health of humans and animals (European Commission 1994; FIFRA 1984). In this context the regulatory agencies in member states of the EU require subchronic studies in rats and dogs, a chronic or carcinogenic study in rats and mice and a chronic study in dogs if there is evidence that the dog is the most sensitive species in subchronic studies, and in particular when the data may be important to predict adverse effects in humans (European Commission 1988; European Commission 1994).

The argument in favour of conducting chronic studies is that they provide a better understanding of the progression in toxic effects over time. However, long-term studies will inevitably produce adverse effects, which may or may not be relevant for risk assessment in humans. Before the data from these animal studies are used in risk assessment, the relevance of such observations to humans must be established scientifically. During the last decade there has been debate between the European Commission and the US FDA on the most appropriate length of studies in dogs for the registration of new drugs (Broadhead et al. 1999; Parkinson et al. 1995). In contrast to the 12-month studies required by the US FDA, in the EC the length of non-rodent chronic toxicity studies for approval of pharmaceutical products is 6 months. Recently agreement has been reached in Europe that 12-month studies in dogs will not be required

Table 11 Herbicides

Compound	Comparison	Affected organs	Target organs taken from the agency	Corresponding findings	Remarks	Corresponding effects in rats or mice
1	52 versus 13 weeks	Testes, spleen, **gallbladder, thymus**, CNS	Thyroid, blood, urinary bladder, testes	Salivation, tremor, **choleliths in gallbladder (thymic atrophy)**, ↓inflammation and spermatogenic activity in testes, pale spleen	Choleliths in 3/8 at highest dose, *author's remark:* effects on gallbladder and thymus not of toxicological relevance	Testes and spleen, effect also in liver
2	52 versus 13 weeks	*Haematopoietic system, **lymphatic system	Liver, kidney	*Haemosiderin in spleen and liver, **lymphofollicular hyperplasia in gastric mucosa	*No haematological findings = no adverse effects, **higher doses used	*Effects also seen in rats, **lymphocyte counts altered in rats
5	104 versus 13 weeks	(Thyroid)	Liver, kidney	↑Weight	No functional or histopathological correlate	
8	52 versus 13 weeks	CNS, mortality	Not defined	Salivation, emesis, tremor, ataxia	Higher doses used in 52-week study, 13 weeks administered orally via capsule	
9	52 versus 13 weeks	Liver	Blood, liver, uterus, testes	Liver weight, liver enzymes no histopathological findings	Liver weight in 13-week study	Hepatic effects in rats and mice (adenoma)
10	104 versus 13 weeks	Haematopoietic system, liver, kidney, thyroid, adrenal, testes, prostate, (spleen)	Not defined		Higher doses used	Haematopoietic and hepatic effects in mice
14	52 versus 13 weeks	Liver	Blood, liver, thyroid	Weight, hypertrophy		Neoplasia in mice
15	52 versus 13 weeks	(Liver, thyroid)	Not defined	↑Liver weight, ↑relative thyroid weight	No functional or histopathological correlate	
16	52 versus 13 weeks	*Haematopoietic system		Anaemia	Accumulation of brown pigments in bile canaliculi after 13 weeks	
17	52 versus 13 weeks	(Spleen)	Blood	↑Weight	No histopathological correlate	Fibrosis in spleen (rats)
19	52 versus 13 weeks	Liver	Liver, kidney	Hepatomegaly, ↑a.ph., ALAT, cholesterol	↑Cholesterol in 13-week study	Hepatic effects in rats and mice (hepatomegaly)
23	55 versus 13 weeks	***Adrenal, **thymus, kidney**	Liver, kidney, blood, eye	**Cortical fatty vacuolation in adrenals, thymic involution**, bile pigments in urine	*Hormonal effects, **nonspecific consequences of general debility	Renal effects in rats (nephropathy)
24	52 versus 13 weeks	Gastrointestinal tract	Kidney, liver, blood, eye	Diarrhoea		
31	52 versus 13 weeks	Thyroid, liver	Blood	Hyperplasia of follicular epithelium, hepatomegaly	Thyroid weight increased in 13-week study, ↑liver weight in 13-week study	Histopathological effects of thyroid and liver in mice and rats
32	52 versus 13 weeks	CNS, thyroid, liver	Blood	Liver enzymes withouthistopatho-logical correlate, thyroid follicular hyperplasia, tremor, ataxia, spasm	Higher doses used in 52-week study	Target liver and thyroid also in rats

Table 11 (Contd.)

Compound	Comparison	Affected organs	Target organs taken from the agency	Corresponding findings	Remarks	Corresponding effects in rats or mice
35	52 versus 13 weeks	Haematopoietic system, liver, kidney	Liver, kidney, blood, eye	MCV, MCH (only after 13 weeks), ↑ liver and kidney weight, renal pigment	Higher doses used in 52-week study	All effects seen in rats and mice
36	52 versus 13 weeks	Thyroid	Kidney	**focal hyperplasia, ↑ weight**	In one male, *author's remark:* questionable substance-specific effect	
39	104 versus 13 weeks	Liver, haematopoietic system	Liver, thyroid	Mild hepatotoxic effects, haemosiderosis		Hepatic effects in rat, haematopoietic effects in rats
40	52 versus 13 weeks	Kidney, (spleen)	Lung	↑Specific gravity of urine, ↑spleen weight	No histopathological correlate	Renal lesions in mice
50	52 versus 13 weeks	Testes, eye, **trachea**	Liver	Testicular degeneration, **minimal to mild tracheal mucosal hyperplasia**, spontaneous corneal lipidoses	Tracheal hyperplasia in 7/10 at high doses and 1/10 at medium dose	Ocular lesions and testicular degeneration in mice
51	52 versus 13 weeks	(Prostate gland)	Liver	↓Prostate weight		Prostate adenomas in rats
52	52 versus 14 weeks	Kidney	Mammary gland	↑Creatinine	No histopathological correlate	
53	52 versus 13 weeks	Haematopoietic system, *kidney	Not defined	RBC parameters at 26 weeks only, hyaline droplets in females	*Higher doses used	Both effects seen in rats
54	104 versus 13 weeks	Liver	Liver	↑a.ph., ALAT, ↑liver weight	↑A.ph. in 13-week study	Adaptive hepatic effects in rats

by the regulatory agencies. Thus, for the registration of drugs in Europe, in addition to long-term studies in rats, a 6-month study in dogs will be sufficient.

We found that in only 7 of 141 pesticide studies was new and relevant information on the toxic properties of the pesticides provided by chronic 52- or 104-week studies that was not seen in subchronic studies in dogs nor in chronic studies in rats or mice. However, it must be borne in mind that due to the design of the studies, the amount of toxicity data from chronic studies is sometimes larger than from subchronic studies, e.g. since more organs are analysed for substance-specific effects in chronic studies than in shorter subchronic studies. If subchronic and chronic studies have been conducted years apart, comparison of the results proved to be difficult for several test substances since testing requirements in the guidelines had changed.

Moreover, a few of the seven studies that provided new information on the toxic properties of pesticides must be evaluated critically for the following reasons. With regard to the changes in testicular weight and degenerative change in the adrenals following administration of Dichlofluanid, gravimetric measurements of testes and histopathology were not performed in the subchronic studies. With regard to the effects of Pyrazophos in chronic studies, the renal effects were minimal. The specific gravity of urine was decreased in a 52-week study but was unchanged after 104 weeks. The calcification of Bowman's capsule was observed only after 104 weeks and the specific gravity of urine was also lower in a rat study. Finally, with regard to the effects of Rifumsulfuron, the mucosal hyperplasia of the trachea was minimal to moderate.

Our data show that most of the organ-specific toxicity of pesticides can be identified in 13-week studies in dogs and that all significant toxic effects are identified after 26 weeks of exposure. Thus, safety testing of pesticides in dogs should be limited to subchronic (13-week) studies since an extension of the duration of the studies does not provide additional essential information.

The two parts of the present study show that according to the NOELs the dog was the most sensitive species in 41% of the chronic and in 52% of the subchronic studies. Taking into account the fact that the LOELs in dogs were lower than the NOELs in the other two species – rat and mouse – the dog was the most sensitive species in about 15% of the chronic and 13% of the subchronic studies. The first part of our study clearly

Table 12 Fungicides

Compound	Comparison	Affected organs	Target organs taken from the agency	Corresponding findings	Remarks	Corresponding effects in rats or mice
1	52 versus 13 weeks	Haematopoietic system	Liver, kidney, adrenal, ovary	RBC parameters	Males only	Also in rats and mice
4	52 versus 13 weeks	**Adrenal, kidney**, pancreas	Liver, skin	**Fatty vacuolation in adrenals, hypertrophy of zona fasciculata, ↑adrenal weight (degenerative change), pigments in renal cells, ↑urine volume, ↓Ca^{2+}, exocrine hypersecretion in pancreas (males) (toxicological significance unclear)**	↑Adrenal and kidney weights, ↓Ca^{2+} in 13-week study, *author's remark:* indication of effect on adrenal and kidney in 13-week study	↑Urinary volume in rats after 13 weeks
6	52 versus 13 weeks	Prostate, spleen, thyroid, testes	Blood	Prostate atrophy, Leydig cell hyperplasia, ↑spleen, thyroid, testis weight	Spleen, thyroid and testes only organ weight without histopathological correlate	Prostate (fibrosis), testes (Leydig cell hyperplasia)
9	103 and 52 versus 13 weeks	Kidney	Liver, kidney	**↓Specific gravity of urine (52 weeks), calcification of Bowman's capsule (104 weeks)**	No alteration in urinalysis after 104 weeks	↑Specific gravity of urine in rats
11	52 versus 13 weeks	Spleen, CNS	Thyroid	Enlargement, emesis	13 weeks administered orally via capsule	↑Spleen weight in rats
12	104 versus 13 weeks	CNS	Liver	Vomiting	13 weeks administered orally via capsule	
13	52 versus 13 weeks	Nose, CNS, **haematopoietic system**	Liver	Nasal dryness (one female), salivation, **RBC parameters, myeloid/erythroid ratio**	No histopathological correlate, *author's remark:* fluazinam produced a generalized nonspecific toxicity with evidence of an increased liver weight	Slight anaemia in rats at 104 weeks
14	52 versus 13 weeks	Haematopoietic system, *liver	Liver	↓Activated partial thromboplastin time, liver weight, bile stasis, ↑N-demethylase	Higher doses used in 52-week study	Single haematopoietic parameters in rats, hepatic neoplasia in rats
15	52 versus 13 weeks	Liver	Liver	↑Liver weight, a. ph, lipidoses	↑A.ph. in 13-week study	Hepatic effects in rats and mice (histopathological)
16	104 versus 13 weeks	Liver, kidney, gastrointestinal tract	Kidney, stomach, liver, blood	Fibrosis, hepatocyte atrophy, degenerative change in proximal tubules, glomerulocytosis, chronic gastritis	Higher doses used in chronic study	Gastrointestinal effects (erosion, ulceration, neoplasia) and renal effects (nephrosis, hyperplasia) in rats and mice, hepatic effects not found in rodents
17	52 versus 13 weeks	Adrenal	Liver, eye, blood	Fatty metamorphosis of cortical adrenal cells, ↑adrenal weight		Degenerative adrenal effects in rats

Table 12 (Contd.)

Compound	Comparison	Affected organs	Target organs taken from the agency	Corresponding findings	Remarks	Corresponding effects in rats or mice
20	52 versus 13 weeks	Kidney	Liver, kidney, thyroid, bone	↑BUN, creatinine, glucosuria, hypertrophy of cortical tubuli	Higher doses used in 52-week study	
23	52 versus 13 weeks	*Adrenal	Liver	*Fatty vacuolation of zona fasciculata	Not observed after 104 weeks	
24	104 versus 13 weeks	CNS, haematopoietic system, kidney	Liver	Spasm, salivation, RBC parameters, ↑kidney weight	13 weeks administered orally via capsule, higher doses used in 104-week study	
25	52 versus 13 weeks	Adrenal, *kidney	Liver, blood	Vacuolation of zona fasciculata, ↑adrenal weight, kidney weight	*Higher doses used in 52-week study	Same renal and adrenal effects in rats
28	52 versus 13 weeks	Haematopoietic system, (thyroid)	Kidney in rats and mice	RBC parameters, pigments in kidney, ↑weight		RBC and urinary effects in rats
29	104 versus 17 weeks	**Testes, adrenal**	Liver, thyroid, bone, kidney	**Testis weight, failure in spermatogenesis (one male only), degenerative changes in cells of zona fasciculata of adrenal**	Studies performed in 1966, histopathology not performed and testicular weights not determined in 13-week study	
30	104 versus 13 weeks	Testes	Liver, testes	Degeneration, ↓weight	Due to bacterial infection	
32	52 versus 13 weeks	Liver	Liver, testes	Functional and histopathological alterations (liver effects in 4-week study, 13-week study from 1968)		Liver effects in rats
34	104 versus 13 weeks	Kidney, liver	Liver, blood, thyroid, kidney	Urinary albumin, ↑BUN, inflammatory liver change, cirrhosis, mortality	↑Relative liver weight in 13-week study	Mortality in mice, renal tubular degeneration in rats, hepatocellular hypertrophy in rats
41	52 versus 13 weeks	**Reproductive system**	Liver, blood, thyroid	**Onset and frequency of oestrus slightly delayed in females**	*Author's remark:* not of toxicological significance since histopathological and gravimetric correlates absent	
44	52 versus 13 weeks	CNS	Thyroid, liver, kidney, blood	Tremor	Administered orally via capsule	
45	52 versus 13 weeks	Haematopoietic system	Liver, thyroid, urinary bladder	↑WBC, ↓clotting time		Haematopoietic effects in rats
47	104 versus 13 weeks	Eye	Eye (dog)	Loss of reflectability of tapetum ludicum	Higher doses used than in 13-week study, rodents do not have a tapetum ludicum	
48	52 versus 13 weeks	Haematopoietic system	Liver, kidney	↑Platelets	Males only	↓Lymphocytes in mice
49	52 versus 13 weeks	Haematopoietic system, liver, (adrenal), **urinary bladder**	Not defined	Anaemia, hepatic cord atrophy, ↑adrenal weight, **small granulomata in urinary bladder and leucocyte infiltration**	↑Liver weight in 13-week study	Haematopoiesis in rats, hepatic and adrenal effects in rats and mice
50	104 versus 13 weeks	Testes	Urinary organs (rats)	**Testicular degeneration**		

Table 12 (Contd.)

Compound	Comparison	Affected organs	Target organs taken from the agency	Corresponding findings	Remarks	Corresponding effects in rats or mice
51	52 versus 13 weeks	**Lymphatic system**	liver, urinary bladder, stomach	**Lymphoid hyperplasia**	*Author's remark:* nonspecific (common observation in Beagle dog)	
56	52 versus 13 weeks	Haematopoietic system, *pancreas	Liver, (thyroid)	Anaemia (RBC parameters, brown pigments in hepatocytes), ↑relative weight	*Higher doses used in 52-week study	Haematopoietic effects in rats (RBC parameters)
58	104 versus 13 weeks	Pituitary		↑Weight without histopathological correlate		

Table 13 Other pesticides

Compound	Comparison	Affected organs	Target organs taken from the agency	Corresponding findings	Remarks	Corresponding effects in rats or mice
1	52 versus 13 weeks	Spleen	Liver, kidney, spleen, blood	Weight	No histopathological effects	↑Spleen weight in rats and mice
2	52 versus 13 weeks	Liver	Kidney, urinary bladder, blood	Hepatocellular degeneration, fibrosis, bile duct proliferation, ↑BSP retention	Histopathological effects in liver after 4 weeks	Histopathological effects in rats and mice (neoplasia etc.)
4	104 versus 13 weeks	Kidney, adrenal, thyroid, spleen, lung	Liver, ChE	Organ weights, pigmentation of kidneys	No functional or histopathological correlate	
5	52 versus 13 weeks	Liver	Liver, blood	Histopathological changes	Some functional changes in 13-week study	Histopathological changes in rats and mice
6	52 versus 13 weeks	**Testes**, thyroid gland	Liver, kidney, thyroid gland	**Reduction in spermatozoa, seminiferous tubular atrophy**, thyroid weight	Thyroid weight without functional or histopathological correlate, ↓Testis weight after 13 weeks, *author's remark:* degenerative testicular changes considered to be spontaneous	
10	52 versus 13 weeks	Gastrointestinal tract, **brain**	kidney, liver, **brain**	Fecal changes, **hippocampus and lateral midbrain effects**	*Author's remark:* biological significance unknown since minimal focal vacuolation few and not associated with any pathological changes or overt neurological signs	
17	52 versus 13 weeks	Pituitary gland	Liver	Relative weight	No histopathological correlate	

showed that studies in rodents can be limited to one species, the rat, since no essential information for the safety assessment of pesticides was provided by studies in the mouse. Although our investigation proves that studies in dogs are essential for hazard identification and risk assessment of pesticides, the information required may be obtained by focusing on only one of the currently established subacute, subchronic and chronic studies. Our analysis of studies in dogs suggests that only in 7 of 141 cases was new relevant information provided by chronic 52- or 104-week studies, which was seen neither in subchronic studies on dogs nor in chronic studies in rats. Our data prove that most of the organ-specific toxicity of pesticides can be found in 13-week studies in dogs and that all significant toxic effects are identified after 26 weeks of exposure. Thus, safety testing of pesticides in dogs should be limited to subchronic (13-week) studies, since an extension of the duration of the studies does not provide additional essential information.

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Appendix 1

New information (target organs and corresponding toxic symptoms) on insecticides (Table 6), herbicides (Table 7), fungicides (Table 8) and other pesticides (Table 9) observed only in chronic studies (52 or 104 weeks) in the dog and comparison with symptoms in rats or mice. Cells in bold type indicate studies in which (at the same dose level) newly affected organs were found in chronic studies but not in subchronic/subacute studies in the dog (the effects were not observed in rats or mice). Test compounds are referred to by numbers for the sake of anonymity.

Appendix 2

New information (target organs and corresponding toxic symptoms) on insecticides (Table 10), herbicides (Table 11), fungicides (Table 12) and other pesticides (Table 13) observed only in chronic studies (52 or 104 weeks) versus subchronic studies (13 weeks) in the dog and comparison with symptoms in rats or mice. Items in bold type indicate studies in which (at the same dose level) newly affected organs were found in chronic studies but not in subchronic/subacute studies in the dog (the effects were not observed in rats or mice). Test compounds are referred to by numbers for the sake of anonymity.

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