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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 (BfS) were analysed to determine whether chronic studies in dogs (52/104 weeks) provide essential additional specific toxicological comparisons 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 chronic/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 that 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 not 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

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 (Ighahasi 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 Ergänzungsverfahren 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 (Industrieverbund 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, molluscsicides, 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