

U.S. Army Center for Health Promotion
and Preventive Medicine

**Wildlife Toxicity Assessment for
Chlordane**

DECEMBER 2005

**Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program**

**USACHPPM Document No: 87-MA02T6-05A
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Wildlife Toxicity Assessment for Chlordane

CAS No. 12789-03-6

Final Report

1. Introduction

Chlordane is a mixture of structurally related cyclodienes (primarily octachloro-tetrahydro-methanoindanes) that was widely used as a broad-spectrum organochlorine-pesticide from 1947 to 1978 (U.S. EPA 2001). Subsequently, chlordane's use was severely curtailed because of increasing concerns about its potential carcinogenicity and reports linking it and other organochlorine compounds to the onset of physiological deficits and reproductive failure in birds (Fry 1995). Thus, from 1978 on, chlordane's only approved use was as an underground termiticide, and even this application was cancelled after 1988 (ATSDR 1994). However, chlordane is still readily detected in environmental media, as it can bioaccumulate. The heavy use of chlordane in the 1950s and 60s and its environmental persistence is evidenced by the estimate that, of the total of 70,000 tons of chlordane that have been produced since 1946, 25-50 percent remain unaltered in the environment to this day (ATSDR 1994).

The CAS number given above refers to "technical" chlordane, a formulation manufactured by the Velsicol Corporation that contains *cis*-chlordane (α -chlordane) and *trans*-chlordane (γ -chlordane) in varying proportions, plus other ingredients. The individual isomers (CAS No. 5103-74-2 for *trans*-chlordane and 5103-71-9 for *cis*-chlordane) are also available commercially in highly purified preparations (>99% by weight). This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of chlordane on wildlife and reports toxicity reference values (TRVs) for chlordane. The TRVs are intended to serve as protective exposure standards for screening-level ecological risk assessments for wildlife inhabiting contaminated sites. The protocol for the development of this assessment the TRVS is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. Toxicity Profile

2.1 Literature Review

Relevant biomedical, toxicological, and ecological databases were electronically searched June 4, 2001, using Dialog® to identify primary reports of studies and reviews on the toxicology of chlordane.

Separate searches were carried out linking the compound to either laboratory mammals, birds, reptiles and amphibians (combined), or wild mammals. In general, a two-tiered approach was used in which all citations were first evaluated as titles and “key words in context.” All available abstracts of those articles selected in the first tier as possibly relevant to TRV development were then evaluated for relevancy and retention for evaluation in the second tier. For chlordane, 129 articles were marked for retrieval from 393 initial hits. Details of the search strategies and the results of each are documented in Appendix A. Secondary references and sources of information on chlordane included the National Library of Medicine’s Hazardous Substances Databank (HSDB 2001), the U.S. EPA's Integrated Risk Information System (IRIS) (U.S. EPA 2001), the Agency's Soil Screening Guidance Technical Background Document (U.S. EPA 1996) and the Health Effects Assessment Summary Tables (HEAST) (U.S. EPA 1997).

2.2 Environmental Fate and Transport

As discussed in ATSDR (1994), a number of lines of evidence link today's presence of chlordane in air to its former use as a pesticide. Its persistence is further supported by the fact that the compound has been detected in indoor air 15 years after a house has been treated with chlordane for termites. Air monitoring data suggest that most of the airborne chlordane exists in the vapor phase.

Chlordane is present in the nation's water bodies as a result of run-off from urban and agricultural uses, though subsequent partitioning to sediment is favored. Alternatively, vaporization can occur depending on the temperature, water turbulence and wind conditions, as well as on the extent and composition of suspended matter.

As might be expected for an agricultural pesticide and termiticide, most of the chlordane detected in the environment exists in soil in which the compound readily absorbs to organic matter and only volatilizes slowly over time.

Degradation of the compound in air occurs as a result of photolysis and oxidation, processes apparently favoring the trans- isomer. Perhaps the most striking feature of its existence in soils is the long residence times reported for the compound in different locations. Mass-balance studies have accounted for the greater part of the originally applied compound to the soil 10 or more years after application, often with little evidence of chemical or biochemical transformations. However, loss of chlordane from light sandy soils is more likely to occur than from soil with high organic content (ATSDR 1994). Biological degradation of chlordane has been demonstrated in some microbial species, including the fungi *Aspergillus niger* and *Phanerochaete chrysosporium*. Nonetheless, overall rates of degradation appear to be very low. Table 1 summarizes physical data relating to the environmental fate and transport of chlordane.

Table 1. Summary of Physical-Chemical Properties of Chlordane

CAS No.	12789-03-6 (technical), 5103-71-9 (cis-), 5013-74-2 (trans)
Molecular weight	409.76
Color	None
Physical state	Viscous liquid
Melting point	106–107°C (cis-), 104–105 °C (trans-)
Boiling point	175°C (at 2mm Hg)
Odor	None
Solubility in water	0.056–1.85 mg/L at 25 °C: miscible with hydrocarbon solvents
Partition coefficients:	
Log K _{ow}	5.54
Log K _{oc}	3.49–4.64
Vapor pressure at 25 °C	(for cis-) 2.2×10^{-5} mm Hg (supercooled liquid), 3.0×10^{-6} (crystal); (for trans-) 2.9×10^{-5} mm Hg (supercooled liquid), 3.9×10^{-6} (crystal)
Henry's Law constant at 25 °C	4.86×10^{-5} atm.m ³ /mole
Conversion factors	1 ppm = 16.76mg/m ³ 1 mg/m ³ = 0.06 ppm

Sources: U.S. EPA (1996), ATSDR (1994)

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Oral Toxicity

2.3.1.1 Mammalian Oral Toxicity - Acute

A number of researchers have published single oral median lethal dose (LD₅₀) values for chlordane. For example, Gaines (1969) surveyed the acute lethality of 98 pesticides and 2 metabolites of DDT in Sherman rats, deriving values for chlordane (grade and vehicle unstated) of 335 and 430 mg/kg for males and females, respectively. These values are consistent with that of 311 mg/kg in male Wistar rats receiving technical grade chlordane in cottonseed oil (Boyd and Taylor 1969). However, Wistar rats receiving protein-deficient diets had significantly lower LD₅₀s in the latter experiment.

A meeting abstract by Harbison (1973) addressing the acute lethality of several pesticides including chlordane in adult versus newborn Sprague-Dawley rats reported respective LD₅₀ values of 344 and 1121 mg/kg for the compound. Truhaut et al. (1974) calculated an oral LD₅₀ value of 350 mg/kg for Wistar rats (sex unstated) receiving a single oral dose of technical chlordane in olive oil. Golden hamsters (with an LD₅₀ of 1720 mg/kg) appeared to be far less susceptible to the acute toxic effects of the compound. Berman et al. (1995) used the simplified up-down procedure to derive an acute oral LD₅₀ of 520 mg/kg for chlordane in female F344 rats.

In a number of studies, laboratory animals received acute chlordane exposures and were monitored for effects other than lethality. For example, Den Tonkelaar and Van Esch (1974) exposed male Wistar and SPF rats to 2, 5, 10, 20, 50 or 200 ppm chlordane in the diet for 2 weeks then measured the specific activities of aniline hydroxylase, aminoantipyrine demethylase, and hexobarbital oxidase in hepatic 10,000 × g supernatants. Aminoantipyrine demethylase appeared to be the most sensitive index of chlordane toxicity, with an apparent dietary lowest-observed-adverse-effect level (LOAEL) for the compound of 10 ppm and a corresponding no-observed-adverse-effect level (NOAEL) of 5 ppm. These dietary levels equate to doses of 0.5 and 0.25 mg/kg-day, respectively, if a default food consumption factor of 0.05 is used. Ogata and Izushi (1991) exposed male Donryu rats to 100 mg/kg "chlordane 2%" in olive oil via gavage once a day for 4 days to assess compound-related fluctuations in clinical chemistry parameters and changes in liver weight and histopathology. As determined 24 hours after the final dose, a number of clinical chemistry parameters were altered in the chlordane-receiving group compared to control, including elevations in triglycerides, cholesterol and gamma-glutamyl transferase activity, as well as a reduction in serum glucose levels. Absolute and relative liver weights were increased due to chlordane, and there was evidence of altered histopathology in the liver of treated rats. The principal metabolites of chlordane detected in the blood were oxychlordane, heptachlor epoxide, and *cis*- and *trans*-nonachlor.

Chatterjee et al. (1981) administered a single dose of 15 mg/kg chlordane (grade unstated) to male Wistar rats and observed greater than 40 percent mortality among the subjects during a 15-day follow-up period. This response was accompanied by increases in liver and kidney weights, histopathological lesions in the liver and kidney, and overall growth retardation among the survivors. However, the response was mitigated in similarly treated animals who received L-ascorbic acid as a dietary supplement. Moser et al. (1995) gavaged female F344 rats with single or multiple doses of pesticides including chlordane (40 percent chlordane plus 60 percent other cyclodiene compounds) at concentrations of up to 291 mg/kg. Survivors were assessed in a battery of neurobehavioral tests that scored such responses as excitability, neuromuscular coordination, motor activity, and grip strength on a semi-quantitative scale. The authors noted a cumulative toxicity that was evident when results from the single-dose studies were compared to those that incorporated a 14-day dosing regimen. The authors suggested NOAELs of 52 and

5 mg/kg for single versus multiple dose effects of chlordane on the neurobehavioral responses of F344 rats.

Barnett and Dorough (1974) subjected both sexes of Sprague-Dawley rats to various acute, subacute and subchronic oral dosing regimen of ¹⁴C-labelled HCS-3260, a 98 percent pure formulation of chlordane containing a 3:1 cis: trans isomer ratio. Fecal elimination of the radioactivity was emphasized although a number of cyclodiene products of chlordane metabolism were tentatively identified in various tissue extracts. For example, adipose tissue contained measurable amounts of oxychlordane, concentrations of which declined during the post-treatment recovery period.

Berman et al. (1995) included chlordane in a screen of 10 chemicals surveyed for toxicological consequences in a 14-day experiment in which 8 female F344 rats/group were dosed. Initially, doses were 0, 5, 16, 52, and 156 mg/kg, although data from the two highest groups were excluded from the reported findings because of early deaths. Survivors were bled to obtain plasma for clinical chemistry analyses and samples of liver, kidney, spleen, thymus, and adrenals were examined histopathologically. Changes in relative liver weight and hepatic centrilobular cytomegaly, as well as reductions in such liver-related clinical chemistry parameters as plasma alkaline phosphatase activity, were observed. Corresponding NOAELs and LOAELs for the responses to chlordane were 5 and 16 mg/kg-day for liver weight increase, 0 and 5 mg/kg-day for histopathological change and 0 and 5 mg/kg-day for plasma alkaline phosphatase reduction.

Another study that has mechanistic implications for the hepatic impact of chlordane in mice was that of Whysner et al. (1998) who used the ³²P-DNA post-labeling technique to look for DNA adduct formation. B6C3F1 mice received either a single oral gavage dose or a 2-week dietary exposure to 50 or 200 ppm chlordane, respectively. The authors used benzidine and 2-acetylaminofluorene in parallel exposures as positive controls to demonstrate the effectiveness of the post-labeling technique, but they obtained only negative results for chlordane in their system.

2.3.1.2 Mammalian Toxicity – Subchronic

Five Osborne-Mendel rats/sex/group were fed casein-sufficient or -deficient diets containing either 0, 125, 250 or 500 ppm chlordane (grade unstated) for 28 days (Casterline and Williams 1971). Clinical signs were monitored daily, and food consumption and body weights weekly. Excised livers were weighed and compared to body weights at termination. Some fluctuations of tissue and serum enzyme activities were noted according to chlordane concentration and dietary status. However, the most marked effects of the pesticide on the rats were the dramatic increases in relative liver weights compared to controls. These were statistically significant at all concentrations of chlordane, irrespective of protein sufficiency/deficiency. Based on the body weight and food consumption data at the lowest dietary

chlordane concentration (125 ppm), a subacute LOAEL within the range 8-14 mg/kg/day is suggested for the compound in Osborne-Mendel rats.

A subchronic toxicity study on chlordane (with and without lead oxide) was used by Al-Omar et al. (2000) to investigate the effects on the testicular function and architecture of Balb/c mice. Twelve males/group received 0, 75 or 275 mg/kg commercial chlordane in corn oil by gavage for up to 35 days. Other groups received lead oxide in addition to chlordane or lead oxide alone. Body weights were recorded weekly, and three mice/group were sacrificed at 2, 3, 4, and 5 weeks for histopathological examination of the testis. A number of structural and functional deficits were observed in mice exposed to chlordane only, including a loss of testicular weight, damaged tubules, and reductions in seminiferous tubule diameter, number of spermatogonia, primary and secondary spermatocytes, spermatids, and Sertoli cells. Lead enhanced the effect of chlordane but was unable to induce the same effects on its own. Given the statistically significant impact of chlordane on these parameters at the lowest concentration, 75 mg/kg-d chlordane represents the LOAEL for chlordane's effects on the male mouse reproductive system.

Mahon and Oloffs (1979) described an experimental protocol where the primary aim was to determine the effect of chlordane within the carbon tetrachloride/rat liver cirrhosis model. The design featured a 10-week exposure to male Long-Evans BLU-LE rats (phase 1) followed by 10 weeks of recovery (phase 2). The author identified #4 as the key group. This group received carbon tetrachloride and chlordane during phase 1 followed by chlordane alone during phase 2. However, for the purposes of this discussion, group #2 provided the relevant data, the animals having consumed 0.05 mg/kg-day of both α - and γ -chlordane daily on a piece of carrot during both exposure phases (20 weeks in all). Some minor changes in relative liver weight were observed in those members of this group who were sacrificed at 10 weeks. However, no such changes were observed in animals receiving chlordane for the full 20 weeks, and no signs of histopathological lesions were apparent at either time point. These data suggest 0.05 mg/kg-day as a subchronic NOAEL for chlordane in Long-Evans rats.

A subchronic dosing regimen has also been used to investigate the possible effects of chlordane on ATPase activities in the brain of male Sprague-Dawley rats. As described in a meeting abstract, Drummond et al. (1980) mixed 0, 25, 50 or 100 ppm chlordane with either iron-deficient or -sufficient lab chow which was then made available to 9 animals/group for 12 weeks. Three animals/group were sacrificed at 4, 8, or 12 weeks after exposure; with "P2" fractions of the brain prepared and assayed for specific Na^+, K^+ , oligomycin sensitive and oligomycin insensitive Mg^{++} ATPase activities. Na^+, K^+ , and oligomycin sensitive Mg^{++} ATPase activities were dose-dependently reduced at all time intervals post-chlordane treatment, whereas oligomycin insensitive Mg^{++} ATPase activity was unaffected. The authors

sought to link the observed biochemical changes to the neurotoxicological effects that have been described in some toxicological studies on chlordane.

Other animal models have been used to demonstrate the toxicological effects of chlordane, including three Holstein cows that received the compound in gelatin capsules daily for 60 days (Dorough and Hemken 1973). The formulation employed was the highly purified HCS 3260 that contained 74 percent α -chlordane and 24 percent γ -chlordane. The dosage was considered equivalent to 50 lbs of feed/day containing 1, 10, or 100 ppm chlordane. Therefore, given an approximate weight of 1400 lbs for each animal, the concentrations were equivalent to approximate doses of 0.036, 0.36, and 3.6 mg/kg-day. The authors did not address the issue of a sub-threshold point of departure for overt toxicity but did demonstrate the appearance of chlordane and its metabolites in milk and fat biopsy tissue. For example, the major product of chlordane in milk was oxychlordane. However, levels of this and other metabolites declined dramatically when exposure was terminated.

2.3.1.4 Mammalian Toxicity – Chronic

In a chronic study by Ingle in 1952, Osborne-Mendel rats (20/ dose group) were fed laboratory chow spiked with 0, 5, 10, 30, 150, or 300 ppm chlordane for 2 years. Clinical signs were evident in those groups receiving 30, 150, or 300 ppm chlordane-dosed chow, including tremors, hyperexcitability, convulsions, prostration, etc. Animals in the two highest dose groups also displayed reduced food consumption, decreased growth and survival, fetal toxicity and increases in liver and kidney weights. At high doses (150 and 300 ppm) there was retardation in growth rate. At these doses there was also an increase in the mortality rate, such that rats receiving 150 and 300 ppm showed significantly higher mortality rates than the lower dose groups. Newborn rats from the 150 and 300 ppm dose groups had definite symptoms of fetal toxicity, including death, when allowed to remain with their lactating mothers. It was also noted that the milk from chlordane-treated mothers did contain a sufficient amount of chlordane to elicit effects in suckling newborns. These effects included death, impaired growth and development. Newborn rats born to chlordane-treated females that were removed and placed with control females showed no signs of toxicity. Histopathological impacts of chlordane were also evident in the liver and kidney, with cellular hypertrophy and bile duct proliferation among the hepatic effects. Some liver damage was evident in those exposed to 30 ppm chlordane, while those receiving 10 ppm were essentially unaffected. The authors considered the doses to be 0, 0.35, 0.65, 1.5, 8.0, and 17 mg/kg-day, which would justify designating 0.65 mg/kg-day as a NOAEL and 1.5 mg/kg-day as a LOAEL for the toxic effects of the compound.

A full-scale bioassay of chlordane for possible carcinogenicity has been published as a National Cancer Institute (NCI) report (NCI 1977) and in the open literature (Reuber and Ward 1979). Fifty Osborne-Mendel rats and B6C3F1 mice/sex/group were exposed to the compound in laboratory chow for

80 weeks, then observed for an additional 29 weeks prior to sacrifice. The researchers used a formulation of technical chlordane that contained 71.7 percent α - and 23.1 percent γ -isomers and changed the concentrations of the compound in the chow at various points during the course of the experiment. These adjustments resulted in time-weighted average (TWA) chlordane concentrations in chow of 0, 203.5, and 407 ppm for the male rats and 0, 120.8, and 241.5 ppm for the females; and 0, 29.9, and 56.2 ppm for the male mice and 0, 30.1, and 63.8 ppm for the females. In common with a number of other NCI-sponsored bioassays of the period, the experimental design incorporated a comparatively small number of concurrent controls (10 rats/sex and 20 mice/sex), but obtained relevant data from pooled controls involved in other NCI-sponsored studies. All animals were observed twice daily for clinical signs, weighed at regular intervals, and palpated for masses at each weighing. Premature decedents and animals sacrificed at term were necropsied, and a full suite of organ and tissue samples were processed for histopathological examination. The U.S. EPA's IRIS summary of this study gave the average daily doses as 10.2 and 20.4 mg/kg-day in male rats and, in female rats, 6.0 and 12 mg/kg-day; and 4.3 and 8mg/kg-day in male mice and 4.3 and 9.1 mg/kg-day in female mice (U.S. EPA 2001). These values were apparently derived using food factors of 0.05 in rats and approximately 0.15 in mice. There were few signs of compound-related histopathological lesions among the rats. However, both sexes of high dose rats displayed reduced body weight gain of 10 percent or greater compared to controls, suggesting a NOAEL for this effect of 6-10.2 mg/kg-day. By contrast to the results in rats, gains in body weight were not reduced in chlordane-treated mice. However, histopathological lesions were noted in the liver in which a dose-dependent increase in hepatocellular carcinomas was reported (2/18, 16/48, and 43/49 in males and 0/19, 2/47, and 34/49 in females, for control, low-dose and high-dose groups, respectively).

A number of toxicity/carcinogenicity studies have been sponsored by the Velsicol Corporation, the manufacturer of chlordane. For example, Khasawinah and Grutsch (1989a) exposed 80 ICR SPF mice/sex/group to 0, 1, 5, or 12.5 ppm technical chlordane in feed for 104 weeks and reported data on the resulting clinical signs, body weight changes, food and water consumption. The authors also looked at hematological and clinical chemistry measurements and urinalyses, organ weights at term, gross pathology, and histopathology. No clinical signs were attributable to chlordane, and no dose-related body weight changes were observed. There were some effects on hematological and clinical chemistry parameters, but these were not consistent and occurred only in the high doses. Overwhelmingly, the primary target organ appeared to be the liver, in which absolute and relative increases in organ weights were evident. In addition, both neoplastic and non-neoplastic changes were observed in the liver of exposed animals, the former including a dose-dependent increase in hepatocellular adenomas and hemangiomas in male subjects, the latter including atrophy, necrosis, and fatty degeneration, among others. While the neoplasm data do not lend themselves to NOAEL/LOAEL designation and while, in general, tumorigenic endpoints are inapplicable to TRVs for wildlife, the non-neoplastic hepatic

responses to chlordane in this animal model suggest a NOAEL of 1 ppm. This is equivalent to a daily dose of 0.15 mg/kg-day (U.S. EPA 2001).

Barrass et al. (1993) examined the role of gene proliferation in the etiology of hepatic tumor development through the use of a complex protocol in which a 2-year hepatocarcinogenic assay was paralleled by a 6-month study that examined serially liver histopathology and thyroid follicular cell proliferation. In the first part of the experiment, 50 ppm dietary levels of chlordane were made available to 100 male C57B1/10J mice inducing 23/79 combined hepatocellular adenomas and carcinomas, compared to 10/400 in historical controls. Non-tumorigenic responses included centrilobular hypertrophy and generalized liver enlargement. The second part of the study featured the administration of chlordane in the diet as before, but for a 6-month duration in which groups of 5 male mice were sacrificed on days 2, 3, 4, 5, 8, 15, 29, 99, and 190 after the start of dosing. Replicating cells were labeled by infusing 5-bromo-2-deoxyuridine (BrdU) at 15 mg/mL via osmotic minipumps implanted subcutaneously 3 days before necropsy on days 4, 5, 8, 18, 29, 190, and 247. Sections of liver, thyroid, and duodenum (as a positive control) were examined for signs of cellular proliferation using anti-BrdU immunostaining. In the liver, the peak hepatocyte labeling index (LI) was observed after 8 days of chlordane treatment with subsequent time-related decreases towards control levels. Treatment-then-withdrawal groups had LIs similar to those of concurrent controls. These data represent a direct demonstration of the ability of liver from C57B1/10J mice to proliferate when challenged with a non-genotoxic carcinogen such as chlordane.

2.3.1.5 Mammalian Oral Toxicity – Other

A number of experimental studies have focused on prenatal exposure of experimental animals to chlordane, including (1) reproductive/developmental and teratogenicity studies in which compound-related impacts to reproductive performance and the incidence of fetal abnormalities were examined, (2) studies on endocrine dysfunction, and (3) experiments assessing chlordane's ability to alter the immune competence of offspring during development. In the first category, a review by Deichmann (1972) describes a 6-generation reproductive study in Swiss mice, reporting a NOAEL of 25 ppm (concentration of chlordane in feed equivalent to a dose of 3.25 mg/kg-day) for effects of the compound on fertility and viability.

More recently, Chernoff and Kavlock (1982) included chlordane in a survey of the teratogenic potential of several compounds in CD-1 mice. Pregnant females were administered 50 mg/kg by gavage

in corn oil on GD (gestation day) 8-12, a treatment that induced few if any reproductive or developmental effects in either dams or progeny.

Various numbers of pregnant female rats (strain unstated) were gavaged during GD 7-17 with either 0, 20, 40, or 80 mg/kg chlordane, the highest dose of which induced deficits in maternal body weight gain and 50 percent fatalities among the dams (Usami et al. 1986). However, when fetuses were examined on GD 20, no statistically significant reproductive, developmental or teratogenic impacts of chlordane were observed, suggesting 80 mg/kg-day as a NOAEL for these effects.

Narotsky and Kavlock (1995) included chlordane in a developmental toxicity screen in which the compound was administered by gavage at 0, 21 or 28 mg/kg-day to pregnant F344 rats during GD 6-19. The dams were allowed to deliver their progeny that were examined on postpartum days (PD) 1, 3, and 6. Litter weights were monitored on PDs 1 and 6, and the number of implants was counted to assess prenatal loss. In contrast to earlier reproductive/developmental experiments on chlordane, some developmental impacts of the compound were evident in the offspring, including dramatic reductions in postnatal viability and pup weight in the absence of any prenatal effects. A LOAEL of 21 mg/kg-day can be based on postnatal effects on pup viability.

The ability of chlordane to affect the endocrine status of experimental animals was addressed in an experiment in which pregnant F2 hybrid mice received 0.16 or 8 mg/kg analytical grade chlordane throughout gestation (Spyker Cranmer et al. 1978). At birth, the pups were weighed, examined for viability, gross defects, and sex, and subsequently assessed for endocrine status (on PD 101). This evaluation included the measurement of corticosterone in plasma, and *in vitro* assessments of corticosterone production in excised adrenals and the capacity of excised liver pieces to metabolize corticosterone. Fifty five percent of offspring born to high-dose dams died so that the findings of endocrine status are essentially confined to the low-dose group. Nonetheless, a complex pattern of results emerged in which the corticosterone concentration in the plasma of low-dose males was elevated although *in vitro* adrenal corticosterone production was unaffected. Total hepatic metabolism was comparable to control values.

Perturbation of steroid hormone levels due to chlordane was also examined by Cassidy et al. (1994), who exposed pregnant Sprague-Dawley rats to 0, 100, 500, or 5000 $\mu\text{mg/kg}$ between GD 4 and lactation day (LD) 21. Pups were similarly treated up to PD 80. At the completion of this exposure regimen, pups were subjected to a number of behavioral tests including their ability to find their way out of a maze, startle response, and the mating responses of males. Serum levels of testosterone and of chlordane metabolites such as heptachlor, heptachlor epoxide, and oxychlordane were measured at PD 85, the latter parameters displaying dose-related increases with treatment. In general, the chlordane-receiving offspring did better in the behavioral tests than did the controls, including the mating trials. These

observations and the fluctuation of testosterone levels according to treatment caused the authors to speculate that cyclodienes such as chlordane may "masculinize" sexually dimorphic functions and behaviors by either mimicking steroid hormone activities or altering their levels.

A study reported by Spyker-Cranmer et al (1982) is typical of several that have sought to show an altered immune competency of offspring exposed *in utero* to chlordane. Pregnant female Balb/c mice were treated throughout gestation at 0, 0.16, or 8 mg/kg to enable contact mediated immune (CMI) responses to oxazolone to be monitored among the offspring. This was achieved by measuring the extent of ear-swelling three days after challenge with the latter compound. The authors concluded that chlordane appeared to reduce the extent of the immune response in the progeny of dams receiving the highest dose. The authors also carried out the Jerne hemolytic plaque-forming cell (PFC) assay in which 120-day-old subjects were immunized with sheep erythrocytes. Freshly isolated spleen cells were then challenged with sheep erythrocytes, the released antibody (IgM) being detected by the addition of complement, which caused zones of lysis in the plated background of sheep erythrocytes surrounding the spleen cells. Once again, positive results (an increase in plaque formation compared to controls) were seen at the higher exposure level, suggesting a NOAEL of 0.16 mg/kg-day. It was thought that chlordane may reduce the activity or number of t-suppressor or t-effector cells. However, these results contrast with those of Johnson et al. (1986), who measured some of the same responses in spleen cells isolated from adult female B6C3F1 mice exposed to 0.1, 1.0, 4.0, or 8.0 mg/kg-day 99 percent γ -chlordane by gavage in corn oil for 14 days. They carried out the Jerne plaque-forming assay, and assessed the proliferation of spleen cells in response to concanavalin A, phytohemagglutinin, and other mitogens. In general, the authors concluded that chlordane might not be a serious threat to immunocompetence, at least as a result of exposure to adults.

The contrast between the immunotoxic effects of chlordane in prenatally exposed versus those exposed as adults was explored in a study by Barnett et al. (1990). These researchers exposed pregnant Balb/c mice to chlordane at 0, 4, or 8 mg/kg for a total of 18 days during pregnancy, the pups being born and nursed in the normal way. A group of non-pregnant adult female mice also were exposed to chlordane for a similar time period. Subjects were monitored for bone marrow hematopoietic activity 14, 100, and 200 days post exposure, using an *in vitro* granulocyte-macrophage colony forming unit (GM-CFU) assay and an *in vivo* colony forming unit assay in the spleen, the latter approach measuring the immunotoxic effects of chlordane on more primitive stem cell precursors. Animals treated prenatally with chlordane showed significant reductions in their bone marrow GM-CFU at 14 days of age and when they had reached adulthood (100 and 200 days). Prenatal treatment with chlordane also reduced the number of spleen colony-forming units in exposed subjects. However, direct treatment of 80-day-old adult mice with chlordane had no effect on these parameters, consistent with the results of Johnson et al. (1986).

Blyler et al. (1994) exposed developing Balb/c mice to chlordane in utero by dosing pregnant females with chlordane at either 0 or 8 mg/kg for a total of 18 gestation days. Myeloid hematopoietic activity of bone marrow cells from 6-week-old offspring was measured *in vitro* through the colony-forming effects of exogenously added recombinant forms of the cytokines granulocyte/macrophage-colony stimulating factor, macrophage-CSF, and interleukin 3 (IL-3). There was a gender-specific reduction in the numbers of bone marrow colony-forming units in response to these challenges (females only), though prenatal treatment with chlordane did not significantly affect the number of viable bone marrow cells in either male or female mice.

2.3.1.6 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

Table 2 summarizes the studies that highlight the effects of chlordane. The liver appears to be the primary target organ of chlordane toxicity in experimental studies (Den Tonkelaar and Van Esch 1974, Ogata and Izushi 1991, Chatterjee et al. 1981, Mahon and Oloffs 1979, Ingle 1952, NCI 1977, Barrass et al. 1993, Malarkey et al. 1995, Berman et al. 1995). Other target organs of chlordane toxicity include the testis (Al-Omar et al. 2000), kidney (Chatterjee et al. 1981) and possibly the brain (Moser et al. 1995, Drummond et al. 1980).

Although the liver seems to be the primary target of chlordane toxicity, for purposes of deriving a TRV, it is difficult to confidently relate hepatic effects to ecologically relevant effects. Other chlordane-induced effects such as testicular effects (Al-Omar et al. 2000), developmental toxicity and reduced weight gains (Ingle 1952; Narotsky and Kavlock 1995) are likely more suitable as a basis for a mammalian TRV.

Al-Omar et al. (2000) showed that 75 and 275 mg/kg chlordane caused a significant reduction in testes weight, diameter of the seminiferous tubules, the number of spermatogonia, and primary and secondary spermatocytes and spermatids in Swiss mice. These results indicate that chlordane is a likely reproductive toxicant although since no functional assays were conducted, it is difficult to determine if the effects on male reproductive parameters would result in a diminished ability to successfully reproduce. There lacks a clear link to functional reproduction, however the results from this study provide useful supporting evidence for reproductive effects of chlordane.

Narotsky and Kavlock (1995) showed a significant decrease in the number of live pups from dams exposed to 21 mg/kg/d chlordane and, in addition, decreased weight gain in chlordane-exposed dams. This study was chosen as a basis for the TRV since there was a clear link between exposure to chlordane and decreased reproductive performance. In addition, significant effects occurred at 21 mg/kg/d, which is lower than effects seen in the Al-Omar et al. (2000) study (75 mg/kg/d). Importantly, although the dose to the pups occurred via the mother, this is the only mechanism by which chlordane could reach the pups. Therefore, the 21 mg/kg/d as received by the dams is suitable as a dose for the pups. Generally,

decreased reproductive output (and lower growth rate) in natural populations is likely to result in deleterious population-level effects, hence, this endpoint is suitable for TRV development based on previously outlined criteria (USACHPPM 2000).

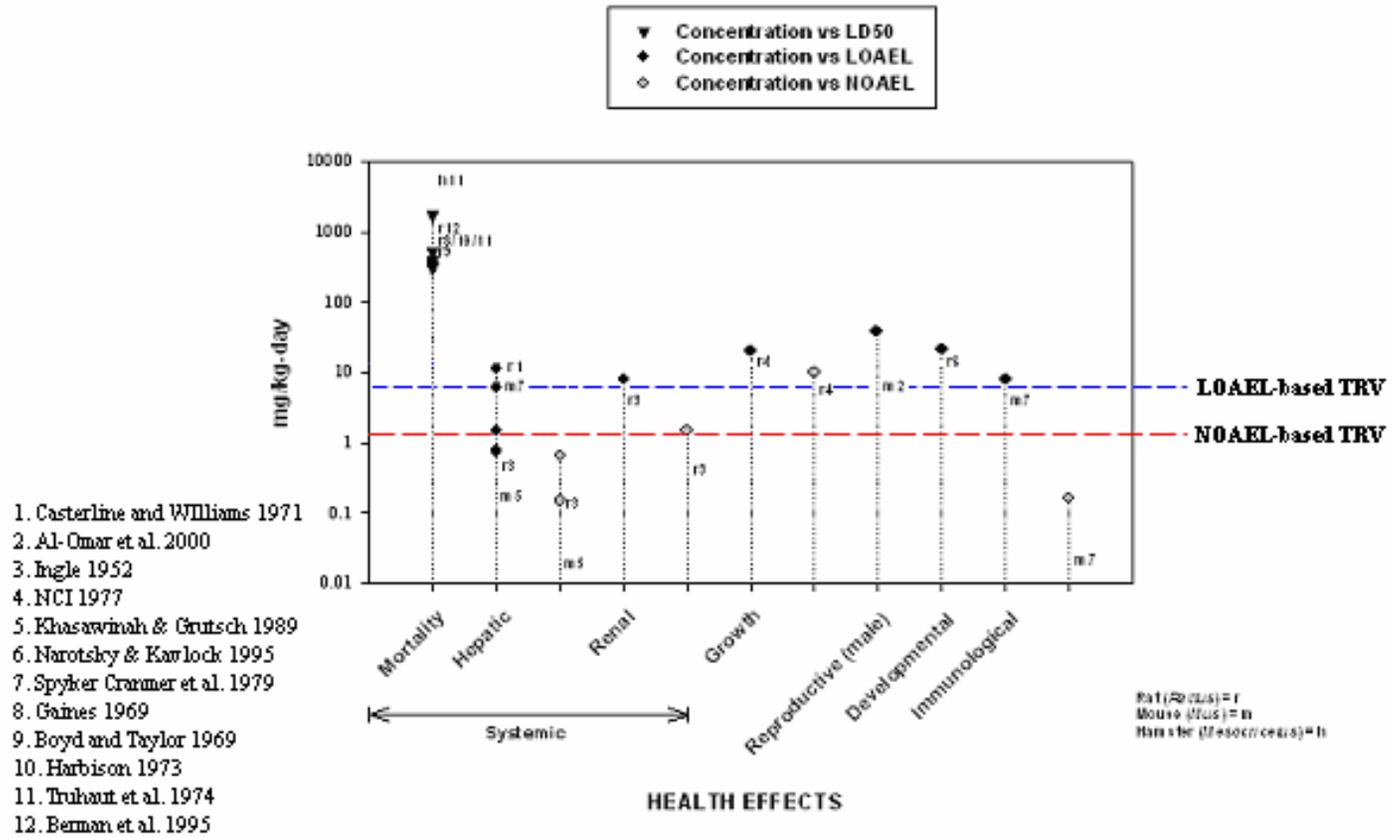
Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Casterline and Williams (1971)	Rat (Osborne-Mendel)	28-d	NA	8-14	Increase in relative liver weight.
Al-Omar et al. (2000)	Mice (Balb/c)	2, 3, 4 and 5-w	NA	75	Structural and functional perturbation of the male reproductive system
Ingle (1952)	Rat (Osborne-Mendel)	2-y	0.65	1.5	Increased mortality, decreased growth at high concentrations, fetal toxicity and hepatic hypertrophy
NCI (1977)	Rat (Osborne-Mendel)	2-y	10.2	20.4	Reduced body weight gain of more than 10%.
Khasawinah and Grutsch (1989)	Mice (ICR SPF)	2-y	0.15	0.75	Hepatic necrosis, atrophy, and fatty degeneration. Absolute and relative increases in liver weights.
Narotsky and Kavlock (1995)	Rat (♀) (F344)	GD 6-19	NA	21	Developmental toxicity (reduced postnatal survival and weight gain in pups)
Spyker Cranmer et al. (1979)	Mice (♀) (Balb/C)	Throughout gestation	0.16	8	Immunocompetence

NA = not applicable GD = gestation day

Figure 1

CHLORDANE: HEALTH EFFECTS TO MAMMALS



2.3.3 Mammalian Inhalation Toxicity

2.3.3.1 Mammalian Inhalation Toxicity – Acute

No data are available.

2.3.3.2 Mammalian Inhalation Toxicity – Subacute

A detailed report on the inhalation toxicology of chlordane contains a subacute component in which 10 Wistar rats/sex/group were exposed to 0, 5.8, 28.2, 154, or 413 µg/l, 8 hours/day, 5 days/week for up to 28 days (Khasawinah et al. 1989). Exposure to chlordane occurred in 0.5 m³ stainless steel and glass chambers. Body weights were monitored on days 0, 3, 7, then weekly. Urinalysis and hematological and clinical chemistry parameters were measured on five animals/sex/group on day 28. Survivors were necropsied at term, excised major organs were weighed, and a full range of organs and tissues were processed for histopathological examination (control versus high-dose). Sections of the lung, liver, and kidney were examined in all groups. Premature deaths and instances of weight loss among the survivors suggest that 154 and 413 µg/l were above the maximum tolerated dose. However, 28.2 µg/l appeared to be at or near the toxicity threshold since clinical signs and a reduction in food consumption were observed in animals exposed to this chlordane concentration. There was no indication of toxicity in animals exposed at 5.8 µg/l, but there were some signs of fluctuation in clinical chemistry parameters in those exposed at 28.2 µg/l, including a reduction in blood glucose. Similarly, while there were no apparent histopathological effects of chlordane in rats exposed to 5.8 µg/l, those receiving 28.2 µg/l displayed hepatocellular hypertrophy. This demarcation between the effects of chlordane at the two exposure levels justifies the choice of 5.8 µg/l as a NOAEL and 28.2 µg/l as a LOAEL.

2.3.3.3 Mammalian Inhalation Toxicity – Subchronic

The report by Khasawinah et al. (1989) also contains an account of subchronic inhalation experiments on chlordane in Wistar rats and cynomolgus monkeys. Varying numbers of Wistar rats and 6 monkeys/sex/group were exposed to chlordane concentrations of 0, 0.1, 1, or 10 µg/l for 90 days, followed, for a subset of rats, by a 90-day recovery period. In addition to the suite of observations and analyses as described for their subacute inhalation study, Khasawinah et al. (1989) (1) measured the levels of chlordane in blood, liver, and adipose tissue, as well as the activity of hepatic cytochrome P450 in exposed subjects, (2) examined the animals' eyes before and in the final week of exposure, and (3) carried out pulmonary function tests on the monkeys before exposure and once during week 12. Rectal temperatures were measured in all monkeys and in five rats/sex in the control and high-dose groups before exposure and periodically throughout the study.

Although there were few compound-related effects of chlordane in monkeys at the concentrations employed, there was some evidence of chlordane-induced toxicity in the intermediate and high dose rats. For example, there was a statistically significant reduction in total and mean corpuscular hemoglobin concentration in males by week 5, a result that was enhanced by week 13, and also apparent in females at the later time point. Other dose-dependent hematological changes were observed, plus a variety of impacts to the liver including increased weight, enlargement, centrilobular hypertrophy, and an increase in the activity of cytochrome P450. These findings justify the choice of 0.1 µg/l as a NOAEL for rats. However, an equivalent value for the monkeys would be >10 µg/l.

2.3.3.4 Mammalian Inhalation Toxicity – Chronic

No data are available.

2.3.3.5 Studies Relevant for Mammalian TRV Development for Inhalation Exposures

There are few inhalation toxicity studies on chlordane. The inhalation study of Khasawinah et al. (1989) demonstrated that the hepatic impacts of chlordane are irrespective of the route of administration. Supporting this are [¹⁴C]chlordane studies that have shown that once absorbed, inhaled chlordane has the same kinetics as orally administered chlordane (Dorough 1978; Nye and Dorough 1976). Thus, the liver was shown to be the target organ in like manner to the oral exposure studies described in Sections 2.3.1 and 2.3.2 of this account. As discussed in Khasawinah (1989), the hepatic toxicity manifest at exposure concentrations in excess of 0.1 µg/l (the suggested NOAEL) were accompanied by dose-dependent increases in the levels of "total" chlordane in adipose tissue, liver, and blood. For the most part, the components observed in rat tissues included oxychlordane, heptachlor epoxide, trans-nonachlor and an unidentified component designated "compound C". Heptachlor, cis-chlordane and trans-chlordane were much less heavily represented.

There is insufficient data on the inhalation toxicity of chlordane to derive a TRV for mammalian inhalation exposures. Given that the kinetics of chlordane is the same for both oral and inhalation exposures it would be possible to use oral toxicity data as a basis for an inhalation TRV. However, there are no data comparing the relative absorption of chlordane from the inhalation and oral routes, hence a TRV for inhalation exposures to chlordane for mammalian wildlife cannot be derived.

2.3.4 Mammalian Dermal Toxicity

Two studies were identified that featured the topical administration of chlordane to experimental animals. In the first, Schop et al. (1990) examined the capacity of the pesticide to induce micronuclei (MN) in femoral bone marrow and nuclear aberrations (NA) in the hair follicles of male CD-1 mice that

had received a single application of the compound in dimethyl sulfoxide (DMSO) at 1/8, 1/16, or 1/32 the dermal LD₅₀. For chlordane, these exposure levels were 125, 250, and 500 mol/kg, each of which was associated with a dose-dependent incidence of NA formation. The results obtained for chlordane in the MN test were less clear-cut, although a positive effect was obtained at the highest dose, equivalent to a LOAEL of 205 mg/kg and a NOAEL of 102 mg/kg. A LOAEL of 51 mg/kg would apply to chlordane in the NA test.

More recently, a meeting abstract by Blaylock and Mehendale (1995) described the topical application of chlordane to female Balb/c mice and the extent of its effects on oxazolone-induced contact sensitivity. The pesticide appeared to have the ability to reduce oxazolone-induced ear swelling whether applied prenatally or at the time of sensitization. The dose-response component of the study suggested that 20 g of chlordane would represent a LOAEL for this effect.

2.3.4.1 Studies Relevant for Mammalian TRV Development for Dermal Exposures

The few studies on the dermal toxicity of chlordane do not provide sufficient information on which to base a dermal TRV.

2.4 Summary of Avian Toxicology

Many studies have surveyed an array of pesticides, polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH), dioxins and dioxin-like residues in the bodily tissues, brains, or eggs of birds, with the underlying intent of correlating the levels of these environmentally important contaminants with symptoms of health impairment, impacts on species abundance, reproductive deficits, etc. In one of a series of dose-response studies of pesticides in birds Stickel et al. (1983) determined the levels of chlordane metabolites in brain and carcass that resulted from feeding a number of different species of birds with measured amounts of chlordane and/or related compounds. Results included estimates of the levels of chlordane (and its metabolites) in the brain that are indicative of (or correlate with) a state of ill-health/failure-to-thrive for a particular species, plus a range of (mostly lower) concentrations of chlordane residues representing "no adverse impact" tissue levels. Many subsequent field studies have used the Stickel criteria to calibrate their field measurements, thereby drawing inferential evidence of the degree of threat to which their subjects were exposed from the prevailing environmental contamination at the time the field samples were collected.

The Stickel et al. (1983) experiments were conducted on a number of bird species that had been captured and kept in large outdoor flight cages equipped with swinging perches. Cage-conditioned birds were provided water *ad libitum*, and their food was spiked to pre-determined levels with the chemical under investigation. In one series of experiments, Stickel et al. (1983) exposed 10 female starlings/group to 200, 300, 400, or 500 ppm HCS-3260 in turkey starter crumbles. Other experimental groups receiving

HCS-3260 included female grackles (200 ppm), male cowbirds (200 ppm), and male red-winged blackbirds (10, 50, 100, and 200 ppm). Additional male grackles received 12.5 ppm oxychlordanes, whereas other groups of male cowbirds and female starlings received nonachlor at 12.5, 25, 50, or 100 ppm. Whole brains and 20-g sub-samples of homogenized carcasses were extracted in solvent and measured for oxychlordanes, heptachlor epoxide, *cis*- and *trans*-chlordanes on a ppm wet weight basis. Some animals receiving the higher concentrations of HCS-3260 (200 ppm and above) showed a range of clinical signs including decreased activity, convulsions, loss of balance and mortality. Importantly, those that died displayed a higher range of concentrations of oxychlordanes in the brain than did survivors. For example, cowbirds that died at 200 ppm HCS-3260 had concentrations of oxychlordanes brain concentrations ranging from 19.1-22.1 ppm wet weight, whereas those that survived to term had ranges of 2.3-4.8 ppm wet weight. Comparative ranges of oxychlordanes in the brains of grackles receiving HCS-3260 were 12.9-25 ppm for dead birds versus 1.3-2.1 ppm for surviving birds. In general, there appeared to be an inverse relationship between dose level and time-to-death, though with a considerable amount of interspecies variation as a result of the chemical insult. The authors demonstrated time- and dose-dependent oxychlordanes accumulation, with residue levels slowly declining ($t_{1/2} = 63$ days) when exposure was discontinued. HCS-3260 was shown to be a precursor of tissue-borne oxychlordanes, whereas technical chlordanes was a precursor of oxychlordanes and heptachlor epoxide.

Although a number of studies have shown that chlordanes is toxic to birds, the main focus of laboratory experiments has been in correlating tissue burdens with toxicity. As a result, no suitable data is available on which to derive an avian TRV. Stickel et al. (1983) is one of the few laboratory toxicity studies involving oral exposure of birds to chlordanes. However, the main focus of this effort was to correlate brain chlordanes levels with mortality and detail on exposure duration and effects was not presented precluding the use of this study for TRV derivation.

2.5 Amphibian Toxicology

No data are available.

2.6 Reptilian Toxicology

There are no feeding studies available that report toxic effects of chlordanes on reptiles. However, studies by Willingham and Crews (1999) and Willingham et al. (2000) used an experimental design that featured the perturbation of the temperature-dependent sex determination of the red-eared slider turtle (*Trachemy scripta elagans*) by the challenge of estrogen or estrogen-like substances such as the organochlorine pesticides. Eggs in stage 17, the temperature-sensitive period, were spotted with 5 L of

organochlorine (including chlordane) in DMSO, with the solvent as a negative control and estradiol in DMSO as a positive control. The sex ratio among the ensuing progeny gave a measure of the degree of resulting "feminization," an excess of which is likely to be detrimental to the population. Greater than 35 percent of the eggs incubated at 28.2°C hatched as female turtles, although eggs incubated at this temperature normally yield male offspring (0 percent females were obtained in control incubations). The ability of chlordane in this system to mimic estradiol and override the temperature dependent sex determination of the red-eared slider turtle demonstrates the possible capacity of this and other organochlorine pesticides to disrupt developmental responses governed by the levels of estrogens. Although these data strongly suggest that chlordane could potentially alter turtle sex ratios, the study design is unsuitable for TRV derivation since the exposure included multiple organochlorine compounds and was did not follow a dose-response design.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Developmental effects in rats of pre- and postnatal maternal exposure to chlordane (Narotsky and Kavlock 1995) were chosen as a basis for the TRV. Chlordane exposed dams showed decreased weight gain and had fewer live litters and fewer live pups on postnatal day 6 compared to control dams (Narotsky and Kavlock 1995). Since the developmental effects were apparent during postnatal maternal exposure, it was hypothesized that exposure of pups occurred via the mother's milk. This result was also supported by a previous study (Ingle 1952). Decreased reproductive output in natural populations is likely to result in deleterious population-level effects, hence, this endpoint is suitable for TRV development based on previously outlined criteria (USACHPPM 2000). Although the developmental effects occurred once pups were born, the weanling stage can be considered a sensitive life stage since it is short in duration and pups are entirely dependent on the mother for survival. Therefore, this exposure is a surrogate for chronic exposure. Because the dose-response data does not include a No-observable-adverse-effect-level (NOAEL), the TRV was derived using the NOAEL-LOAEL approach and the results are presented in Table 3. The LOAEL for the developmental and growth effects was 21 mg chlordane/kg-d, which is the value for the LOAEL-based TRV. The NOAEL-based TRV is 2.1 mg chlordane/kg-d obtained from by dividing the LOAEL by an uncertainty factor of 10 (USACHPPM 2000). This TRV is assigned a medium confidence rating due to a shortage of longer-term studies on other mammalian species.

Table 3. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
NOAEL-based	2.1 mg/kg-d	Medium
LOAEL-based	2.1 mg/kg-d	Medium

4. Important Research Needs

Data clearly indicates that chlordane is toxic to mammalian, avian and reptilian wildlife. However, few studies are available that provide data suitable for TRV development. The major research need regarding chlordane toxicity is more studies focused on relating dose to toxic effect. Mainly, oral toxicity studies on avian, reptilian and perhaps amphibian species are needed. In addition, to develop higher confidence TRVs, an emphasis on chronic toxicity data would be most useful.

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APPENDIX A

LITERATURE REVIEW

The following files were searched in DIALOG:

File 155 MEDLINE; File 156, TOXLINE, File 5 BIOSIS, File 35 Dissertation Abstracts, File 76 Life Sciences Collection, and File 185 Zoological Record.

The search strategy for **Amphibians & Reptiles**:

- ◆ The expression chlordane and the CAS numbers for the following isomers/formulations: technical chlordane, non-stereospecific chlordane, cis-chlordane, and trans-chlordane.
- ◆ AND (amphibi? or frog or frogs or salamander? or newt or newts or toad? or reptil? or crocodil? or alligator? or caiman? snake? or lizard? or turtle? or tortoise? or terrapin?)
- ◆ RD (reduce duplicates)

The search strategy for **Birds**:

- ◆ The expression chlordane and the CAS numbers for the following isomers/formulations: technical chlordane, non-stereospecific chlordane, cis-chlordane, and trans-chlordane.
- ◆ And chicken? or duck or duckling? or ducks or mallard? or quail? or (japanese()quail?) or coturnix or (gallus(domesticus) or platyrhyn? or anas or aves or avian or bird? or (song()bird?) or bobwhite? or (water()bird) or (water()fowl)
- ◆ AND (reproduc? or dietary or systemic or development or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- ◆ RD (reduce duplicates)
- ◆ NOT (human? or culture? or (cell()line) or gene or vitro or inject or subcutane? or skin? or cancer? or salmonella or carcin? or tumo?)

WILDLIFE TOXICITY ASSESSMENT FOR CHLORDANE

- ◆ NOT (bioaccumulate? or bioconcentrat? or soil or media or fish or lake or wetland? or ocean or atmosphere?)

The search strategy for **Wild Mammals:**

- ◆ The expression chlordane and the CAS numbers for the following isomers/formulations: technical chlordane, non-stereospecific chlordane, cis-chlordane, and trans-chlordane.
- ◆ AND (didelphidae or opossum? or soricidae or shrew? Or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae) or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or myocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?
- ◆ AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- ◆ RD (reduce duplicates)

The search strategy for **Laboratory Mammals:**

- ◆ The expression chlordane and the CAS numbers for the following isomers/formulations: technical chlordane, non-stereospecific chlordane, cis-chlordane, and trans-chlordane.
- ◆ AND (rat or rats or mice or mouse or hamster? or (guinea()pig?) or rabbit? or monkey?)
- ◆ AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- ◆ NOT (human? or culture? or subcutaneous or vitro or gene or inject? or tumo? or inhalation or carcin? or cancer?)/ti,de

WILDLIFE TOXICITY ASSESSMENT FOR CHLORDANE

- ◆ NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)
- ◆ RD (reduce duplicates)

The strategy outlined above yielded 52 hits for chlordane with reptiles/amphibians, 158 articles with birds, 55 with wild mammals and 128 articles with laboratory mammals.

All abstracts from the DIALOG search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

As noted above and in Section 2.1, 393 hits on chlordane were obtained in the initial search, of which 129 were selected (Tier 2) as being relevant to this survey of the impacts of chlordane on wildlife.