A Comparison of the Results of Studies on Pesticides from 12- or 24-Month Dog Studies with Dog Studies of Shorter Duration

Karl P. Baetcke, Whang Phang, and Vicki Dellarco*

Health Effects Division, Office of Pesticide Programs,

U.S. Environmental Protection Agency, Washington, D.C. 20460

4/7/05

This document is for review by the

U.S. EPA FIFRA Scientific Advisory Panel

Meeting May 5-6, 2005

Table of Contents

List of Tables

Executive Summary

The Office of Pesticide Programs has established chronic RfDs for 304 pesticides as of October 2004. One hundred and sixteen (116 or 38%) of the chronic RfDs are based on results from dog studies. A retrospective analysis of the results of dog studies of different duration conducted with 77 of these 116 pesticides was undertaken to ascertain whether data from 13-week dog studies alone would suffice, in the absence of chronic dog studies (1 year or more), for the identification of NOAELs and LOAELs for the derivation of chronic reference doses (RfDs). Interim data (13-weeks or less) from chronic dog studies and data from subchronic dog studies (*e.g.,* 28-day) were also compared with the data from terminal sacrifices in the chronic studies. The analysis showed that for 58 of the 77 pesticides (75%), the results of the chronic studies and studies of lesser duration provide comparable NOAELs and LOAELs. For the remaining 19 pesticides, where the doses administered in the 13-week and chronic dog studies differed, the apparent NOAELs and/or LOAELs were lower in the chronic study than in the 13-week study. Evaluations of dose response data and consideration of interim data from 1- to 2-year studies or results from studies of other than 13-weeks duration showed that NOAELs and LOAELs would likely be equivalent for 11 of the 19 pesticides if the 13-week studies had employed the same dose levels as the 1- or 2-year study. For another 2 pesticides, the database contained a study that would provide NOAELs/LOAELs comparable to the NOAELs/LOAELs identified in the chronic dog study. For an additional pesticide, the lack of sufficient clinical chemistry, hematologic, and histopatholgy data limits meaningful comparisons of the results of the 13-week and chronic dog studies. For an additional 2 pesticides, there is a lack of detection of effects in the 13-week study that would be expected to occur early on (cholinesterase inhibition and effects on thyroid hormones that normally are observed at $\leq$13-weeks), which suggests that the finding of different NOAELs/LOAELs is not related to the different duration of the 13-week and chronic studies but rather due to variability in the measurement of cholinesterase and hormone activity. There are only 3 pesticides where there are indications that a chronic dog study would potentially be more appropriate than a 13-week study for the selection of NOAELs and LOAELs. Thus, the present analysis indicates that a 13-week dog study would be adequate for identification of a NOAEL or LOAEL that would be similar to that established from a chronic dog study for all except 3 pesticides (3/77 or 4%) of the cases evaluated.

1. Introduction

The need for multiple dog studies of different duration has long been a subject of debate among regulatory agencies. Currently, there is no international standard regarding the duration of dog studies. The Office of Pesticide Programs (OPP), US Environmental Protection Agency (USEPA) requires that the results of a rodent and nonrodent (*i.e.,* dog) subchronic (13-week) study and a chronic (2-year rat and a 1-year dog) study be submitted to support the registration of a food use pesticide or for non-food use pesticides if exposure is likely to result in repeated exposure over a significant time. The U.S. Food and Drug Administration has an established standard duration of 1-year for dog studies although exceptions are made to allow a 6-month study for pharmaceutical. The European Union (EU) requires a 6-month non-rodent study for approval of pharmaceuticals and a 6-month dog study is considered sufficient for the registration of most compounds in Japan (Spielmann and Gerbracht, 2001; DeGeorge *et al*., 1999; Contrera *et al*., 1993). For plant protection products, the EU always requires a 90-day dog study and a 1-year dog study may be required if the dog is shown to be more sensitive than the rat in a 90-day study; a proposal under consideration by the EU is to require only 1 dog study. Canada requires a 1 year dog study for pesticides.

The value of testing pharmaceuticals in rodent and non-rodent species for longer than 13 weeks or 6 months has been addressed in a number of reports. Lumley *et al*. (1992) evaluated the minimum duration of chronic animal toxicity studies needed to detect adverse responses and to define safety margins between the proposed use levels and adverse responses for pharmaceutical compounds. The analyses were based on results primarily from rat, dog, and primate studies, but also from other species. Additional toxicities were identified in longer than 6 months for 9 of 75 case studies with 6-month study results; additional toxicities were also identified in 12-month studies from 21 of 80 case studies that were not observed in 1 or 3 month studies. There were no cases where data from 12-month studies or longer had an effect on development of a pharmaceutical for clinical use. As to the results of toxicity studies conducted specifically with dogs, all significant effects were identified within 6 months for 98% of the pharmaceuticals (55 of 56 case studies). Based on the results of this retrospective analysis, it was suggested that animal toxicity studies lasting 6 months would be sufficient for routine toxicity testing of therapeutic agents.

Contrera *et al*. (1993) conducted a retrospective analysis on the results of thirty 6- and 12- month duration non-rodent toxicity studies on pharmaceutical compounds. Ten of the 30 studies were 1-year dog studies with 6-month interim sacrifices. New toxicological findings (*i.e.,* an effect observed at 12 months that was not seen or recognized as significant at 6 months) were identified in 3 of the 10 studies. Both 6-month and 1-year studies were compared for 27 dog studies; new findings were identified in 5 of the 27 study pairs. Based on these comparisons, they concluded that the requirement for a 12-month non-rodent study be maintained because 12- month dog studies provided information of regulatory significance that was not generally detected in 6-month studies. They recommended, however, that the issue be reconsidered when additional data on 6-month and 12-month dog studies became available.

The same group analyzed 117 pharmaceuticals in the Center for Medicines Research toxicology data base and determined that dog studies >6 months demonstrated additional effects in only 13 of the 117 (11%) compounds (Parkinson *et al*., 1995). For most of the chemicals, the significant effects were seen within 3 months. In cases where additional toxicities were identified after 3 months, similar responses were seen in the rat studies. While these authors suggested that dog studies longer than 3 months provide relatively little new toxicological information, they concluded that a six month dosing period in the dog should be the only non-rodent bioassay that is routinely required for evaluating chronic toxicity.

Participants at an International Conference on Harmonization (ICH) workgroup reviewed data on dog studies (17 case studies) to arrive at a consensus on the best duration time for non-rodent toxicity studies (DeGeorge *et al*., 1999). Members of the workgroup from the European Union, the United States, and Japan evaluated results only from dog studies with data showing significant new findings that occurred beyond a 6-month dosing period. For only a small number of cases (3 of the18) was there agreement that no new findings were detected beyond 6 months of treatment. This workgroup concluded that the results of their analysis supported a 9-month duration for non-rodent toxicity studies because most of the toxicities observed in the case studies could have been detected by 9 months.

Gerbracht and Spielman (1998) conducted a retrospective analysis of studies on 216 pesticides. The data examined was from studies submitted for regulatory purposes to the Federal Institute of Health Protection of Consumers and Veterinary Medicine, Germany. The analysis was designed to examine the relevance of studies in dogs on regulatory testing of pesticides. The authors reported no significant differences in species-specific organ toxicities among rats, mice and dogs in 13-week and 52- or 104-week studies. However, haematoxic effects were more often detected in dogs. They reported that the dog was generally more sensitive to the toxic effects of insecticides than rats or mice and but that the rat appeared to be more sensitive than the dog to the toxic effects of herbicides. Overall, they concluded that studies in both rats and dogs are important for the safety assessment of pesticides.

More recently, Spielmann and Gerbracht (2001) performed a comprehensive analysis of data, also submitted to the Federal Institute of Health Protection of Consumers and Veterinary Medicine, Germany, from dog studies on 172 pesticides. The focus of this analysis was on whether dog studies >13 weeks provided important additional information provided by studies of shorter duration. They reported that "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." They further stated that "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 recommendation for a study of 13-weeks duration was supported by the finding that in only 7 of 141 pesticide studies was new and relevant information on the toxic properties of the pesticide provided by chronic dog studies that was not seen in dog studies of shorter duration or in studies with rats or mice.

2. Methods

The Science Information Branch, Health Effects Division, Office of Pesticide Programs, U.S.EPA periodically prepares a tabulation of acute and chronic reference doses (RfDs) that have been established for risk assessments. The RfD is defined as an estimate, within an order of magnitude, of exposure assumed to be without appreciable risk for adverse noncancer health effects. In the risk characterization step, the RfD values are compared to potential or known environmental exposure levels. RfDs for noncancer effects are generally based on identifying a no-observed-adverse-effect-level (NOAEL) for a critical effect which is usually determined from laboratory animal studies. The NOAEL is typically divided by two uncertainty factors (UF) to account for uncertainties inherent in the extrapolation from laboratory animal species to humans (the interspecies UF) and for potential variations in sensitivity among members of the human population (the intraspecies UF). Additional uncertainty factors may be applied to the NOAEL to address the comprehensiveness and quality of the database available (i.e., database UFs). The tabulations of RfDs includes the species and study used for selection of an RfD, the NOAEL and LOAEL from the key study, a description of the endpoint used to establish an RfD, as well as other pertinent information (*e.g.,* uncertainty factors applied to the NOAEL to establish an RfD and the exposure duration and relevant population to which the RfD should be applied for a risk assessment).

The current retrospective analysis began with a review of an internal data base of RfDs established by the Health Effects Division, Office of Pesticide Programs through October 2004. The species identified in this data base used to derive 304 chronic RfDs was considered as the initial source of information. Of the 304 chronic RfDs, 116 (38%) were based on data from a dog study. The data base on these 116 pesticides was subjected to an initial analysis to determine whether the available data were sufficient to compare the results of $\geq$1 year dog studies with dog studies of shorter duration. Final selection of pesticides to be included in the retrospective analysis was made after considering the following criteria: (1) availability of a dog study with a duration of $\geq$ 1 year and a dog study with a duration of 13-weeks or (2) availability of results from a dog study of $\geq$1year duration that included clinical or hematology measurements at 13-weeks or less. New pesticides not yet registered or pesticides that have had their registrations cancelled were not included in this analysis. Data Evaluation Reports (DER's) and reports of the Hazard Identification, Assessment, and Review Committee (HIARC) were evaluated for additional information on these inclusion factors and for additional details on dose-response and effects data.[1]

After consideration of these factors for inclusion of a pesticide in the retrospective analysis, EPA identified 77 pesticides for inclusion in this retrospective analysis. 42

[1]DER - Data Evaluation Record is the record of the Office of Pesticide Programs review and conclusions for a submitted study. The DER includes data on clinical chemistry, hemotologic, and urinalysis measurements and tabulations of histopathologic findings. The HIARC report is the record of toxicological peer review for the chemical. It summarizes the conclusions of the committee about the toxicological aspects of the risk assessment (*e.g.,* doses, endpoints, and uncertainty factors for use in the risk assessment and rationale to support them as well as the overall toxicity profile and hazard characterization for the chemical).

pesticides were identified as being amenable to comparisons of results from 1 year or longer and dog studies of 13-weeks or less duration. An additional 35 pesticides for which no 13-week studies are available but shorter-term and interim term clinical chemistry or hematologic measurements are available were also included for comparison with terminal data from chronic studies. For most of these 35 pesticides, no interim histopathologic data are available. In these cases, comparisons of LOAELs were confined to effects on hematologic or clinical chemistry parameters. Also, for those chemicals that are inhibitors of cholinesterase activity, the percentage decreases in brain cholinesterase activity are not provided because brain cholinesterase activity cannot be measured at interim time points.

Results of studies with dogs provide data that are used for hazard identification as well as hazard characterization. Consideration is given to effects across all dose-levels that may be relevant to the uncertainty factors applied to a NOAEL during the derivation of a RfD and that are in addition to the standard interspecies and intraspecies uncertainty factors. The rationale for application of additional uncertainty factors to the NOAEL derived from a chronic dog study was also evaluated, in order to ascertain whether additional findings observed in a chronic dog study were the sole bases for applying an additional uncertainty factor.

3. Results

Table A1 (in Appendix) shows the NOAELs and LOAELs from 42 pesticides for which long-term and 13-week studies were available. For 23 of these (bolded in Table A1, in Appendix), NOAELs and LOAELs were comparable (i.e., no more than $\geq$1.5-fold differences) at the 13-week and 1-2 year time points. For all 35 pesticides for which 13-week studies were not available, the same NOAELs and LOAELs were found at 13-weeks (or less) in 1-year studies and at 1-year (Table A2, in Appendix). Thus, 58/77 (75%) of the dog studies evaluated had comparable NOAELs/LOAELs at both timepoints. The initial evaluation in Table A1, in the Appendix, indicated that 19 of the 77 pesticides where chronic RfDs were based on a dog study have NOAELs or LOAELs in 13-week studies higher ($\geq$1.5-fold) than NOAELs or LOAELs established from chronic dog studies. Because the different NOAELs or LOAELs in the chronic and 13-week studies of these 19 pesticides may be associated with differences in the treatment doses selected in the chronic and 13-week studies, additional analyses using dose-response data were performed on results of studies on these pesticides.

Table A3 (in Appendix) presents dose-response data for the 19 pesticides and comments regarding comparisons of the results of dose-response data from chronic dogs studies and 13-week or shorter dog studies or from interim results from 1-year dog studies. The analysis of the dose-effect data indicated that for 11 of the 19 pesticides, it was likely that comparable NOAELs/LOAELs would be identified if these pesticides were evaluated in well conducted 13-week and chronic dog studies. The observed differences in NOAELs or LOAELs between 13-week and chronic studies for these 11 pesticides were judged to be associated with differences in dose selection and dose spacing, inter-experimental variability (interim data in the chronic study indicates the same NOAEL/LOAEL should be observed in a 13-week study) or sensitivity of the

study (*i.e.,* the effects were of low incidence or magnitude and the power of the dog studies were limited because of the use of 4 dogs/sex/dose).

Table 1, below, provides a summary of effects data and further evaluation of 8 of the remaining 19 pesticides for which a 1-year dog study appeared to be the critical study. The lack of sufficient clinical chemistry, hematologic, and histopatholgy data on Fosetyl Al limits meaningful comparisons of the results of the 13-week and chronic dog studies. A study performed on 2 of the 8 pesticides with rats would provide NOAELs/LOAELs comparable to the NOAELs/LOAELs identified in the chronic dog study (Cypermethrin and Diflufenzopyr). In the 13-week study for 2 additional pesticides, there is a lack of detection of effects in the 13-week study that would be expected to occur early on (Triazamate - cholinesterase inhibition; Ethylene thiourea - effects on thyroid hormones, both effects that normally are observed at ≤13-weeks), which suggests that the finding of different NOAELs/LOAELs is not related to the different duration of the 13-week and chronic studies but to a lack of sensitivity of the study. Further, application of an additional uncertainty factor to the point of departure for the 13-week study with Triazamate, for lack of a NOAEL, would yield a point of departure as protective as the 1-year dog study.

Thus, there are only 3 pesticides (Etoxazole, Hexazinone, and Tebuconazole) where there are indications that a chronic dog study, and not a 13-week dog study, would result in the selection of a lower NOAEL for the derivation of an RfD. Overall, the present analysis indicates that a 13-week dog study would be adequate for identification of a NOAEL that would be similar to that established from a chronic dog study with the possible exception of 3 of the 77 pesticides evaluated.

Table 1. Pesticides with lower NOAELs in ≥ 1year dog studies than in 13-week dog studies.

Chemical	Differences in NOAELs and LOAELs (13-week study versus 1-year study)	Primary basis for selection of 1-year study	Comments
Cypermethrin	4X and 2X	At the LOAEL, tremors observed at low incidence	Low incidence of effects at LOAEL in 1-year study and observation of effects in 1 male at NOAEL in 13-week study, although considered transitory, suggests sample size (sensitivity) of the study may account for selection of different NOAELs and LOAELs In the absence of the 1-year study, a 90-day rat neurotoxicity study would be used for a chronic risk assessment. An additional 3X uncertainty factor would currently be applied to the rat study to account for lack of a chronic study. In the rat 90-day neurotoxicity study, decreased motor activity, increased foot splay, and decreased body weights were observed at the LOAEL of 26.3 mg/kg/day; the NOAEL for the study is 5 mg/kg/day; these NOAELs/LOAELs are not biologically significantly different than the NOAELs/LOAELs reported for the 1-year dog study (6.0/18.1)
Diflufenzopyr	2.2X and 1.34X	Effects on hematopoietic system of higher incidence in 1 year study	Increased incidences of effects at the LOAEL in 1 year study compared to incidences in 13 wk study. The other most appropriate study in the data base is a rat reproduction study with a NOAEL of 27.3 and a LOAEL of 113.1 based on reduced body weight gain and increased seminal vesicle weights (1-year dog study NOAEL, 26 and LOAEL 299). Thus, use of the rat reproduction study to derive a chronic RfD would be as protective as the 1-year dog study.
Ethylene thiourea	33.4X and 33.2X	Thyroid effects (hypertrophy and decreased hormone levels)	Dose spacing differences between two studies make comparisons difficult. Because decreases in thyroid hormones are typically seen early (as early as 14-days) unclear why 13-week study would not be sufficient for selection of a NOAEL/LOAEL. No acceptable 2-year rat study available; no other study with NOAELs/LOAELs comparable or lower than that from 1-year dog study
Etoxazole	1X and 2.3X	Hepatocellular toxicity	It is likely that actual NOAEL would be lower in 1-year study than in 13-week study based on increased incidence of liver toxicity or increases in liver enzymes. However, if the 13-week dog study was used for the chronic RfD, NOAEL would be the same as in the 1-year dog study. The most sensitive study in the data base other than the chronic dog study is the 13-week dog study.

Chemical	Differences in NOAELs and LOAELs (13-week study versus 1-year study)	Primary basis for selection of 1-year study	Comments
Fosetyl Al	1.1X and 2.6X	Focal/trace spermatocytic and/or spermatidic giant cells	Limited details on clinical chemistry, hematology, or histopathology limit meaningful comparions with 13-week study. In a 2-year rat study, the only effect reported (non-neoplastic) was an increase in urinary protein at a LOAEL of 1500 mg/kg/day; the NOAEL is 400 mg/kg/day versus NOAEL/LOAEL 250/500 in the 1-year dog study. The 2-year rat study is the only other study in the data base that would be suitable for derivation of an RfD.
Hexazinone	5X and 3.3X	Hepatocellular toxicity	Effects on ALP at 37.6 mg/kg/day in the 1-year study greater (males) or comparable (females) to the effects on ALP at 122.5 mg/kg/day in the 13 week study (NOAEL 25.9). 1 year study provides data that would lead to selection of a lower NOAEL (5.0 mg/kg/day) for derivation of a chronic RfD 2-year chronic study performed with rats has a NOAEL of 10.2 versus a NOAEL of 5.0 in the chronic dog study. The LOAEL in the chronic rat study of 53.3 is based on decreased body weights and food efficiency; the LOAELs in the rat and dog studies are comparable (53.3 and 37.6, respectively), but the effects were more extensive and severe in the 1-year dog study. Thus, use of the chronic rat study would not provide as protective a point of departure for derivation of a chronic RfD
Tebuconazole	2.6X and 8.8X	Adrenal toxicity	Higher incidence of adrenal hypertrophy at 4.4 mg/kg/day (LOAEL) in the 1-year study than at 38.8 mg/kg/day (LOAEL) in the 13-week study. The NOAEL in the 1-year study is 2.94 mg/kg/day. In a 2-year rat study, the NOAEL is 7.4 mg/kg/day (lowest dose tested) and the LOAEL is 22.8 mg/kg/day based on body weight depression, decreased hemoglobin, hematocrit, MCV, and MCHC, and increased liver enzymes in females; dose-related depressions in female adrenal weights were noted at all dose levels in association with dose-related decreases in adrenal cortical degeneration. The rat chronic study would yield significantly higher values for the NOAEL (>2-fold) and LOAEL (>5-fold) if used to establish a chronic RfD. No other study in the data base has a lower NOAEL/LOAEL than the chronic rat study. Thus, the 1-year dog study is more appropriate for selection of a chronic RfD.

Chemical	Differences in NOAELs and LOAELs (13-week study versus 1-year study)	Primary basis for selection of 1-year study	Comments
Triazamate	No NOAEL in 13-week study/1.3X	Brain and plasma cholinesterase inhibition	No inhibition of the more critical effect (brain cholinesterase inhibition) in the 13-week study. However, use of the 13-week dog study with the application of an additional uncertainty factor (for lack of a NOAEL) would yield a lower point of departure than the chronic dog study (0.01 versus 0.164 mg/kg/day). The NOAEL/LOAEL in a 2-year rat study is 0.45/11.5 mg/kg/day based on plasma cholinesterase inhibition (31-65%) and RBC cholinesterase inhibition (16-29%). Thus, 2-year rat study would not provide as sensitive an endpoint as the 1-year or 13-week dog study for establishing a chronic RfD.

The identification of new effects observed in 1- or 2-year dog studies that were not seen in 13-week dog studies or in chronic studies performed with rats or mice was not undertaken in the current retrospective analysis because this aspect of toxicity studies in dogs was extensively evaluated previously (Spielmann and Gerbracht, 2001). However, consideration of the severity of effects noted at any dose in a rodent or non-rodent (dog) study may also bear on the uncertainty factors that are applied to a NOAEL prior to assignment of a chronic RfD. Thus, although the focus of this retrospective analysis was primarily on comparing NOAELs and LOAELs from the results of 13-week and chronic dog studies, an additional analysis was undertaken to determine whether the characterization of effects noted in a chronic dog study would lead to the application of an additional uncertainty factor during the derivation of a RfD. Among the 77 chronic dog studies evaluated, extra uncertainty factors have been applied to 11 pesticides during the derivation of RfDs. Table 2 (below) provides information on the magnitude of the uncertainty factors applied and comments regarding the bases for application of additional factors and whether, in the absence of a chronic dog study, an additional uncertainty factor would still have been applied when deriving an RfD. As shown in Table 2, in no case was an additional uncertainty factor applied based on effects seen in a chronic dog study that was not seen in other rodent or 13-week dog studies. Thus, it is apparent from the information provided in Table 2 that the presence or absence of the chronic dog study would not affect uncertainty factors. Note that the extra uncertainty factors applied to carbofuran and carbaryl reflect the absence of the establishment of a NOAEL in the chronic dog study, which is a function of dose selection and not identification of additional toxicities of concern.

Table 2. Chemicals with Data Base Uncertainty Factors (UFs) Applied to Derivations of RfDs

Chemical	Uncertainty Factor	Comment
Amitraz	1000	Extra 10X applied for data gap for lack of Developmental Neurotoxicity Toxicity study; concern raised in 13 week dog study for signs of CNS depression observed in 13-week dog study and in in rat and rabbit studies;
Bifenthrin	300	Extra 3X applied for lack of Developmental Neurotoxicity study; neurotoxicity also observed in 13-week dog study (tremors) and in acute, subchronic and reproductive studies performed with rats
Ethylene thiourea	1000	Extra 10X applied for lack of comparative thyroid hormone study in immature animals; severe thyroid hypertrophy and reduced T3 and T4 also observed in 13-week study
Mepiquat chloride	300	Extra 3X applied for lack of Developmental Neurotoxicity study; tonic/clonic convulsions also seen in 13-week study
Carbaryl	300	Extra 3X applied for lack of a NOAEL
Carbofuran	1000	Extra 10X applied for lack of NOAEL and for data gap for reproduction study
Cypermethrin	1000	Extra 10X applied for lack of Developmental Neurotoxicity study; neurotoxicity also observed in acute and subchronic neurotoxicity studies; tremors and irregular gait observed at 11 weeks in 1-year dog study at 33.9 mg/kg/day, which is about 2X the LOAEL
Fenpropathrin	1000	Extra 10X applied for lack Developmental Neurotoxicity study; tremors seen over entire duration of 1-year dog study and signs of neurotoxicity also observed in studies performed with rats, mice, and rabbits
Halosulfuron methyl	300	Extra 3X applied for lack of Developmental Neurotoxicity study; study required because of effects observed in a developmental toxicity study, not in the dog study
Parathion	300	Extra 3X applied for lack of a NOAEL
Pentachloro-phenol	300	Extra 3X applied for lack of a NOAEL

4. Discussion

The purpose of the current retrospective analysis of the results of subchronic and chronic studies conducted with dogs was to ascertain whether data obtained from 13-week dog studies would be adequate, in the absence of a chronic dog study, for identifying NOAELs and LOAELs that would be used to derive chronic reference doses (RfDs) in support of pesticide registrations. Given that RfDs are used for risk assessments on environmental chemicals that are intended to protect human health, this retrospective analysis focused on a comparison of NOAELs and LOAELs that were identified from subchronic and chronic dog studies for each of the 77 pesticides. A second objective of this retrospective analysis was to evaluate whether effects found at any dose in a chronic dog study would impact the uncertainty factors that would be applied to a NOAEL before deriving a chronic RfD.

NOAELs or LOAELs established from 1-year dog studies were found to be lower than NOAELs or LOAELs established from 13-week or 6-month studies for 19 of 77 pesticides for which dog studies were used to derive chronic RfDs by the Health Effects Division, Office of Pesticide Programs. However, a comparison of dose-effect data, evaluations of interim data from 1-year studies, or data from shorter term studies indicates that for only 3 of the 19 pesticides do the data raise the issue that the chronic dog study would lead to a lower RfD than the toxicity data base would lead to without the availability of that study.

This current analysis also evaluated and compared the effects of a 13-week and a 1-year dog study on the characterization of effects at any dose that might lead to the application of additional uncertainty factors that would be incorporated in the derivation of an RfD. In no case was an effect identified in a chronic dog study that would not be seen in a shorter duration dog study or in rodent studies and that would also support the application of additional uncertainty factors. Thus, this retrospective analysis of short- and long-term dog studies indicates that reliance on dog studies with a 13-week duration is not likely to alter EPA's estimates of chronic RfDs.

The conclusions drawn from this analysis of EPA's pesticide data base on dog studies regarding the adequacy of NOAEL's established from 13-week dog studies are consistent with the findings reported by Spielmann and Gerbracht (2001). They compared subchronic and chronic dog studies with 172 pesticides and found no significant differences in the LOAELs between the two study types (correlation $r = 0.78$-0.84). Their initial analyses revealed organ specific effects that were observed in chronic dog studies but not in subchronic dog studies (30 of 50 fungicides, 25 of 44 herbicides, 17 of 38 insecticides, and 10 of 16 other pesticides). Further analyses indicated that in only 7 of 141 studies with dogs there was significant, additional information provided by chronic dog studies that was not provided in 13-week dog studies or in studies with rats or mice. These authors, who conducted an in-depth review of studies on the 7 pesticides, found that differences in subchronic and chronic studies with these pesticides could be accounted for by a number of factors (*e.g.,* differences in study design and interpretation of results, and differences in the extent of histopathological examinations in subchronic and chronic studies). They concluded that

extension of a dog study beyond 13 weeks does not provide additional, essential information.

There have been a number of papers published on the duration of dog studies for pharmaceuticals (Lumley et al., 1992; Contrera *et al* 1993; Parkinson et al., 1995; DeGeorge et al., 1999). These papers focused on retrospective analyses of the results of 6 and 12 month studies. An examination of these papers on pharmaceuticals is supportive of the overall conclusion that long term toxicity studies in the dog rarely provide either qualitatively new toxicological information or quantitative information not already gained from other required studies, i.e. a short term study in the dog in conjunction with short and long term studies in the rat but there are important exceptions. For example, an International Conference on Harmonization workgroup, based on a retrospective analyses of dog studies performed with pharmaceuticals, recommended a 9-month duration for non-rodent (dog) toxicity studies (DeGeorge *et al.,* 1999). This recommendation was based on the finding that in 11 of 17 case studies, data from studies of 9-12 months were needed in order to identify additional effects (e.g., death, morbidity, kidney toxicity, gingival hyperplasia, gastric irritation) not seen at 6-months or the 9- to 12-month studies indicated a shift in the dose-response for toxic effects (whether or not margins of safety would be different using the results 6-month and 1-year dog studies was not specifically addressed but a shift in the dose-response was noted for 2 of the 17 case studies). Also, Contrera *et al. (1993)* reported an exacerbation and/or extension of 6 month observations in 12-month observations for 8 dog studies conducted with 30 pharmaceuticals; 5 of the 30 case studies were judged to provide significant new information that supported extension of dog studies from 6 to 12 months; 1 of the 5 and 1 additional case study showed that extension of the dog study beyond 6-months resulted in a lowering of the margin of safety. Caution should be exercised, however, before comparing results of these analysis of toxicity tests performed in dogs with pharmaceuticals with results of dog studies conducted with pesticides. In the case of pharmaceuticals, it is important to characterize all possible adverse effects that may occur in humans treated with pharmacological doses. Thus, the recommendations to extend dog studies on pharmaceuticals beyond 6-months appears to be based largely on additional effects that were observed at any dose in chronic dog studies but not in 6- or 9-month dog studies; there appear to be few examples in the literature reports where “new findings” affected the margins of safety. In contrast, results of dog studies with pesticides are used primarily to identify dose-levels below which no adverse health effects are likely to occur in humans exposed to environmental levels of a pesticide. Although additional effects observed above a LOAEL may support the application of additional uncertainty factors when RfDs are derived, the current retrospective analysis did not identify a single case where only the results from a dog study would lead to the application of an additional uncertainty factor to a NOAEL. Thus, the results of this current retrospective analysis of studies on pesticides, when considered with the results of the analysis by Spielmann and Gerbracht (2001), show, with few exceptions, that a 13-week dog study is as adequate as a 1-year dog study for identification of a NOAEL.

Acknowledgements

We thank Doug Wolf, National Health and Environmental Effects Research Laboratory, Nancy McCarroll, Office of Pesticde Programs, U.S. Environmental Protection Agency, David Lai, Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency and William Sette, Office of the Science Advisor, Office of Research and Development, U.S. Environmental Protection Agency for their critical reviews of this manuscript.

References

Appleman, L. M., and Feron, V. J. (1986). Significance of the dog as “second animal species” in toxicity testing for establishing the lowest ‘no-toxic-effect level’. J. Appl Toxicol 6(4):271-279.

Contrera, J.F., Aub, D., Barbehenn, E., Belair, E., Chen, C., Evoniuk, G., Mainigi, K., Mielach, F., and Sancilio, L. (1993). A retrospective comparison of the results of 6 and 12 month non-rodent toxicity studies. Adverse Drug React. Toxicol. Rev. 12(1):63-76.

CFR, Code of Federal Regulations 40, Protection of the Environment, Part 158.340, Office of the Federal Register National Archives and Records Administration, July 1, 2003.

DeGeorge, J., Meyers, L., Takahashi, M., and Contrera, J. (1999). The duration of non-rodent toxicity studies for pharmaceuticals. Toxicological Sciences 49:143-155.

Gerbracht, U. and Spielmann, H. (1998). The use of dogs as second species in regulatory testing of pesticides. I. Interspecies comparison. Archiv. Toxicol. 72(6):312-329.

Lumley, C. E., Parkinson, C., and Walker, S. R. (1992). An international appraisal of the minimum duration of chronic toxicity studies. Human and Experimental Toxicology 11:155-162.

Parkinson, C., Lumley, C. E. and Walker, S. R. (1995). The value of information generated by long-term toxicity studies in the dog for the nonclinical safety assessment of pharmaceutical compounds. Fund. Appl. Toxicol. 25 (1):115-123.

Spielmann, H., and Gerbracht, U. (2001). The use of dogs as second species in regulatory testing of pesticides. Part II: Subacute, subchronic and chronic studies in the dog. Arch Toxicol. 75(1):1-21.

Appendix

Table A1. NOAELs & LOAELs (mg/kg/day) in 13-Week and 1- or 2-Year Dog Studies (42 Chemicals)

Chemical	Duration/Route	NOAEL	LOAEL	Effects seen at LOAEL
Azafenidan	1-yr/diet	0.3	0.87	Hepatocellular hypertrophy, multiple nuclei, pigment in liver cell, and increased ALP
	13-wk/diet	0.3	2.0	Hepatocellular hypertrophy, increased ALT & ALP
Amitraz	2-yr/capsule	0.25	1.0	Increased blood sugar concentration; slight hypothermia
	13-wk/capsule	0.25	1.0	CNS depression, hypothermia, increased blood sugar, neutrophilia in bone marrow, and hyperplasia of zona glomerulosa with decrease in zona fasiculata and reticularis
Baythroid (cyfluthrin)	1-yr/diet	2.43	10.64	Clinical signs, abnormal gait, abnormal postural reactions
	13-wk/diet	2.36	13.9	Gait abnormalities, vomiting, decreased body weight gain
Bensulide	1-yr/diet	0.5	4	Decreased body weight gain, brain and plasma cholinesterase inhibition
	13-wk/diet	<1	1	Increased absolute liver weights, plasma cholinesterase inhibition
Bifenazate	1-yr/diet	1.01	8.95	Increased platelets, leucocytes, MCV, reticulocytes, segmented neutrophils; hyperplasia in femur, rib, and sternum; pigment deposits in liver and kidney
	13-wk/diet	0.9	10.4	Decreased hematocrit, hemoglobin, and RBC's; increased MCV, MCH, reticulocytes, and platelets; increased bilirubin; pigment deposits in Kuppfer cells
Bifenthrin	1 yr/capsule	1.3	2.7	Tremors
	13 wk/capsule	2.21	4.42	Tremors
Bispyibac sodium	1-yr/capsule	10	100	Bile duct hyperplasia, hepatocellular granulation
	13-wk/capsule	100	600	Clinical signs, bile duct hyperplasia (2/4 males versus 0/4 controls and 3/4 females versus 2/4 controls)
	28-day/capsule	<100	100	Bile duct hyperplasia observed (2/2 males and 0/2 females) (same dose-level as 1 year study)
Bromoxynil phenol	1-yr/capsule	1.5	7.5	Decreased body weight gain
	13-wk/capsule	1.0	5.0	Decreased body weight gain, liquid feces

Chemical	Duration/Route	NOAEL	LOAEL	Effects seen at LOAEL
Cadusafos	1-yr/capsule	0.001	0.005	Plasma cholinesterase inhibition
	13-wk/capsule	<0.01	0.01	Plasma cholinesterase inhibition
Chlorethoxyfos	1-yr/diet	0.06	0.62	Plasma, brain, and RBC cholinesterase inhibition
	13-wk/diet	0.02	0.19	Plasma and brain cholinesterase inhibition
Clethodim	1-yr/capsule	1.0	75	Increased absolute and relative liver weights, changes in hematology and clinical chemistry (hematology and clinical chemistry changes consistent only at 300 mg/kg/day
	13-wk/capsule	25	75	Increased absolute and relative liver weights(Note: same LOAEL as 1-year)
Cypermethrin	1-yr/diet	6.0	18.1	Gait abnormalities, tremors, death
	13-wk/diet	26.4	34.3	Tremors and decreased body weights
Dicloran	2-yr/diet	2.5	75	Reduced body weight gain, increased liver, kidney, and spleen weights, changes in hematologic and clinical chemistry parameters, histopathology in the liver
	13-wk/diet	2.5	75	Reduced body weight gain, increased liver, kidney and spleen weights, changes in hematologic and clinical chemistry parameters, histopathology in the liver
Diflubenzuron	1-yr/capsule	2.0	10.0	Increased methemoglogin and sulfhemoglobin
	13-wk/dietary	2.0	6.24	Increased methemoglobin
Diflufenzopyr	1-yr/diet	26	299	Bone marrow hyperplasia, hemosiderin deposits in liver, kidney, and spleen, reticulocytosis
	13-wk/diet	58	403	Erythroid hyperplasia in bone, extramedullary hematopoiesis in liver
Ethylene thiourea	1-yr/diet	0.18	1.99	Thyroid follicular dilation and hypertrophy
	13-wk/diet	6.02	66.23	Mortality, thyroid hyperplasia, increased thyroid weights, and reduced T3 and T4
Etoxazole	1-year/diet	4.62	23.5	Increased ALP and liver weights; centrolobular hepatocellular swelling
	13-wk/diet	5.33	53.7	Vomiting and mucous stools; centrolobular hepatocellur swelling; acinar cell atrophy in the prostate; increased ALP, triglycerides, albumin and globulin
Fenhexamid	1-yr/diet	17	124	Decreased RBC's and hematocrit, increased Heinz bodies, increased adrenal weights
	13-wk/diet	34	239	Increased Heinz bodies, decreased RBC's and hematocrit

Chemical	Duration/Route	NOAEL	LOAEL	Effects seen at LOAEL
Flucarbazone	1-yr/diet	35.9	183	Decreased body weight gain, increased N-demethylase, decreased T4 hormone
	13-wk/diet	33.8	162	Decreased T4, increased thyroxine binding, microsomal enzyme induction, histopathology in liver and stomach
Fosetyl Al	2-yr/diet	250 (10,000 ppm)	500 (20,000 ppm)	Degeneration of testes
	13-wk/diet	274 (10,000 ppm)	1309 (50,000 ppm)	Decreased serum potassium
Hexaconazole	1-yr/capsule	2.0	10.0	Fatty liver and increased liver weights
	13-wk/capsule	5.0	25.0	Decreased cholesterol and triglycerides, fatty liver (differences in NOAELs between 1-year and 13-week studies may be accounted for by differences in dose selection; at 90 days in 1-year study liver toxicity indicated by increased ALP occurred at 10 mg/kg)
Hexazinone	1-yr/diet	5.0	37.6	Increased ALP, (increased AST decreased albumin, increased globulin only seen at highest dose, 167 mg/kg/day)
	13-wk/diet	26	123	Decreased body weight gains and increased ALP
Iodosulfuran	1-yr/diet	7.25	43.7	Gross and histopatholgy in hemopoietic system
	13-wk/diet	8.1	49	Hemopoietic effects in bone and spleen, clinical chemistry and hemotologic changes
Isoxadifen ethyl	1-yr/diet	3.3	24.0	Increased creatinine, decreased specific gravity, increased partial thromboplastin time, renal tubular vacuolation
	13-wk/diet	1.2	6.1	Fat deposits in collecting ducts of kidney, changes in hematologic parameters, aspermia, decreased body weight gain
Mepiquat chloride	1-yr/diet	58.4	95.3	1 year-sedation, abdominal and lateral positioning, tonic/clonic convulsions at 170 mg/kg/day (13-week study used to establish LOAEL)
	13-wk/diet	32.4	95.3	Body weight loss, abdominal and lateral positioning, sedation, tonic/clonic convulsions hematologic effects
Metolochlor	1-yr/diet	9.7	32.7	Decreased body weight gains
	13-wk/diet	9.7	29.6	Decreased body weight gains
Paraquat	1 yr/diet	0.45	0.93	Chronic pneumonitis
	13wk/diet	0.5	1.5	Increased lung weights, alveolitis, alveolar collapse

Chemical	Duration/Route	NOAEL	LOAEL	Effects seen at LOAEL
Phorate	1-yr/capsule	0.05	0.25	RBC and brain cholinesterase inhibition
	105 day/capsule	0.05	0.25	RBC cholinesterase inhibition
Phostebupirim	1-yr/diet	0.02	0.13	RBC, plasma, and brain cholinesterase inhibition
	13-wk/diet	0.018	0.15	RBC, plasma, and brain cholinesterase inhibition
Prallethrin	1-yr/capsule	5.0	10	Increased cholesterol, phopholipid, and ALP; subendocardial red discoloration in the left ventricle of the heart
	13-wk/capsule	3.0	10	Tremors, mortality, decreased albumin/globulin ratio, increased cholesterol and phospholipids
Primisulfuron methyl	1-year/diet	25	250/125 High dose reduced at week 10	Decreased erythrocytes, hemoglobin, and hematocrit; increased platelets (all before week 10); pale livers; hepatocellular vacuolation; thyroid hypeplasia; high dose reduced at week 10 because of decreases in body weights
	13-wk/diet	25	250	Decreased body weights, food consumption, and food efficiency; decreased erythrocytes, hemoglobin, and hematocrit; increased platelets and prothrombin time; decreased thyroid and parathyroid weights; parafollicular cell hyperplasia
Prosulfuron	1-yr/diet	1.84	20.2	Decreased albumin, decreased RBCs,, lipofucsin accumulation in liver
	13-wk/diet	5.3	57	Decreased hemoglobin, hematocrit, RBC's, and albumin; increased liver weights; hemosiderin deposits in stomach, spleen, and lymph nodes
Rimsulfuron	1-yr/diet	81.8	342.4	Increased absolute liver and kidney weights
	13-wk/diet	9.63	193	Increased absolute liver and kidney weights
Spinosad	1-yr/diet	2.68	8.22	Increased aspartate amino transferase, alanine amino transferase, and triglycerides; arteritis and granular cell vacuolation (parathyroid)
	13-wk/diet	4.89	9.73	Vacuolated cells in spleen, lymph follicles of multiple tissues, acinar cells of pancreas, and atrophy of gastric mucosa of stomach.
Sulfosate	1-yr/gavage	10	50	Salivation, emesis, and hydrocephalus
	13-wk/gavage	10	50	Salivation, emesis, hydrocephalus
Tebuconazole	1-yr/diet	2.94	4.39	Hypertrophy of adrenal zona fasciculata cells
	13-wk/diet	73.4	351.8	Decreased body weights and body weight gains, increased N-demethylase activity

Chemical	Duration/Route	NOAEL	LOAEL	Effects seen at LOAEL
Tebufenozide	1-yr/diet	1.8	8.7	Decreased body weight gain (males) bone marrow hyperplasia, spleen hematopoiesis and sinusoidal engorgement, increases in absolute liver weights, increased pigment in Kupffer cells, decreased RBC's, hemoglobin, hematocrit, increased Heinz bodies, methemoglobin, MCV
	13-wk/diet	2.1	20.1	Decreased hemoglobin, RBC, hematocrit and elevated MCV; increased Heinz bodies, increased liver weights, increased pigment in Kupffer cells, hematopoiesis in spleen and increased spleen weights
Thiophanate methyl	1-yr/capsule	8	40	Decreased T4 levels in males at 6 months; increased TSH; increased thyroid weights in males; follicular cell hypertrophy
	13-wk/capsule	<50	50	Follicular cell hypertrophy; in females, decreased T3 and T4; increased TSH
Tralkoxydim	1-yr/capsule	0.5	5.0	Increased liver weights, increased ALT, periportal fatty changes
	13-wk/capsule	0.5	5.0	Increased liver weights, decreased albumin, triglycerides, and total protein, increased N-demethylase activity
Triazamate	1-yr/diet	0.0164	0.0236	Brain cholinesterase inhibition
	13-wk/diet	<0.03	0.03	Plasma cholinesterase inhibition (brain and RBC cholinesterase not affected)
Triadimenol	2-yr/diet	3.75	15	Increased ALP and N-demethylase activity
	13-wk/diet	<3.75	3.75	Decreased body weight gain, increased ALP
Zoxamide	1-yr/diet	48	255	Decreased body weight gain, increased liver and thyroid weights, increased ALP; (at 6 months in 1-year - increased ALP at 255; ALP not measured at earlier time points)
	13-wk/diet	55	322	Increased liver weights (effects on liver weight in the 13-wk. study at the same dietary level used in the 1-year study - 7500 ppm)

Table A2. NOAELs & LOAELs from 1- or 2- yr dog studies with interim data (1 day to 3 months) (35 chemicals)

Chemicals	Duration	NOAEL mg/kg/day	LOAEL mg/kg/day	Effects seen at LOAEL
Acetochlor	1 yr	2.0	10	Increased ALT (70%), GGT (at 13 weeks only), OCT (122%), and triglycerides (only at 12 weeks); decreased glucose (9%)
	12 weeks	2.0	10	Increased ALT (32%), GGT (33%), OCT (48%) and triglycerides (34%); decreased glucose (11%)
Azinphos methyl	1 yr	0.15	0.69	Diarrhea, RBC cholinesterase inhibition (RBC cholinesterase inhibition observed at 4 weeks used for RfD)
	4 weeks	0.15	0.69	RBC cholinesterase inhibition
Bentazon	1 yr	3.1	13.1	Anticoagulation effects (at week 13) and blood in feces (1/6 males at week 4)
	4-13 weeks	3.1	13.1	Anticoagulation effects and blood in feces
Carbaryl	1 yr	<3.1	3.1	Plasma (23% in females) and brain cholinesterase inhibition
	5 weeks	1.43	3.83	Plasma cholinesterase inhibition (22% in females)(5 week study used to identify NOAEL)
Carbofuran	1 yr	<0.25	0.25	Plasma cholinesterase inhibition (12% in males)
	3 days	<0.25	0.25	Plasma cholinesterase inhibition (18% in males)
Chlorpropham	1 yr	5	50	Increased thyroid weight (95%), enlarged irregular follicles with clear staining follicles (3 males and 4 females); no T4 decrease at 1-year at 50 mg/kg/day but T4 decreased 43% at 350 mg/kg/day)
	14 weeks	5	50	Decreased T4 (33%)
	28 day	5	50	Histopathology in thyroid (1/1 of each sex)
Clofentezine	1 yr	1.25	25	Increased organ weights, histopathology in liver, increased cholesterol (33%)and triglycerides (44%)
	4 weeks	1.25	25	Begining at 4 weeks in 1 year-increased cholesterol (34%) and triglycerides (27%)
Coumaphos	1 yr	0.025	0.775	RBC (47%), plasma (76%), brain cholinesterase inhibition
	90 days	0.025	0.775	RBC (42%) and plasma (63%) cholinesterase inhibition
Cyhalothrin	1 yr	0.1	0.5	Muscle tremors & convulsions (2/6 males at week 52)
	2 weeks	0.1	0.5	Muscle tremors/ataxia (1/6 males and 1/6 females beginning at 2 days)
Dichlorvos	1 yr	0.05	0.10	RBC (53%), plasma (53%), and brain cholinesterase inhibition
	12 days	0.05	0.10	RBC (54%) and plasma (48%)cholinesterase inhibition

Chemicals	Duration	NOAEL mg/kg/day	LOAEL mg/kg/day	Effects seen at LOAEL
Dicofol	1 yr	0.12	0.82	Inhibition of ACTH stimulated release of cortisol (21%)
	13-weeks	0.12	0.82	Inhibition of ACTH stimulated release of cortisol (30%)
Disulfoton	1 yr	0.013	0.094	RBC (38%), brain, corneal, and retinal cholinesterase inhibition
	13 weeks	0.013	0.094	RBC (49%) and plasma cholinesterase inhibition
Diquat dibromide	1 yr	0.5	2.5	Cataracts , lens opacity (2/4 females); decreased adrenal and epididymis weights
	8 weeks	0.5	2.5	Lens opacity (1/4 females)
Ethion	1 yr	0.05	0.52	Plasma cholinesterase inhibition (56%)
	3-weeks	0.05	0.52	Plasma cholinesterase inhibition (53%)
Ethoprop	1-yr	0.01	0.025	Plasma cholinesterase inhibition (males, 6%; females, 32%) (NOAEL based on 5 month study); at 6 weeks in 1-year study, plasma cholinesterase inhibition (males,15%; females, 28%)
	6-weeks	0.01	0.025	Plasma cholinesterase inhibition (males,15%; females, 28%); also, plasma cholinesterase inhibition at week 12 in a 5-month study (males31%; females, 29%); no plasma cholinesterase inhibition at 0.01)
Fenamiphos	1 yr	0.01	0.03	Plasma cholinesterase inhibition (males,26%; females,26%)(NOAEL established from 6-month study)
	3 months	0.01	0.03	Plasma cholinesterase inhibition (males, 25%; females, 21%)
Fenitrothion	1-yr	0.125	0.25	Plasma cholinesterase inhibition (males, 15%; females, 26%)
	4-weeks	0.125	0.25	Plasma cholinesterase inhibition (males, 14%; females, 22%)
Fenpropathrin	1 yr	2.5	6.25	Tremors (4/4 of each sex)
	Entire time	2.5	6.25	Tremors (incidences not provided)
Fludioxonil	1-yr	3	35	Decreased body weight gain (43%)
	3-months	3	35	Decreased body weight gain (31%)
Halosulfuron methyl	1yr	10	40	Decreased body weight gain (16%)
	0 to 13 weeks	10	40	Decreased body weight gain (7%)
Imazalil	1-yr	2.5	20	Increased liver weights, increased ALP (80%), decreased body weight gains (60% during weeks 0-12); vomiting (occasional in 4/4 of each sex during week 46 and later
	0-12 weeks	2.5	20	Decreased body weight gains, increased ALP (111%); vomiting (4/4 males up to day 29)

Chemicals	Duration	NOAEL mg/kg/day	LOAEL mg/kg/day	Effects seen at LOAEL
Iprovalicarb	1-yr	2.6	24.7	Hepatocellular hypertrophy (4/4 each sex, minimal), increased absolute liver weights (24%), increased ALP (155%)
	28 day study	3.0	31.5	Hepatocellular hypertrophy (2/2 of each sex), vacuolated hepatocytes (2/2 of each sex), increased ALP (88%)
Linuron	1-yr	0.77	3.5	Increased methemoglobin (no statistically significant increases at 1 year)- and sulfhemoglobin (0.7% versus 0.3% in contols)
	3 months	0.77	3.5	Increased methemoglobin (1.1% versus 0.1% in controls)- and sulfhemoglobin (0.6% versus 0.3% in controls)
Methidithion	1 yr	0.15	1.33	Increased ALP (132%) and SGTP (641%); RBC (76%) cholinesterase inhibition, chronic hepatitis, distended bile canaliculi, elevated hepatic enzymes
	3 months	0.15	1.33	Increased ALP (181%) and SGTP (664%); (83%) cholinesterase inhibition
Nitrapyrin	1-year	3.0	15.0	Increased ALP (137%), cholesterol (50%), and absolute liver weights; liver hypertrophy
	3 months	3.0	15.0	Increased ALP (30%) and cholesterol (38%)
Oxydemeton methyl	1 yr	0.0125	0.125	Brain cholinesterase inhibition (males, 15%; females 12%); RBC cholinesterase inhibition (males, 11%; females, 15%); plasma cholinesterase inhibition (males, 19%; females, 15%)
	3 months	0.0125	0.125	Plasma cholinesterase inhibition (16% in males and 10% in females)
Parathion	1 yr	<0.01	0.01	RBC (22%), plasma (27%), and brain cholinesterase inhibition
	2 months	<0.01	0.01	RBC (26%) and plasma (25%) cholinesterase inhibition
Pentachlorophenol	1 yr	<1.5	1.5	Increased liver weights, increased stomach lymphocytic mucosal inflammation, increased ALP (85%)
	13 weeks	<1.5	1.5	Increased ALP (49%)
Prohexadion	1 yr	80	200	Renal pathology, increased urinary volume (34%), sodium (69% at week 39), choloride (at week 12), and potassium (at week 13)
	13 weeks	80	200	Increased urinary volume (90%), chloride 62%), sodium (94%), and potassium (52%)
Pyridiben	1 yr	0.5	1.0	Decreased body weight gains (29%)
	13 weeks	0.5	1.0	Decreased body weight gains ((27%)
Sulprofos	2 yr	0.25	2.5	RBC (53%), plasma (47%), and brain cholinesterase inhibition
	1 month	0.25	2.5	RBC (68%) and plasma (54%) cholinesterase inhibition

Chemicals	Duration	NOAEL mg/kg/day	LOAEL mg/kg/day	Effects seen at LOAEL
TCP	1-yr	12	48	Increases in liver enzymes ALP (127%) and ALT (94%)
	3 months	12	48	Increases in liver enzymes ALP (55%) and ALT (85%)
Terbufos	1 yr	0.005	0.015	Plasma cholinesterase inhibition (28-day study used for RfD)
	28 day study	0.005	0.015	Plasma cholinesterase inhibition (basis of RfD)
Triadimefon	2-yr	11.4	33.7	Increased ALP (25%); decreased GPT (30%)
	13 weeks	11.4	33.7	Increased ALP (17%) and decreased GPT (19%)
Tribufos	1 yr	0.1	0.4	Plasma cholinesterase inhibition (33%)
	13-weeks	0.1	0.4	Plasma cholinesterase inhibition (28%)

Table A3. Pesticides with Lower NOAELs or LOAELs (≥ 1.5) in 1-year than in 13-Week Dog Studies (19 Chemicals)

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Azafenidan 1-year diet		Liver histopathology and changes in enzyme levels (ALT and ALP) somewhat more pronounced in 1 year versus 13 week study at LOAEL of 0.87 in 1-year study and LOAEL of 2.02 in 13-week study. However, interim data at 12 weeks in 1-year study indicate that same NOAEL and LOAEL would be established at 12-weeks and 1-year based on changes in enzyme parameters. If performed at 12-weeks in the 1-year study, likely hepatocellular hypertrophy would have been observed based on the changes in enzyme levels. Thus, differences in LOAELs between 1-year and 13-week studies may be attributed to inter-experimental variability or dose selection
0	Hepatocellular hypertrophy, pigment in liver cells, or multiple nuclei in liver not observed in any dogs	
0.16	Hepatocellular hypertrophy (0/5); pigment in liver cells (males, 2/5; females, 1/5); multiple nuclei in liver (0/5 of each sex)	
0.30 (NOAEL)	Hepatocellular hypertrophy (0/5 of each sex); pigment in liver cells (4/5 of each sex); multiple nuclei in liver (0/5 of each sex)	
0.87 (LOAEL)	Hepatocellular hypertrophy (3/5 of each sex); pigment in liver cells (5/5 of each sex); multiple nuclei in liver (males, 5/5; females, 3/5); increased ALT (males, 175%; females, 209%); increased ALP (50% in males, no increase in females)	
3.53	Hepatocellular hypertrophy (5/5 of each sex); pigment in liver cells (5/5 of each sex); multiple nuclei in liver (5/5 of each sex); increased ALT (males, 320%; females 379%); increased ALP (males, 233%; females, 84%)	
11.01	Hepatocelluar hypertrophy (5/5 of each sex); pigment in liver cells (5/5 of each sex); multiple nuclei in liver (5/5); increased ALT(518%); increased ALP (302% and 105% in males and females)	
12 weeks in 1-year		
0.30 (NOAEL) **0.87 (LOAEL)**	Increased ALT (males, 230%; females, 187%); increased ALP in males (34%; no increase in females))	
3.53	Increased ALT (males, 557%; females, 338%); increased ALP (males, 141%; females67%)	
11.01	Increased ALT (males, 722%; females 587%); increased ALP (males, 221% females 137%)	
13-week/diet		
0	Hepatocellular hypertrophy, pigment in male liver cells or multiple nuclei in liver cells not observed	
0.34 (NOAEL)	Hepatocellular hypertrophy (0/4 males and 0/4 females); pigment in male liver cells (1/4); increased ALT (31% in females, not statistically significant but consistent with dose response)	
2.02 (LOAEL)	Hepatocellular hypertrophy (1/4 male and 2/4 females); pigment in liver cells (4/4 in each sex); increased ALT (males, 150%; females, 344%)	
4.0	Hepatocellular hypertrophy (3/4 males and 1/4 females); pigment in male liver cells (4/4 in each sex); multiple nuclei in liver (2/4 and 0/4 in males and females); increased ALT (males, 440%; females, 278%); increased ALP in females (43%)	
8.64	Hepatocellular hypertrophy (2/4 males and 4/4 females); pigment in liver cells (4/4 in each sex); multiple nuclei in liver cells(2/4 and 4/4 in males and females); increased ALT (460% and 678% in males and females); increased ALP in females (222%)	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Bifenthrin 1-year/capsule 0 0.66 **1.3 (NOAEL)** **2..7 (LOAEL)** 4.4 13-week/capsule 0 **2.21 (NOAEL)** **4.42 (LOAEL)** 8.84 17.7	 Tremors in 1/4 males and 2/4 females beginning at 16 weeks Tremors in all animals of both sexes No tremors in any dogs Tremors in 1/4 male dogs (investigator claims due to handling and not treatment related; seen occasionally in untreated dogs in other studies; reviewer agreed not treatment related) Tremors in 3/4 males and 3/4 females Tremors in all animals of both sexes Tremors in all animals of both sexes	Increased incidence of tremors (only critical effect) observed at the LOAEL in the 13-week study compared to the incidence of tremors observed in the 1-year study indicates that the actual NOAELs and LOAELs in the two studies would not be significantly different. Differences between NOAELs and LOAELs for the two studies may be attributed to differences in dose selection

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Bispyibac sodium 1 year/capsule 0 **10 (NOAEL)** **100 (LOAEL)** 750 13 week/capsule 0 30 **100 (NOAEL)** **600 (LOAEL)** 28 day/ capsule 0 **100 (LOAEL)** 300 600	 Hyperplasia of the intrahepatic bile ducts (0/4 in both sexes) Hyperplasia of the intrahepatic bile ducts (0/4 in both sexes) Hyperplasia of the intrahepatic bile ducts(1/4 males and 1/4 females) Hyperplasia of the intrahepatic bile ducts(4/4 males and 3/4 females) Proliferation of the intrahepatic bile ducts (0/4 males and 2/4 females) Proliferation of the intrahepatic bile ducts (0/4 males and 1/4 females) Proliferation of the intrahepatic bile ducts (0/4 males and 1/4 females) Proliferation of the intrahepatic bile ducts (2/4 males and 3/4 females) Bile duct hyperplasia (2/2 males) Bile duct hyperplasia (2/2 males and 2/2 females) Bile duct hyperplasia (2/2 males and 2/2 females)	Bile duct hyperplasia (2/2 males) observed in 28-day/capsule study at same dose-level (100 mg/kg/day) as 1-year study (incidence, 1/4 males and 1/4 females); no other significant observations at the LOAEL in the 1-year, 13-week study, or 28-day study; given the background incidence (e.g., 2/4 females in control females in the 13-week study) and the results of the 28-day study, the difference in LOAELs for the 13 week and 1-year study may be a result of inter-experimental variability.

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Cadusafos		Plasma cholinesterase inhibition comparable at 13-weeks and at termination of 1-year study. NOAEL in 13-week study would likely equal or approach NOAEL in 1 year study if dose selections were comparable
1-year/capsule		
0		
0.0002		
0.001(NOAEL)	Plasma cholinesterase inhibition (8% in females)	
0.005 (LOAEL)	Plasma cholinesterase inhibition (23% in females and 13% in males)	
0.020	Plasma cholinesterase inhibition (26% in females and 39% in males)	
13 weeks in 1-year		
0.0002		
0.001	No plasma cholinesterase inhibition	
0.005	Plasma cholinesterase inhibition (22% in females and 5% in males)	
0.020	Plasma cholinesterase inhibition (23% in females and 25% in males)	
13-week study		
0		
0.01 (LOAEL)	Plasma cholinesterase inhibition (22% in males and 22% in females)	
0.03	Plasma cholinesterase inhibition (29% in males and 24% in females)	
0.09	Plasma cholinesterase inhibition (56% in males and 54% in females)	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Clethodium		Same LOAEL and same percentage increase in liver weights at the LOAEL in 13-week study and in 1- year study; differences in NOAEL's can be accounted for by dose selection.
1 year/capsule		
0		
1.0 (NOAEL)		
75.0 (LOAEL)	Increased absolute liver weights (16%)	
300.0	Increased absolute liver weights (60%); hematology and clinical chemistry changes consistent only at 300 mg/kg/day	
13-week/capsule		
0		
1.0		
25.0 (NOAEL)		
75.0 (LOAEL)	Increased absolute liver weights (16%)	
125.0	Increased absolute liver weights (34%)	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Cypermethrin 1-year/diet 0		Gait abnormalities, salivation, death; not observed at 3 months at 18.1 in 1-year study; however, effects at 33.9 and 37.0 in 1-year study and 13-week study comparable: tremors and irreglar gait observed in 2/4 males at 11 or 16 weeks at 33.9 mg/kg/day in 1 year study; 2/4 females and 1 male exhibited tremors during course of 13-week study; low incidence of effects at LOAEL in 1-year study and observation of effects in 1 male at NOAEL in 13-week study, although considered transitory, suggests sample size (sensitivity) of the study may account for selection of different NOAELs and LOAELs; however, use of 1-year for selection of a NOAEL can not be discounted.
2.9		
6.0 (NOAEL)	No effects	
18.1 (LOAEL)	Tremors and irregular gate in 1 male observed on 2 of 133 days before death beginning on week 19; tremors and irregular gate in 2nd dog on 3 occasions (test weeks 39 and 44) during study (369 days); no tremors or irregular gate observed in females	
33.9	Tremors observed in males on up to 54 days of 368 days beginning at week 11; irregular gait observed in males on up to 94 days of 368 days beginning at week 11; tremors in females (2 dogs) observed on up to 132 days beginning at week 33; irregular gait observed in females (2 dogs) on up to 148 days beginning at week 33; body weights decreased from 18 to 24% from week 36 to week 52	
13-week/diet 0		
10.4	No effects	
20.7	No effects	
24.6 (NOAEL)	1 male exhibited tremors on days 66 and 67 (considered transitory and not related to treatment)	
37.0 LOAEL	Two females and 1 male exhibited tremors beginning on day 49; one male and 1 female exhibited tremor activity on two occasions (male) or during weeks 8, 9, or 12 during detailed physical of the female; decreases in body weights in males and females (10% and 18%, respectively)	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Diflufenzopyr		
1 year/diet		No interim pathology in 1-year study.
0	Incidences for hyperplasia, reticulocytosis, and hemosiderin deposits not stated	
26 (NOAEL)	Incidences for hyperplasia, reticulocytosis, and hemosiderin deposits not stated	Increased incidences of effects at the LOAEL in 1 year study compared to incidences in 13 wk study indicate 1 year study more definitive.
299 (LOAEL)	Bone marrow hyperplasia (all animals); reticulocytosis (4/4 males and 2/4 females); hemosiderin deposits in the kidney (1/4 males)	
529	Bone marrow hyperplasia (all animals); reticulocytosis (all animals, statistically significant at 13 weeks); hemosiderin deposits in the kidney (2/4 females) and in the liver (2/4 males and 3/4 females)	
13 week/diet		
0	No effects reported	
58 (NOAEL)	No effects reported	
403 (LOAEL)	Bone marrow hyperplasia (2/4 males and 1/4 females), hemosiderin deposits in the liver (1/4 females), extramedullary hemopoiesis in the liver (2/4 males and 1/4 females)	
1131	Bone marrow hyperplasia (all animals); extramedullary hemopoiesis in the liver (all animals); reticulolcytosis (2/4 males and 1/4 females)	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Ethylene thiourea 1-year/diet 0		No apparent reduction in thyroid hormones was observed at the LOAEL (1.99 mg/kg/day) in the 1-year study, whereas, although not statistically significant, T4 was reduced by 8% to 10% at 0.39 mg/kg/day and by 18-19% at weeks 4 and 8 at 6.02 mg/kg/day in the 13-week study; the dose-response effects on T3 across dose-levels suggest the effect at 1.99 may be biologically significant and treatment related. Thyroid weight were increased up to 170% at 20 mg/kg/day in the 1-year study and by 1500% at 66 mg/kg/day in the 13 week study. Based on (1) the similarities in hormone measurements at 20.13 mg/kg/day at 1-year and at 13-weeks during the 1-year study, and (2) a comparison of the effects on hormone levels at 66.23 and 6.02 mg/kg/day in the 13-week study versus effects on hormone levels at 20.1 mg/kg/day in the 1-year study, it appears differences between the 2 studies may be a consequence of dose selection or inter-experimental variability
0.18 (NOAEL)		
1.99 (LOAEL)	No apparent reductions in T3 or T4; follicular dilation and hypertrophy (males 1/4 and females 1/4);	
20.13	T3 reduced (males 9%; females 24%) T4 reduced (males 27%; females no reduction); increased thyroid weights (males, 170%; females72%); follicular dilation and hypertrophy (males 2/2 and females 2/3);	
13-weeks in 1-year 0.18	No apparent reductions in T3 or T4	
1.99	T3 reduced (males 21%; females 10%); T4 reduced (males 46%; females 10%)	
20.13	(Note: details on thyroid histopathology observed at all doses not provided in the Data Evaluation Record)	
13-week/diet 0		
0.39	although not statistically significant, T4 reduced 8% and 10% at weeks 4 and 8 in males, respectively; see additional doses for evidence of dose-response increases in effects	
6.02 (NOAEL)	No increase in thyroid weights; although not statistically significant, T4 reduced 19% and 18% at weeks 4 and 8 in males, respectively	
66.23 (LOAEL)	mortality (2/4); statistically significant thyroid changes: reduced T3 (males, 71% at week 8; females, 66% at week 8); reduced T4 (males, 91% at week 8; females, 90% at week 8); increased thyroid weights (male, 1200%; females, 1400%); diffuse hyperplasia of the thyroid in all dogs	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Etoxazole		Comparable severity or incidence of effects at LOAEL of 23.5 in 1-year study and LOAEL of 53.7 in 13-week study. Increases in ALP at 13-weeks at 23.5 mg/kg/day less than at 1-year (males) but more in females. Increases in ALP greater in both males and females at highest dose tested at 1-year compared to 13-week interim data. Overall, data indicate actual LOAEL would be better defined using 1-year study.
1-year/diet		
0		
4.62 (NOAEL)	Increased liver weights (16% in males)	
23.5 (LOAEL)	Increased ALP (males 262%; females 75%); increased liver weights (males 29%; females 34%); centrolobular hepatocellular swelling (4/4 each sex)	
116.0	Increased ALP (males 586%; females 955%); increased liver weights (males 75%; females 76%); centrolobular hepatocellular swelling (4/4 each sex)	
13 weeks in 1-year		
4.62 (NOAEL)		
23.5 (LOAEL)	Increased ALP (males 155%; females 107%)	
116.0	Increased ALP (males 371%; females 599%)	
13-week/diet		
0		
5.33 (NOAEL)	No increase in liver weights	
53.7 (LOAEL)	Increased ALP (males, 196%; females, 107 %); increased liver weights (21-23%); centrolobular hepatocellular swelling (4/4, each sex)	
268	Increased ALP (males, 196%; females, 855); increased liver weights (33-51%); hepatocellular swelling (4/4 each sex)	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Fenhexamid 1 year/diet 0		At 13 weeks in 1-year study, magnitude of effects of decreased RBC's and hematocrit, and increased Heinz bodies comparable magnitude of changes in same parameters at 124 mg/kg/day at termination of 1-year study; difference in NOAELs between 1-year and 13-week study can be associated with inter-experimental variability
17.0 (NOAEL)	Increased adrenal weights in females (8%)	
124.0 (LOAEL)	Decreased RBC's (9% and 17% in males and females) and hematocrit (8% and 15% in males and females); increased Heinz bodies (4 versus 0 control males and 30 versus 0 control females); increased adrenal weights in females (15%)	
918.0	Decreased RBC's (males, 22%; females, 18%) and hematocrit (males, 18%; females,15%); increased Heinz bodies (36 versus 0 control males and 46 versus 0 control females); increased adrenal weights in females (25%)	
13 weeks in 1 year		
17 (NOAEL)		
124 (LOAEL)	Decreased RBC's (males, 9%; females, 16%) and hematocrit (males,6%; females, 14%), increased Heinz bodies (3.0 versus 0 control males and 8 versus 0 control females)	
918.0	Decreased RBC's (males,19%; females, 21%) and hematocrit (males, 16%; females, 18%); increased Heinz bodies (45 versus 0 control males and 37 versus control females)	
13 week/diet 0		
34 (NOAEL)		
239 (LOAEL)	Decreased RBC's (males, 10%; females, 16%) and hematocrit (males, 9%; females, 14%); increased Heinz bodies (18 versus 0 control males and 18 versus 0 control females); adrenal weights apparently not affected	
1748	Decreased RBC's (males, 15%; females, 14%) and hematocrit (males, 14%; females, 10%); increased Heinz bodies (50 versus 0 control males and 94 versus 0 control females); adrenal weights apparently not affected	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Fosetyl Al		1981 and 1982 studies with limited details on clinical chemistry, hematology, or histopathology observations. Data Evaluation Record states testicular effects were slight. Insufficient detail to make meaningful comparisons
1-year/diet		
0		
250 (NOAEL)		
500 (LOAEL)	spermatocytic and/or spermatidic giant cells (2/6, graded as focal/trace)	
1000	spermatocytic and/or spermatidic giant cells (6/6, graded as numerous focal to multifocal/trace)	
13-week/diet		
0		
55		
274 (NOAEL)		
1309 (LOAEL)	Decreased serum potassium (13% in males, and 18% in females; potassium also decreased at weeks 4 and 8)	
Hexaconazole		1-year study classified as supplementary: deficient in that the colony of dogs used was of questionable health (26 of the 32 dogs had some degree of pnuemonia); missing clinical chemistry and hematology data make data interpretation difficult; did not satisfy guideline requirements. Thus, some doubt meaningful comparison of 2 studies are possible. However, severity of effects noted at LOAEL in 3-month study suggest that effects would be seen at or approaching 10 mg/kg/day, the same LOAEL as in the 1-year study.
1 year/ capsule		
0		
2.0 (NOAEL)		
10.0 (LOAEL)	Fatty liver (3/4 males); increased liver weights (16%)	
50.0	Fatty liver (4/4 males and 4/4 females); increased liver weights (70%)	
13 week/ capsule		
0		
5.0 (NOAEL)		
25.0 (LOAEL)	Decreased cholesterol (males, 38%; females, 29%) and triglycerides (males, 14%; females, 34%); increased AAT (males, 251%; females, 163%), fatty liver (number of dogs affected not given)	
75 reduced to 50.0 at 10 days	Decreased cholesterol (males, 68%; females, 54%) and triglycerides (males, 49%; females, 51%); increased AAT (males, 285%; females, 229%) liver weights increased (males, 34%; females, 48%)	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Hexazinone		Effects on ALP at 37.6 mg/kg/day in the 1-year study greater (males) or comparable (females) to the effects on ALP at 122.5 mg/kg/day in the 13 week study. 1 year study provides data that would lead to selection of a lower NOAEL for establishment of a chronic RfD
1-year/diet		
0		
5.0 (NOAEL)		
37.6 (LOAEL)	Increased ALP (males,3091%; females, 94%)	
167.0	Increased ALP (males, 1271%; females 459%); increased AST (males,40%; females, 41%); increased ALT (males, 176%; females, 159%); single cell necrosis (liver) (3/5 males and 3/5 females); decreased body weights (males, 22%, females, 33%)	
at 13 weeks		
37.6	Increased ALP (males, 96%; females, 23%)	
167.0	Increased ALP (males, 246%; females, 207%); no increases in AST; increased ALT (males, 118%); body weights decreased (males, 24%, females, 28%)	
13-week/diet		
0	Note: because control animals weigh less than animals in all treatment groups, body weight gains used for comparisons	
5.1	Increased liver weights (males, 10%)	
25.9 (NOAEL)	Increased liver weights (males, 21%)	
122.5 (LOAEL)	Increased ALP (males, 214%; females, 124%); no apparent increases in AST or ALT; increased liver weights (males, 26%, females, 33%); proteinuria (1/4 of each sex); body weight gains decreased (0.7 controls versus minus 0.9 kg in males and minus 0.3 kg in females)	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Prosulfuron		Comparable effects detected at 3 months in 1-year study and at the end of the 1-year study at the same LOAEL; effects at 48.8 mg/kg/day (1-year study) and 54.0 mg/kg/day (13-week study) comparable; thus, differences in NOAELs between 1-year and 13-week studies may be accounted for by differences in dose selection.
1 year/diet		
0	No lipofucsin accumulation in the liver	
0.31	No lipofucsin accumulation in the liver	
1.84 (NOAEL)	No lipofucsin accumulation in the liver	
20.2 (LOAEL)	Decreased albumin (males, 6%; females, 12%); decreased RBC's (males, 0%; females, 11%); lipofucsin accumulation in the liver (3/4 males and 1/4) females; increased liver weights (females 27%, not statistically significant)	
48.8	Decreased albumin (males, 16%; females,15%); decreased RBC's (males, 10%; females, 12%); hemoglobin decreased 16% in females); hematocrit decreased 16% in females; lipofucsin accumulation in the liver (2/4 males and 3/4 females); increased liver weights (females 34%, not statistically significant)	
3 months in 1 year		
1.84 (NOAEL)		
20.2 (LOAEL)	Decreased albumin (males, 0%; females, 12%); decreased RBC's (males, 8%; females, 7%); hemoglobin decreased 16% at 1-month	
48.8	Decreased albumin (males, 13%; and females, 12%); decreased RBC's (males, 7%; females 6%); hemoglobin decreased 10% at 1-month	
13 week/diet		
0		
0.59		
6.5 (NOAEL)		
54.0 (LOAEL)	Decreased albumin (males, 19%; females, 16%); decreased hemoglobin (males, 12%; females, 14%); decreased hematocrit (males, 8%; females, 12%); decreased RBC's (males, 14%; females, 17%); increased liver weights (females 42%, statistically significant); hemosiderin deposits in stomach (0/4 males and 1/4 females), spleen (1/4 males and 1/4 females)	
120.0	Decreased albumin (males, 37%; females, 23%); decreased hemoglobin (males, 22%; females, 16%); decreased hematocrit (males, 18%; females, 12%); decreased RBC's (16%); increased liver weights (females 53%, statistically significant); hemosiderin deposits in stomach (1/4 males and 0/4 females) and spleen (1/4 males and 1/4 females)	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Spinosad		The primary effects of spinsad are vacuolated cell aggregations in lymphoid tissues. The tissues affected and the incidences reported are equivalent at 8.46 mg/kg/day in the 1-year study and at 9.73 mg/kg/day in the 13-week study. The increases in ALT and AST observed in the 1-year study at the LOAEL of 8.46 mg/kgday are transitory, not accompanied by hepatocellular histopathology, and are of questionable significance. Also, as stated in the Data Evaluation Report, there was no evidence of progression in severity of the effects on lymphoid tissue based on a comparison of the 1-year and 13 week study.
1-year/diet		
0		
1.44		
2.68 (NOAEL)		
8.46 (LOAEL)	Vacuolated cell aggregations in lymphoid tissue (spleen, faucial tonsil, lymph node or intestine)(4/4 males and 2/4 females); increased ALT (males only, 140% at 26 weeks; no increase at week 52) and AST (males only, 56% at 26 weeks, no increase at week 52). Increased triglycerides (males only, 38% at week 26, no increase at week 52) arterites in the epididymis (1/4 males) or cerebral meninges (1/4 females); slight vacuolation of the parathyroid (2/4 males)	
13-week/diet		
0		
4.89 (NOAEL)		
9.73 (LOAEL)	Vacuolated cell aggregation in lymphoid tissues (cervical, mesenteric, faucial tonsil, ileum, colon, or rectum lymph nodes)(4/4 males and 4/4 females); vacuolated acinar cells of the pancreas (2/4 males and 0/4 females)	Given the similarity of effects noted on lymphoid tissues at the LOAEL in each of the studies, it is likely the actual NOAELs would be comparable.
33.4	Vacuolated cell aggregation in lymphoid tissues (4/4males and 4/4 females). Vacuolated cells of the parathyroid (4/4 males and 4/4 females); vacuolated nerve cells in the brain (3/4 males and 1/4 females); increased ALP (males, 34%, females, 163%); increased ALT (males, 91%, females, 405%); increased AST (males, 49%, females, 1,025 %). Kupffer cell proliferation (3/4 males and 3/4 females); increased triglycerides (33% males and 25% females); decreased albumin (22% males and 22% females); testicular arteritis (2/4)	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Tebuconazole 1 year/diet (1989) 0	Hyertrophy of adrenal zona fasiculata (0/4 males and 1/4 females)	1-year study provides more definitive information on effects on adrenal and there are pronounced differences in NOAELs/LOAELs between the 1-year and 13-week studies. In the 1-year study, all animals were observed to have hypertrophy of the adrenal zona fasiculata at a dose level of 4.39 mg/kg/day, whereas only 2/4 females and no males were observed to have this effect at a dose level of 38.8 mg/kg/day in the 13 week study.
2.94 (NOAEL)	Hyertrophy of adrenal zona fasiculata (0/4 males and 0/4 females)	
4.39 (LOAEL)	Hypertrophy of adrenal zona fasciculata, minimal to mild (4/4 males and 4/4 females); fatty changes in the zona glomerulosa (3/4 males and 2/4 females) and lipid hyperplasia in the cortex (2/4 males and 0/4 females	
13 week/diet 0	Vacuolation of the adreanals zona fasiculata (0/4 males and 1/4 females)	
7.5 (NOAEL)	Vacuolation of the adrenal zona fasiculata (0/4 males and 1/4 females); increased N-demethylase activity (31%)	
38.8 (LOAEL)	Decreased body weights (6%), increased N-demethylase activity (180%); vacuolation of the adrenal zona fasiculata (0/4 males and 2/4 females); **Note: the increased incidence of vacuole formation in the adrenals was considered to be an adaptive response and was not considered an effect at the LOAEL**	
207.6	Decreased body weight (17%); vacuolation of the adrenal zona fasiculata (1/4 males; no information on females); increased N-demethylase activity (355%); increased ALP (males, 160%; females, 52%); cataracts in 0/4 males and 3/4 females	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Tebufenozide 1-year/diet 0 0.6 **1.8 (NOAEL)** **8.0 (LOAEL)** 53.0 13-week/diet 0 **2.1 (NOAEL)** **20.1 (LOAEL)** 202.2	 Sinusoidal engorgement (2/4 males and 1/4 females); bone marrow hyperplasia (1/4 males and 4/4 females); increased pigment in Kupffer cells (1/4 males and 1/4 females); decreased RBC's (16%); decreased hemoglobin (10%, statistically significant only in males); decreased hematocrit (14%); increased Heinz bodies (12-fold) Sinusoidal engorgement (4/4 males and 4/4 females); bone marrow hyperplasia (4/4 males and 4/4 females); spleen hematopoiesis (3/4 males and 4/4 females); increased pigment in Kupffer cells (3/4 males and 3/4 females); decreased RBC's (15% consistent over the course of the study); decreased hemoglobin (10%); decreased hematocrit (12%); increased Heinz bodies (66-fold); increased methemoglobin (89% in males) Sinusoidal engorgement (4/4 males and 4/4 females); bone marrow hyperplasia (0/4 males and 0/4 females); spleen hemopoiesis (1/4 males and 3/4 females); increased pigment in Kupffer cells (4/4 males and 4/4 females); decreased RBC's (8%, statistically significant); decreased hemoglobin (8%); hematocrit decreased <10%, not statistically significant; increased Heinz bodies (65-fold) Bone marrow hyperplasia (4/4 males and 4/4 females); spleen hemopoieses (4/4 males and 4/4 females); sinusoidal engorgement (4/4 males and 4/4 females); increased pigment in Kupffer cells (4/4 males and 4/4 females); decreased RBC;s (13%, statistically significant); decreased hemoglobin (7%); hematocrit decreased <10%, not statistically significant; increased Heinz bodies (190-fold); increased methemoglobin (171%)	Decreased hematocrit (14% at 8mg/kg/day and 12% at 53 mg/kg/day) statistically significant during a single of 5 time periods during the course of the study) and MCV (115% at 8 mg/kg/day and116% at 53 mg/kg/day) statistically significant only at highest dose during the course of the study) Bone marrow hyperplasia observed at 8.0 mg/kg/day in 1-year study and at 202.2 mg/kg/day in 13-week study. However, increased incidence of sinusoidal engorgement and pigment in Kuppffer cells and increase in Heinz bodies at LOAEL in 13-week study when compared to incidence of these lesions in the 1-year study indicates that actual LOAEL in 13 week study would approach that of the 1-year study.

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Thiophanate methyl 1-year/capsule 0		Incidence of thyroid follicular cell hypertrophy comparable at the LOAELs in 1-year and 3-month studies. Variability in T3 and T4 measurements limit quantitative comparisons of 2 studies. Differences in LOAELs not biologically significant;
8 (NOAEL)		
40 (LOAEL)	Thyroid follicular cell hypertrophy (0/4 males and 2/4 females); increased thyroid weights (males, 33%, not statistically significant; females, 44%,); decreased T4 (at 6 months, males,70%; females, no decrease)	
200	Thyroid follicular cell hypertrophy (4/4 males and 3/4 females); thyroid follicular cell hyperplasia (1/4 males and 1/4 females); increased thyroid weights (males, 41%; females, 39%, neither statistically significant); decreased T4 (at 6 months, males, 79%; females, no decrease)	Incidence of thyroid follicular cell hypertrophy comparable in 1-year and 3-month studies at 200 mg/kg/day; incidence of thyroid follicular cell hyperplasia greater in 3-month study than in 1-year study at 200 mg/kg/day; increased thyroid weights comparable in males but not females in 1-year and 3-month studies . Unlikely if selection of same doses in two studies would have resulted in significantly different NOAELs Also, in a 2-year rat study, the NOAEL/ LOAEL was identified as 8.8 mg/kg/day and 54.4 mg/kg/day based on hepatocellular hypertrophy, increased severity of nephropathy, increased thyroid weights, follicular cell hypertrophy and hyperplasia, and decreased T3 and T4 hormone levels. Given the similarities in effects observed in the 1-year dog study and the 2-year rat study and the comparability of the NOAELs/LOAELs in the 2 studies, selection of the rat study for establishment of a chronic RfD, absent a 1-year dog study, would be appropriate.
3-month/capsule 0		
<50 (NOAEL); 50(LOAEL)	Thyroid follicular cell hypertrophy (1/4 males and 1/4 females); decreased T3 (males, no decrease; females, 14%, not statistically significant)	
200	Thyroid follicular cell hypertrophy (3/4 males and 2/4 females); thyroid follicular cell hyperplasia (1/4 males; 0/4 females); increased thyroid weights (males, 31%, not statistically significant; females, no effect); decreased T3 (males, no decrease; females, 26%); decreased T4 (males, no decrease; females, 47%)	
800 (lowered to 400 at 50 days)	Thyroid follicular cell hypertrophy (4/4 , males and 4/4 females); thyroid follicular cell hyperplasia (4/4 males; 3/4 females); increased thyroid weights (males, 49%, not statistically significant; females, no effect); decreased T3 (males, 22%, not statistically significant; females, 25%); decreased T4 (males, no effect; females, 31%, not statistically significant)	

Chemical/ Duration/Route Doses (mg/kg/day)	Effects	Comment
Triazamate		Effect at 0.39 mg/kg/day on plasma cholinesterase inhibition and RBC cholinesterase inhibition in males at 13-weeks in 1-year study and at termination in 1-year study comparable, indicating 13-weeks duration sufficient to establish NOAEL/LOAEL..
1 year/diet		
0		
0.0025		
0.0078		
0.0164 (NOAEL)		
0.0236 (LOAEL)	Brain cholinesterase inhibition (males only 12%, not statistically significant); no plasma or RBC cholinesterase inhibition	NOAEL not established in 13-week study but may have approached or equaled NOAEL in 1-year study based on plasma cholinesterase inhibition (i.e., plasma cholinesterase inhibited at 0.03 mg/kg/day in 13-week study but not at 0.02 mg/kg/day in 1-year study. However, based on inhibition of brain cholinesterase activity, 1-year study more definitive. Unknown why no brain cholinesterase inhibition observed at any dose in 13-week study.
0.39	Brain cholinesterase inhibition (males 33% and females 20%); plasma cholinesterase inhibition (males 39% and females 13% not statistically significant); no statistically significant reductions in RBC cholinesterase activity	
4.42	Brain cholinesterase inhibition (males 39% and females 47%); plasma cholinesterase inhibition (males 72% and females 79%); RBC cholinesterase inhibition (males 33% and females 18%)	
13-weeks in 1-year		
0.0236	No cholinesterase inhibition	
0.39	Plasma cholinesterase inhibition (males 31% and females no inhibition)	
4.42	Plasma cholinesterase inhibition (males 67% and females 62%); RBC cholinesterase inhibition (males 23% and females no inhibition)	
13 week/diet	(No statistically significant reduction in RBC or brain cholinesterase activity at any dose)	
0.03 (LOAEL)	Plasma cholinesterase inhibition (males none and females 26%)	
0.31	Plasma cholinesterase inhbition (males 61% and females 61%)	
3.1	Plasma cholinesterase inhibition (males 81% and females 84%)	
11.0	Plasma cholinesterase inhibition (males 89% and females 90%)	