TOXICOLOGICAL REVIEW

of

CHLORDANE (TECHNICAL)

(CAS No. 12789-03-6)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

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U.S. Environmental Protection Agency
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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard identification and dose-response information in the Integrated Risk Information System pertaining to chronic exposure to chlordane. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of chlordane.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response (U.S. EPA, 1995a). Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the individual assessments and to aid and guide the risk assessor in the ensuing steps of the risk assessment process. For other general information about this assessment or other questions relating to the Integrated Risk Information System, the reader is referred to EPA's Risk Information Hotline at (202) 566-1676.

1.0 INTRODUCTION

Chlordane was first produced in 1947 and was used as an insecticide for agricultural crops and livestock, for lawns and gardens, and also for underground treatment around the foundation of homes. In 1978, because of concern over cancer risk, evidence of human exposure and danger to wildlife, EPA canceled its use on food crops and phased out its other above-ground uses. From 1983 to 1988 its only approved use was as a termiticide around home foundations, and all uses were canceled after 1988. However, residues still exist in soils and sediments and chlordane bioaccumulates in fatty tissue of fish and humans; this bioaccumulation is a source of current concern.

This Toxicological Review is the background document on chlordane from which the three 1997 summary assessments on the Integrated Risk Information System (IRIS), the oral reference dose (RfD), inhalation reference concentration (RfC), and cancer assessment, are derived. These RfD and cancer summaries replace the information entered on the IRIS in 1993, and the RfC summary is new.

The RfD and RfC provide information on long-term toxic effects other than carcinogenicity. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of milligrams per kilogram per day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC is analogous to the oral RfD. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory). It is expressed in units of milligrams per cubic meter.

The carcinogenicity assessment provides information on three aspects of the carcinogenic risk assessment for the agent in question: (1) the EPA classification (2) quantitative estimates of risk from oral exposure and (3) inhalation exposure. The classification reflects a weight-of-evidence judgement of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of the application of a low-dose extrapolation procedure and is presented as the risk per milligrams per kilogram per day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ of air breathed. The third form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000 or 1 in 1,000,000.

Development of these hazard identifications and dose-response assessments for chlordane has followed the general guidelines for risk assessments as set forth by the National Research Council (1983). Other EPA guidelines that were used in the development of this assessment include the following: Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), (new) Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991), Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996b), Interim Policy for Particle Size and Limit

Concentration Issues in Inhalation Toxicity (U.S. EPA, 1994a), (Proposed) Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1995b), Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994b), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. EPA, 1988a) and Use of the Benchmark Dose Approach in Health Risk Assessment (U.S. EPA, 1995c).

The literature search strategy employed for this compound was based on the Chemical Abstract Service Registry Number (CASRN) and at least one common name. As a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, and MEDLINE and MEDLINE backfiles.

Information submitted by the public as a consequence of the Federal Register notification on the development of the IRIS file for this chemical (U.S. EPA, 1996b) was also utilized and considered in the development of this document.

2.0 CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

The U.S. EPA (1979) considers technical chlordane (CAS No. 12789-03-6) to be composed of 60% octachloro-4,7-methanotetrahydroindane (the *cis* and *trans* isomers) and 40% related compounds.

The term chlordane in association with CAS No.57-74-9 refers to a mixture of chlordane isomers, other chlorinated hydrocarbons and numerous other components. For example, the mixture used by the National Cancer Institue (NCI) in its 1977 bioassay was described as 94.8% chlordane (*cis* [or alpha]-chlordane, 71.7%; *trans* [or gamma]-chlordane, 23.1%) with heptachlor, 0.3%; *trans*-nonachlor, 1.1%; *cis*-nonachlor, 0.6%; chlordene isomers, 0.25%; 3% other compounds, and hexachlorocyclopentadiene, 0.25% (NCI, 1977).

Technical chlordane, CAS No. 12789-03-6, is a mixture of chlordane and chlordane related compounds having a lower percentage of the *cis* and *trans* isomers and a larger percentage of other compounds relative to mixtures with the above CAS number. Dearth and Hites (1991) identified 147 different compounds in a preparation of technical chlordane. The identity and percent of total for the 12 most abundant compounds were: *cis*-chlordane, 15%; *trans*-chlordane, 15%; *trans*-nonachlor, 9.7%; octachlordane, 3.9%; heptachlor, 3.8%; *cis*-nonachlor, 2.7%; "compound K," 2.6%; dihydrochlordene, 2.2%; nonachlor III, 2%; and three stereoisomeric dihydroheptachlors totaling 10.2%. These 12 compounds comprise 67% of the mixture, and the remaining 33% of the mixture consists of 135 other compounds. Infante et al. (1978) reported another production sample of technical chlordane to have a composition of 38 to 48% *cis*- and *trans*-chlordane, 3 to 7 or 7 to 13% heptachlor, 5 to 11% nonachlor, 17 to 25% other chlordane isomers, and a small amount of other compounds. Unless otherwise indicated all studies described in this document were carried out with technical grade chlordane.

Other physical characteristics of chlordane relevant to this assessment include its solubility and vapor pressure. The log octanol/water partition coefficient has been estimated at 5.54 (ATSDR, 1994), indicating that it is insoluble in water and has the propensity to accumulate in fatty tissues. The vapor pressure for pure chlordane has been reported as 1E-5 and for technical grade chlordane 4.6E-4 mm Hg at 25 °C (IARC, 1991). Based on these values (and a molecular weight of 409), a saturated vapor concentration for pure chlordane would be around 0.2 mg/m^3, and for technical chlordane (same assumed molecular weight) around 10 mg/m^3. In inhalation studies carried out at or above this concentration the chlordane would be in both vapor and aerosolized particulate form, and at concentrations near or less than this value the chlordane would be predominately in vapor form. This is relevant as the form in which the compound is delivered (i.e., particle or vapor) affects the manner of calculating dose (U.S. EPA, 1994b).

At 25 °C and assumed molecular weight of 409, conversion factors are ppm (v/v) × 16.75 = mg/m^3 and mg/m^3 × 0.0597 = ppm (v/v).

3.0 TOXICOKINETICS RELEVANT TO ASSESSMENTS

The primary toxicokinetic determinant of chlordane and its metabolites is their partitioning into and persistence in fat tissue. Animal studies show rapid and efficient uptake of chlordane and relatively complete elimination of individual doses. Oxychlordane, an epoxide metabolite of chlordane, appears to be the most persistent metabolic chlordane residue in both animal and human tissues. Oxychlordane and heptachlor epoxide, the latter from heptachlor which is present in nearly all mixtures of chlordane (Section 2.0), are the metabolites of chlordane that are considered of primary toxicological significance.

Animal studies indicate that single doses of chlordane administered orally are well absorbed, extensively metabolized, distributed throughout the body, almost completely eliminated in 7 days, and leave residues in several tissues, predominately fat. Male and female Sprague-Dawley rats were orally administered single radiolabeled doses of a 3:1 mixture of *cis*- and *trans*-chlordane isomers and the individual isomers and their tissue concentrations and elimination monitored (Barnett and Dorough, 1974). The chemical nature of the excreted radiolabel was determined chromatographically. Single doses of the isomers were almost completely (> 90%) eliminated after 7 days, mostly via feces, with only 2 to 6% of this total eliminated in the urine. Approximately 15% of the radiolabel in the feces was as the administered compound, the remainder being mostly dechlorination products. Of the residues remaining in the tissues, the epoxide metabolite (oxychlordane) was the principal residue in all tissues examined, accounting for 32 to 35% of the total residue content present in the kidney and 53 to 63% in fat. Nye and Dorough (1976) examined the fate of endotracheally administered radiolabeled chlordane in anesthetized female Sprague-Dawley rats. Administration of the insecticide was as an aerosol (isomeric composition not given) and blood levels, tissue levels after 1 h, and excretion of radiolabel were studied. The chemical nature of the excreted radiolabel was not determined. No radiolabel was detected in exhaled air. Chlordane residues appeared rapidly in the blood, reaching maximum concentrations 2 to 5 min after administration. Blood levels began to dissipate immediately, but then slowly subsequent to the maximum. No elimination constants were calculated. One hour after administration, the lungs contained 24%, the liver 20%, the kidney

0.3%, and the bladder 0.1% of the total dose. Elimination was primarily via the feces with about 52% of the dose eliminated after 6 days, whereas the urine accounted for another 12% (for a total of 64% as compared to > 90% total in oral studies in 7 days).

Lipid partitioning of chlordane and its metabolites has been documented in both humans and animals. Concentrations of chlordanes (*cis*-chlordane, *trans*-chlordane, oxychlordane, and *trans*-nonachlor) detected in human cadaver liver samples were 17-fold higher when expressed on a fat rather than a wet weight basis (average concentration of 0.05 mg/kg fat versus 0.003 mg/kg wet weight) (Mussalo-Rauhamaa, 1991). This value is in concordance with the results of Khasawinah (1989) who reported this ratio to be 200 to 300/1 in rats and monkeys after 90 days of inhalation exposure to technical chlordane. Taguchi and Yakushiji (1988) found elevated chlordane residues, including oxychlordane and heptachlor epoxide, in the milk of women exposed to chlordane as a consequence of home termite treatment. After 56 days of feeding isomeric mixtures of chlordane (3:1, *cis*-:*trans*-) to rats, Barnett and Dorough (1974) observed concentrations of residues in the fat to be approximately 3-fold more than the concentration of chlordane in the feed, whereas concentrations in other organs were far less than the dietary concentrations levels (only 1/8 as high as liver).

The lipid partitioning nature of chlordane may also facilitate dermal absorption. Hirai and Tomokuni (1993) noted higher skin surface/blood ratios of chlordane and its metabolites among hospital outpatients (n = 196) whose homes had been treated compared with those whose homes had not been treated with chlordane.

Evidence from humans and animals indicates that oxychlordane is the most persistent chlordane metabolite. Oxychlordane and heptachlor epoxide have been reported in a large majority of adipose tissue samples (90 to 92%) taken at surgery from humans (Nomeir and Hajjar, 1987). In four pest control workers, plasma levels of components or metabolites of chlordane (*cis*- and *trans*-nonachlor, oxychlordane, heptachlor epoxide) were monitored over a period of eight mo after discontinuance of chlordane exposure subsequent to 6 to 8 years of employment (Takamiya, 1990). In all four workers concentration was highest for *trans*-nonachlor with oxychlordane, *cis*-nonachlor and heptachlor epoxide being present at about the same concentration. In three of the four workers, the plasma levels of these metabolites did not drop during the 8 mo exposure-free monitoring period. Among 51 pest control operators, *trans*-nonachlor was detected in 37% with a mean concentration range of 0.55 ppb, oxychlordane in 22% with a mean of 0.29 ppb, and heptachlor epoxide in 20% with a mean of 0.29 ppb (Saito et al., 1986). In male and female Sprague-Dawley rats that were fed isomeric mixtures (3:1, *cis*-:*trans*-) of chlordane at 1, 5, and 25 ppm for up to 56 days, fat concentrations of oxychlordane were essentially unchanged 56 days after withdrawal of the chlordane feeding, whereas other metabolites underwent nearly complete depuration in this period. Because of long retention time in adipose tissue, oxychlordane is believed to be more toxic than its parent isomers, which are eliminated relatively rapidly from the body (Satoh and Kikawa 1992); therefore, oxychlordane may be a major contributor to chlordane toxicity.

Oxychlordane was a minor residue (14 to 19% of the total) in tissues (adipose, plasma, liver, and red blood cells) of monkeys exposed to aerosolized chlordane (10 mg/m^3, 8 h/day, 5 days/week) for 90 days, whereas it was a major component (36 to 51% of the total) in these

same tissues from rats similarly exposed (Khasawinah, 1989). Thus, rat tissues appear to have a much greater level of metabolic activity towards the precursors of oxychlordane (i.e., chlordane) than do monkey tissues. In a subgroup of rats examined 90 days after this exposure, the figure for oxychlordane had risen to 85 to 92% of the total as a result of its selective retention over other residues. *Trans*-nonachlor, a component of technical chlordane, was the major residue in the sampled monkey tissues comprising 38 to 62% of the total; for rats the corresponding figure was 2 to 13%. Minor quantities of other unidentified chlordane metabolites are excreted in the urine and feces (Barnett and Dorough, 1974) and also may be stored in the body fat for long periods of time. Alpha (*cis*)- and gamma (*trans*)-chlordane tend to concentrate in muscle of mice following oral administration (Satoh and Kikawa, 1992).

Adeshina and Todd (1991) developed a methodology to estimate daily human doses of chlordane from all routes of exposure. Although their method implies that the fat concentration after steady state conditions have been reached is directly proportional to the daily intake, human data on dieldrin, which they cite as a chlordane analogue, indicates a nonlinear relationship. This discrepancy can not be resolved until the long time (9 to 12 mo or longer in humans) required to attain asymptotic levels (or steady state) in fat is taken into account.

The major metabolites of *cis*- and *trans*-chlordane following incubation of the compounds with human liver microsomal preparations are oxychlordane, chlordene chlorhydrin, and monohydroxylated dihydrochlordene (Tashiro and Matsumura, 1978). The metabolite pattern was similar for both isomeric forms.

4.0 HAZARD IDENTIFICATION

4.1 Studies in Humans

4.1.1 Noncancer Effects in Humans

Kilburn, K.H. and J.C. Thornton. 1995. Protracted neurotoxicity from chlordane sprayed to kill termites. Environ. Health Perspect. 103(7-8): 690-694.

Batteries of neurophysiological and neuropsychological tests were performed on 216 adult occupants (109 women and 97 men) of an apartment complex, the exterior of which had been sprayed with an unknown concentration of chlordane 7 years earlier. Chlordane levels were assayed for 3 to 4 years after the initial spraying and showed indoor concentrations of chlordane as high as 13.6 µg/ 929 cm^2 (wipe samples) and indoor air levels reported as above 0.5 µg/ m^3 for 8-h samples. The duration of the individuals' residence at the complex was not noted. Blood and fat samples from eight of these residents (number actually tested not given) showed measurable and elevated levels of heptachlor, oxychlordane, and *trans*-nonachlor. Testing and questionnaire administration were done in either Spanish or English. The exposed group was compared with 174 unexposed referents matched on age and educational level. Neurophysiological results showed significant ($p < 0.002$) slowed reaction times in both English tested (414 ms occupants versus 309 ms referents, simple; 639 ms occupants versus 564 ms referents, choice) and Spanish tested (456 ms occupants versus 334 ms referents, simple; 694 ms occupants versus 609 ms

referents, choice) subjects. Balance sway speed was significantly increased ($p < 0.01$) in both English tested (0.95 cm/s occupants versus 0.82 cm/s referents with eyes open; and 1.48 cm/s occupants versus 1.26 cm/s referents with eyes closed) and Spanish tested (0.96 cm/s occupants versus 0.81 cm/s referents with eyes open; 1.40 cm/s occupants versus 1.17 cm/s referents with eyes closed) subjects. Neuropsychological tests also showed deficits among exposed occupants relative to the referent population; both immediate and delayed verbal recall were significantly lower ($p < 0.04$) in both English tested (scores of 8.7 occupants versus 10.5 referents for immediate; 6.7 occupants versus 8.3 referents for delayed) and Spanish tested (scores of 8.4 occupants versus 10.4, immediate; 6.4 versus 8.5, delayed) occupants. Tests of cognitive function showed significant deficits between exposed and referent populations in vocabulary scores in both English tested (16.0 occupants versus 18.5 referents, $p = 0.0254$) and Spanish tested (11.6 occupants versus 17.5 referents, $p = 0.0001$) subjects. Tests also showed significant deficits between exposed and referent populations on digit symbol scores in English tested (48.5 occupants versus 57.5 referents, $p = 0.00005$) and Spanish tested (38.3 occupants versus 46.9 referents, $p = 0.0046$) subjects. Profiles of mood scores including those for tension, depression, anger, vigor, fatigue and confusion, were all significantly higher ($p < 0.0005$) for subjects of both languages than the corresponding referent populations. Confounding factors which could affect the results, such as alcohol consumption, illicit drug use, and neurological or psychiatric diseases, were addressed and dismissed as not having any influence on the results mostly because these factors were not different from those in the referent population. However, no dose-response information, no definitive exposure information (either levels or duration of exposure), or information about co-exposure to other neurotoxicants, such as lead, could be gleaned from this report and no effect levels were assigned.

These results show significant impairment of both neurophysiological and psychological functions in a nonoccupational population exposed to uncertain levels of chlordane, predominately through the inhalation route via indoor air. The most notable changes were slowing of reaction time, balance dysfunction, reductions in cognitive function, and deficits of immediate and delayed recall. These results indicate that neurological effects are a relevant endpoint in humans chronically exposed to airborne chlordane concentrations around 0.0005 mg/m^3. However, no dose-response information and no definitive exposure information (either levels or duration of exposure) could be gleaned from this report, and no effect levels can be assigned.

Alvarez, W.C. and S. Hyman. 1953. Absence of toxic manifestations in workers exposed to chlordane. Arch. Ind. Hyg. Occup. Med. 8: 480-483.

Twenty-four male workers at a chlordane manufacturing plant underwent examinations that included chest X rays, liver function tests (sulfobromophthalein dye excretion, thymol turbidity tests, total bilirubin in blood), hemoglobin concentration, and urinalysis. Unspecified neurological examinations were also performed. The workers had been employed from periods of 2 mo to 5 years and had been exposed to chlordane through both inhalation and dermal absorption, although no exposure levels are given. Chest X-ray films revealed minimal shadows in the lungs of 7 workers, although these were not considered to be from chlordane exposure. No evidence of injury to the liver, kidneys, or blood-forming organs was indicated by any of the tests. No indications of any neurological disturbances were noted.

Fishbein, W.I., J.V. White, and H.J. Isaacs. 1964. Survey of workers exposed to chlordane. Ind. Med. Surg. 33: 726-727.

Fifteen male workers at a chlordane manufacturing plant underwent liver function tests (sulfobromophthalein dye excretion), EKGs, urinalysis, and had a chest X ray taken. A routine physical examination and medical history were also carried out. The duration of employment for 14/15 of the workers was from 9 to 16 years, the other being employed for only 2 years. Four air samples for chlordane were taken that ranged from 1.2 to 1.7 $\mu g/m^3$. All organ function tests and results from physical examinations were normal. No gastrointestinal or nervous system disturbances were noted, although it is not apparent to what extent they were examined for these symptoms.

Menconi, S., J.M. Clark, P. Langenberg, and D. Hryhorczuk. 1988. A preliminary study of potential human health effects in private residences following chlordane applications for termite control. Arch. Environ. Health 43(5): 349-352.

A cross-sectional study of 85 chlordane-treated houses (self-selected, totaling 261 people) was performed via a questionnaire. Indoor air sampling from 107 homes (including those 85 answering the questionnaire) allowed for three categories of exposure levels: $< 1\ \mu g/m^3$, 1 to 5 $\mu g/m^3$, and $> 5\ \mu g/m^3$. These levels were reported by the authors as unrelated to time after application, as chlordane levels decline very slowly. The range of time between completing the questionnaire and the last termite control application, 1 to 24 years (mean 3.8 years.), may be viewed as duration of exposure. Incidence rates of acute and chronic conditions from the questionnaires were compared to rates from the 1979 U.S. Health Interview Survey. Incidence rates for a number of conditions including anemia, migraine, bronchitis, sinusitis, and dermatitis were found to be significantly elevated ($p < 0.05$) over those noted in the Health Interview Survey. When smoking, age, and sex were controlled for the increased rates for sinusitis, bronchitis, and migraine remained significant ($p < 0.05$) for the upper two exposure categories. However, this study population was self-selected and not random and any results are subject to bias. No effect levels are assigned to this study.

Aldrich, F.D. and J.H. Holmes. 1969. Acute chlordane intoxication in a child. Case report with toxicological data. Arch. Environ. Health 19(1): 129-132.

Garrettson, L.K., P.S. Guzelian, and R.V. Blanke. 1985. Subacute chlordane poisoning. J. Toxicol. Clin. Toxicol. 22(6): 565-571.

Olanoff, L.S., W.J. Bristow, J. Colcolough, Jr., and J.R. Reigart. 1983. Acute chlordane intoxication. J. Toxicol. Clin. Toxicol. 20(4): 291-306.

These three studies report central nervous system effects including headaches, irritability, excitability, confusion, incoordination, muscle tremors, seizures, convulsions, and coma following acute oral exposure to insecticidal formulations of chlordane. In one incident (Aldrich and Holmes, 1969), intermittent clonic convulsions, incoordination, hyporeflexia, and increased excitability were exhibited by a 4-year-old girl who ingested a 45% chlordane emulsifiable concentrate at an estimated dose of 0.15 mg/kg.

Fleming, L.E. and W. Timmeny. 1993. Aplastic anemia and pesticides: An etiologic association? J. Occup. Med. 35: 1106-1116.

This review of 30 years of medical literature identified 280 cases of aplastic anemia associated with pesticide exposure including numerous organochlorines (including heptachlor/chlordane), carbamates, and organophosphates. For 90 of these cases, information about age, sex, and latency was available and, of these, four involved chlordane/heptachlor with the average age of 50 years and average latency 2 mo. These case studies indicate that insecticides (including chlordane/heptachlor) are associated with myelotoxicity but nearly always under conditions of acute high doses. Few cases, if any, appear to be associated with long-term low-level exposures. Only qualitative and anecdotal information about exposure levels is available.

McConnachie, P.R. and A.C. Zahalsky. 1992. Immune alterations in humans exposed to the termiticide technical chlordane. Arch. Environ. Health 47(4): 295-301.

Twenty-seven individuals who had been acutely exposed to technical chlordane either at home (23) or at work (4) underwent an immunological evaluation. Information given indicated that durations of exposure ranged from 3 days to 15 mo, and the length of time from exposure (beginning or end of exposure not indicated) to testing ranged from 4 mo to 10 years. Analysis of fat biopsies in 14 of the 27 individuals revealed detectable levels of *trans*-nonachlor and/or chlordane. All of these individuals complained of health problems (not described) that they attributed to chlordane. The exposed individuals differed significantly ($p > 0.01$) from a referent nonexposed population ($n = 118$) in distribution of lymphocytes and in their mean response to decreased function (decreased response to several foreign antigens). Test for autoantibodies were positive in 11 of 12 individuals tested although the rate in the referent nonexposed population is not given. These results indicate an association of immunologic dysregulation and acute exposure to technical chlordane.

4.1.2 Cancer Effects in Humans

4.1.2.1 Case-control studies

Cantor, K.P., A. Blair, G. Everett, R. Gibson, L.F. Burmeister, L.M. Brown, L. Schuman, and F.R. Dick. 1992. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res. 52: 2447-2455.

Cantor et al. (1992) compared 622 white men recently diagnosed with non-Hodgkin's lymphoma (NHL) in Iowa and Minnesota with 1,245 population-based controls matched with respect to age, vital status at the time of interview, and state of residence. Detailed interviews

over a period from 1981 through 1984 elicited information about their pesticide handling practices. Chlordane was one of 12 chemicals evaluated as animal (livestock) insecticides; the others were coumaphos, DDT, dichlorvos, famphur, flyspray (NOS), lindane, malathion, methoxychlor, nicotine, rotenone, and toxaphene. It was also one of 15 chemicals evaluated as crop insecticides; the others being aldrin, carbofuran, carboxyl, copper acetoarsenate, DDT, diazinon, dieldrin, fonofos, heptachlor, lindane, malathion, phorate, turbofos, and toxaphene. They found that among NHL cases the odds of chlordane use specifically as an animal insecticide were significantly greater than among controls (OR = 1.7, 95% CI = 1.0 to 2.9; 31 cases and 38 controls). The odds of chlordane use more generally as either an animal or a crop insecticide was also significant (OR = 1.8, CI = 1.1 to 3.1; 30 cases). The pattern of odds ratios for other chemicals studied were similar to chlordane, in that livestock use had greater odds ratios than crop use; this consistency indicated to the authors that the increased odds ratios were due to greater exposure with livestock than crops rather than some random error. In discussing the limitations of their study, the authors point out the difficulty of attributing the results to individual pesticides when the exposures were multiple, and they also point out the possibility that important associations may have been missed due to nondifferential exposure misclassification because of the difficulties in accurate recall of past pesticide exposures. They concluded that chlordane, as well as some other specifically mentioned pesticides, has an important role in the etiology of NHL among farmers.

Brown, L.M., A. Blair, R. Gibson, G.D. Everett, K.P. Cantor, M. Schuman, L.F. Burmeister, S.F. Van Lier, and F. Dick. 1990. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res. 50: 6585-6591.

In a study of similar design to that of Cantor et al. (1992), the authors conducted a population-based case-control study of leukemia among 578 white men with leukemia and 1,245 controls living in Iowa and Minnesota. No significantly elevated odds ratios for leukemia were associated with chlordane exposures. Chlordane was one of 17 crop insecticide exposures and one of 16 animal insecticide exposures evaluated.

Woods, J.S., L. Polissar, R.K. Severson, L.S. Heuser, and B.G. Kulander. 1987. Soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenoxyherbicide and chlorinated phenol exposure in western Washington. J. Nat. Cancer Inst. 78(5): 899-910.

Woods et al. (1987) published a population-based case-control study of 128 soft-tissue sarcoma cases and 576 NHL cases among men in western Washington state, where phenoxyherbicides and chlorinated phenols are widely used in the agricultural, forestry and wood products industries. There were 694 controls in the study and the case diagnoses were from 1981 through 1984. They investigated other chemical handling practices, including the use of chlordane, that potentially could modify or independently affect the risk of these diseases. Although their main interest was herbicides and phenols, one of their findings was an elevated, but not statistically significant, odds ratio of NHL among farmers who worked with chlordane (OR = 1.46, CI = 0.8 to 2.8). The pooled odds ratios for all occupations studied was 1.6. (0.7 to 3.8). This study is mentioned here in connection with chlordane use by farmers, not because it is strong evidence of chlordane risk, but because it supports the findings of Cantor et al. (1992), discussed above.

Zahm, S.H., D.D. Weisenburger, P.A. Babbitt, R.C. Saal, K.P. Cantor, and A. Blair. 1988. A case-control study of non-Hodgkin's lymphoma and agricultural factors in eastern Nebraska. Am. J. Epidemiol. 128: 901.

This abstract reported the results of a population-based case-control study of NHL in relation to agricultural factors in eastern Nebraska. Among the 385 cases and 1,432 controls in the study, the use of chlordane was one specific factor significantly associated with NHL (OR = 2.2, lower 95% confidence bounds is above 1.0). The use of three other insecticides was also significantly associated with NHL, and the authors also mentioned the use of three herbicides as other factors. As with the other studies in farmers, multiple exposures of individuals to chemicals in addition to the specific chemical identified is possible.

Zahm, S.H., D.D. Weisenburger, P.A. Babbitt, R.C. Saal, J.B. Vaught, K.P. Cantor, and A. Blair. 1990. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1(1): 349-356.

This subsequent publication of the results for eastern Nebraska showed a 50% excess risk of NHL associated with using the herbicide 2,4-D, and that the risk was not altered by considering exposure to chlorinated hydrocarbons as a class. Chlordane was not mentioned specifically.

Brown, L.M., L.F. Burmeister, G.D. Everett, and A. Blair. 1993. Pesticide exposures and multiple myeloma in Iowa men. Cancer Causes Control 4: 153-156.

Brown et al. (1993) published a population-based case-control study of multiple myeloma among 173 white men in Iowa compared with 650 controls in order to evaluate possible contribution of agricultural risk factors and exposure to individual pesticides. In 173 men with multiple myeloma (and 650 controls), no statistically significant elevated risks for multiple myeloma were found for farmers or individuals who handled either classes of pesticides or specific pesticides. Chlordane was one of seven animal insecticides evaluated, but no information about potential exposure levels was available.

Pesatori, A.C., J.M. Sonntag, J.H. Lubin, D. Consonni, and A. Blair. 1994. Cohort mortality and nested case-control study of lung cancer among structural pest control workers in Florida (United States). Cancer Causes Control 5: 310-318.

Pesatori et al. (1994) updated an earlier study of licensed structural pest control workers in Florida (Blair et al., 1983). The original cohort of 3,827 men was started in 1965 and was followed until 1977, and the 1994 study updated the follow-up period to 1982. In view of the elevated lung cancer mortality found in the original study, they conducted a nested case-control study of 65 lung cancer cases; 294 controls were interviewed. The interviews gathered information on tobacco use, occupation, work practices, and dietary habits. They found in the case-control study that tobacco use, diet, and other occupations had little effect on the excess lung cancer associated with occupational pesticide exposure. This excess was larger among those licensed before the age of 40 and those licensed for more than 20 years. Because of the small sample size, the use of individual pesticides could not be evaluated, but carbamates and phenoxyacetic acids were significantly elevated and use of inorganics, organobromides,

organochlorines, and organophosphates were nonsignificantly elevated in these lung cancer cases. Use of chlordane specifically was not associated with lung cancer.

4.1.2.2 Occupational cohort studies

Wang, H.H. and B. MacMahon. 1979a. Mortality of workers employed in the manufacture of chlordane and heptachlor. J. Occup. Med. 21: 741-744.

Wang and MacMahon (1979a) studied the mortality patterns of male workers in two plants, one in Illinois that manufactured chlordane and another in Tennessee which manufactured heptachlor and endrin. The authors did not have a measure of exposure to chlordane. The employees working from the start-up of the plants (1946 for the chlordane plant and 1952 for the other plant) until 1976 were eligible for the study. In the 113 deaths at the two plants, there was no statistically significant excess of deaths from cancer, even in those followed for more than 20 years after the beginning of employment.

Brown, D.P. 1992. Mortality of workers employed at organochlorine pesticide manufacturing plants - an update. Scand. J. Work Environ. Health 18: 115-161.

Ditraglia, D., D.P. Brown, N. Namekata, and N. Iverson. 1981. Mortality study of workers employed at organochlorine manufacturing plants. Scand. J. Work Environ. Health 7(suppl.): 140-146.

Brown (1992) reported follow-up data through 1987 of a cohort mortality study of white male workers employed at four organochlorine pesticide manufacturing plants, two of which were the same plants studied by Wang and MacMahon (1979a). The original study (Ditraglia et al., 1981) included all employees who worked at least 6 mo prior to 1964 and vital statistics were followed until 1976. There was no excess cancer mortality in either the original study or in Brown's follow-up study. The small number of deaths in the chlordane plant workers (159) available for analysis precludes the ability to make definitive conclusions about the effects of chlordane exposure.

Shindell, S. and S. Ulrich. 1986. Mortality of workers employed in the manufacture of chlordane: an update. J. Occup. Med. 28: 497-501.

Cancer mortality was studied in a cohort of 800 male employees who worked at a U.S. chlordane manufacturing plant for at least 3 mo during the years 1946 through 1985. Death certificates were available for 161 of the 181 deaths that occurred within the study period, and "reliable causes of death" were reported for an additional 11 deaths. Compared with U.S. death rates, no elevated risks for death from all causes or cancer were found in the cohort or in subcohorts comprised of either production workers or nonproduction workers. Average blood concentrations of oxychlordane and heptachlor epoxide measured in 1976 and 1979 among workers (n = 42 and 70) showed average levels were higher by 34 to 113% in production versus nonproduction workers. Although there was no elevated risk for death from heart disease in the cohort (76 observed versus 84.2 expected), elevated risk was found for death from stroke (20 observed versus 11.7 expected; $p < 0.01$).

Infante, P.F. and C. Freeman. 1987. Cancer mortality among workers exposed to chlordane. J. Occup. Med. 29(11): 908-909.

In a letter to the editor, Infante and Freeman (1987) challenged the results of Shindell based on calculation of expected rates according to published studies of this cohort, asserting there was an increasing trend of the standard mortality rate (SMR) with increasing time of employment. Shindell responded in the same 1987 publication with a table showing the expected rates and stating that there was no trend. These data, along with the results of a linear regression performed by the authors, is shown in Table 1.

Wang, H.H. and B. MacMahon. 1979b. Mortality of pesticide applicators. J. Occup. Med. 21: 741-744.

Wang and MacMahon (1979b) studied the prospective mortality of male pesticide applicators employed by three nationwide companies for at least 3 mo between 1967 and 1976. Of the 16,126 men in the study, there were 311 deaths with no statistically significant excess cancer mortality. A follow-up of this cohort to 1984 was published by MacMahon et al. (1988). A total of 1,082 deaths were ascertained and 994 death certificates were obtained. Expected numbers of death by cause were calculated based on U.S. national rates and compared with observed deaths in the cohort. Only one category of death, lung cancer, showed a significantly elevated SMR in the total cohort (SMR = 135; 90% CI = 114 to 158), and this excess was restricted to those employed for less than five years. Attributing that finding to chlordane exposure is unlikely, since there was no increase in lung cancer among termite control operators, the group most likely to be exposed.

Table 1. Observed and expected deaths (based on U.S. rates) from respiratory or other cancers in white male workers in a U.S. chlordane manufacturing plant, 1946-1985.

Years Employed (5-year intervals)	Respiratory Cancer			Other Cancer		
	Observed	Expected	SMR	Observed	Expected	SMR
0-4	3	6.1	49	7	11.1	63
5-9	1	1.3	77	3	2.6	115
10-14	2	1.5	133	4	3.2	125
15-19	2	1.2	167	3	2.5	120
20-24	2	1.6	125	2	2.8	71
25-29	1	1.7	59	2	2.7	74
30-34	1	1.0	100	3	1.0	300
35+	0	-	-	1	0.3	333

Total	12	14.4	83	25	26.3	95

Note: Linear regression analysis of employment duration class (up to 35 years) versus SMRs for respiratory cancer or other cancers found no statistically significant trend for increasing SMR for respiratory cancer ($p = 0.67$) or other cancers ($p = 0.20$) with increasing duration of employment.

Source: Shindell and Ulrich (1986).

4.1.2.3 Case reports

Epstein, S.S. and D. Ozonoff. 1987. Leukemias and blood dyscrasias following exposure to chlordane and heptachlor. Teratogen. Carcinogen. Mutagen. 7(6): 527-540.

Epstein and Ozonoff (1987) presented 25 cases of blood dyscrasias (thrombocytopenic purpura, aplastic anemia, and leukemia) following acute exposure to chlordane/heptachlor. Four of these were leukemia cases. In 16 of the 25 cases, no exposure in addition to chlordane or heptachlor was reported and over 75% of these involved homeowners following termite treatment, and garden and lawn applications. No other exposure information is given. These results were included in the review of Fleming and Timmeny (1993), discussed above.

Infante, P., S.S. Epstein, and W.A. Newton, Jr. 1978. Blood dyscrasias and childhood tumors and exposure to chlordane and heptachlor. Scand. J. Work Environ. Health 4: 137-150.

The authors presented clinical case histories of five children under the age of seven with neuroblastoma, all of whom had been exposed to chlordane in household situations, either in utero (three cases) or post-natally. They also presented three cases of aplastic anemia, with evidence of exposures to other pesticides in addition to chlordane in two of them. Three cases of leukemia were presented also, two of which had no known exposure to other agents besides chlordane. (Also discussed in the review by Fleming and Timmeny [1993], above.)

Chadduck, W.M., S.M. Gollin, B.A. Gray, J.S. Norris, C.A. Araoz, and A.F. Tyrka. 1987. Gliosarcoma with chromosome abnormalities in a neonate exposed to heptachlor. Neurosurgery 21(4): 557-559.

Chadduck et al. (1987) discussed the case of an infant boy with gliosarcoma, an uncommon pediatric malignant brain tumor. The mother had consumed large amounts (over 2 quarts/day) of milk from a local dairy throughout pregnancy which was found to be contaminated with heptachlor. The authors stated that causal attribution to heptachlor was not possible from this case, but that further study was needed.

Caldwell, G.G., S.B. Cannon, C.B. Pratt, and R.D. Arthur. 1981. Serum pesticide levels in patients with childhood colorectal carcinoma. Cancer 48: 774-778.

The authors obtained chemical exposure histories of nine adolescent colon cancer patients treated at a hospital in Tennessee during the years 1974 through 1976. Eight of them had lived on farms and had been exposed to pesticides. Analysis of the serum of patients and their families for

DDT, dieldrin, beta-hexachlorocyclohexane, and heptachlor epoxide showed no overall differences from 24 adolescent controls living in the same region, and both patients and controls had levels similar to Mississippi state averages. Therefore, the data provide no evidence that these pesticides contribute to this rare form of cancer.

Teufel, M., K.H. Niessen, J. Sartoris, W. Brands, H. Lochbühler, K. Waag, P. Schweizer, and G.V. Oelsnitz. 1990. Chlorinated hydrocarbons in fat tissue: Analyses of residues in healthy children, tumor patients and malformed children. Arch. Environ. Contam. Toxicol. 19: 646-652.

This study found no differences in fatty tissue concentrations of chlorinated hydrocarbons (including heptachlor and heptachlor epoxide) among three groups of children: 183 healthy children, 33 children with congenital malformations or benign tumors, and 46 children with malignant tumors. This study does not provide data to support an association between chlordane or heptachlor and cancer.

Falck, F., A. Ricci, M.S. Wolff, J. Godbold, and P. Deckers. 1992. Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. Arch. Environ. Health 47: 143-146.

This study found no statistically significant differences in levels of chlordane residues (heptachlor epoxide, oxychlordane, *trans*-nonachlor) in breast fat from 20 women with malignant breast disease compared with 20 women with benign breast disease (predominately nonproliferative fibrocystic changes). They did find higher levels of DDE and PCBs in cancer patients than in women with benign disease. The study fails to make any association between chlordane residue levels and breast cancer incidence.

4.2 Subchronic and Chronic Studies and Cancer Bioassays in Animals—Oral and Inhalation

Khasawinah, A.M., C. Hardy, and G. Clark. 1989a. Comparative inhalation toxicity of technical chlordane in rats and monkeys. J. Toxicol. Environ. Health 28: 327-347.
(The 90-day rat study.)

Wistar rats (35 to 47/sex/group) were exposed to 0, 0.1, 1.0, or 10 mg/m^3 technical chlordane, 8 h/day, 5 days/week, for 13 weeks (adjusted to continuous 24 h exposures to 0.024, 0.24, or 2.4 mg/m^3), followed by a 13-week recovery period. The control group was exposed to an air-only chamber. Monkeys were exposed concurrently with the rats. The test material was produced by stainless steel concentric jet atomizers. The polydisperse aerosol of chlordane was introduced into a elutriating glass column in which nonrespirable droplets of chlordane were removed by sedimentation and impaction. Evaporation of chlordane also occurred in an elutriating column, enhancing the proportion of chlordane as vapor. The aerosol at the top of the column was then introduced into the exposure chambers. The concentration of chlordane in the chamber was determined at least three times during each 8-h exposure. Chlordane concentration was determined by summation of six peaks termed as characteristic of technical chlordane from gas chromatography analysis, which included both chlordane isomers, *trans*-nonachlor, and heptachlor. Particle size distribution based on mass deposition was determined at least once per

week during the study and reported as 91.9 and 82.9% less than 5.5 µm in aerodynamic diameter for the high- and intermediate exposure groups, respectively. Ancillary information on particle sizing and characterization was included in an unpublished version of this study (Velsicol Chemical Corporation, 1984). A three-stage liquid impinger was used to test particle size distribution twice each week for the first week and once each week after that for a total of 17 determinations. These data showed that, at 1 mg/m^3 chlordane, 51.4% of the particle mass was <2 µm, 31.5% was between 2 and 5.5 µm, and 17.2% was >5.5 µm. This information was used to obtain an approximate mass median aerodynamic diameter (MMAD) of 1.8 µm and a corresponding particle distribution estimate (sigma g) of 3.1, which allow estimation of a regional deposited dose ratio (RDDR) (Section 5.2.3; U.S. EPA, 1994b). Optical counting and sizing of particles from air samples taken from both the low-dose and control chambers showed that nearly all particles were less than 0.5 µm in diameter and that counts were highly variable. This finding gives little evidence of particulate chlordane exposure at the lowest dose level. As the vapor state would predominate at this concentration (vapor pressure supporting >0.2 mg/m^3, see Section 2), and as 0.5 µm is in the range of diffusion, the concentration of chlordane at this lowest concentration is considered to be totally vapor.

Rats were sacrificed at 9 (5/sex/group) and 13 weeks (15/sex/group) of exposure, and 13 weeks postexposure (9/sex/group). Blood chemistry and urinalysis were performed before and at 5 and 13 weeks of exposure. There were no exposure-related effects on mortality, clinical signs (except sensitivity to touch at the highest concentration), food consumption, body weight, ophthalmoscopy, rectal temperature, or urinalysis. White blood cell (WBC) parameters were affected in female rats at Week 13; elevated total WBC and lymphocyte counts occurred in the 1 mg/m^3 (WBC, 10.8 versus $7.1 \times 10^3/mm^3$ controls; lymphocyte, 8.23 versus $5.45 \times 10^3/mm^3$ controls) and 10 mg/m^3 (WBC, 11.5 versus $7.1 \times 10^3/mm^3$ controls; lymphocyte, 8.60 versus $5.45 \times 10^3/mm^3$ controls) females, and increased neutrophil count (2.61 versus $1.42 \times 10^3/mm^3$ controls) occurred in the 10-mg/m^3 females. Hematological changes were noted predominately in females and included decreased mean corpuscular hemoglobin concentration at all exposure levels (8%) that was not concentration-related and reduced hemoglobin at 1 (8%) and 10 mg/m^3 (9%). None of these effects are considered adverse as they are all minor changes, do not follow any clear concentration-response, and are all within normal ranges for these parameters: WBC 3 to $15 \times 10^3/mm^3$, lymphocyte 4 to $10 \times 10^3/mm^3$, neutrophils 1.1 to $4 \times 10^3/mm^3$ (Mitruka and Rawnsley, 1981). Thrombocytopenia was also noted in females exposed to the two highest exposure concentrations where platelet counts were decreased 25 and 35% ($p < 0.01$) respectively, from the control count. This decreased count was not accompanied by any significant increase in clotting time even at the highest concentration tested and the difference was no longer apparent 13 weeks postexposure. At 13 weeks of exposure there were several blood chemistry indications of hepatic functional alteration restricted to the highest level of chlordane. These included a significant decrease in glucose (83% of control for females), increased globulins (114% of control for males), increased total protein (104% of control for males), decreased albumin (95% of control for males), an increase in cholesterol (158% of control for females), and an altered albumin/globulin ratio.

Histopathology was performed on all tissues, including nasal passages (number of levels not noted), pharynx, larynx, trachea, lungs, and bronchi, in the control and high concentration group. No effects were observed on the respiratory system. Because effects (apparent increased

incidence of liver and thyroid enlargement at the highest dose) were observed in the liver and thyroid during the 9-week interim sacrifice, these organs were examined in all groups at 13 weeks of exposure and 13 weeks postexposure. At the end of the exposure period, there was a statistically significant increase ($p < 0.05$) in the incidence of rats with centrilobular hepatocyte enlargement (or hypertrophy), which was observed in all rats of the 10-mg/m^3 group (minimal severity) and in 5/15 males and 5/15 females of the 1 mg/m^3 group (slight severity). No animals were affected in the control or 0.1 mg/m^3 groups. A polynomial model was fit to these data to estimate the air concentration corresponding to a 10% incidence of hypertrophy; the result is 0.64 mg/m^3. This information is used in Section 5.3.3 to compare with the oral dose giving the same toxic effect in the Khasawinah and Grutsch (1989b) study. At 27 weeks (13 weeks postexposure) only one female rat in the 10 mg/m^3 group had slight hepatocyte enlargement. Increased liver weights ($p < 0.01$) were observed for male and female rats exposed to 10 mg/m^3 at weeks 9 and 14, but weights were similar to those of the controls by week 27. Exposed rats also exhibited a concentration-related increase in both hepatic cytochrome P-450 concentration and microsomal protein. This response of induction involving reversible liver cell hypertrophy and liver enzymes is considered to be an adaptive response indicative of systemic exposure to chlordane. Kidney weights were significantly elevated ($p < 0.05$) in the 1 and 10 mg/m^3 males at 9 weeks, and in females and males exposed to 10 mg/m^3 at week 14 ($p < 0.01$), but no histopathological lesions were observed. Males also exhibited slightly increased height of the follicular epithelium of the thyroid in 1/15 animals exposed to 1 mg/m^3, and in 11/15 animals exposed to 10 mg/m^3, although no abnormalities were noted in animals examined 13 weeks postexposure. The lowest-observed-adverse-effect level (LOAEL) of 10 mg/m^3 chlordane was determined based on the wide range of biochemical indicators indicating hepatic functional alteration at this concentration. The no-observed-adverse-effect level (NOAEL) was 1 mg/m^3.

Khasawinah, A.M., C.J. Hardy, and G.C. Clark. 1989b. Comparative inhalation toxicity of technical chlordane in rats and monkeys. J. Toxicol. Environ. Health 28: 327-347. (The 28-day rat study.)

In a 28-day range-finding study, Wistar (Crl:Cobs WI Br strain) rats (10/sex/group) inhaled 0, 5.8, 28.2, 154, or 413 mg/m^3 technical chlordane, 8 h/day, 5 days/week. Body and organ weights and food and water consumption were measured and hematology, urinalysis, and microscopic examination were performed. Because of deaths (incidence not reported) occurring early in the study (females dying earlier than the males), animals in the 154 and 413 mg/m^3 groups were sacrificed after exposures 11 and 3, respectively. At 154 mg/m^3, abnormal respiratory movements and excessive salivation occurred in some animals, occasionally followed by convulsions and death. Reduced weight gain and food consumption were also observed in the groups exposed to the two highest concentrations. No deaths or significant weight loss were observed in the 5.8 and 28.2 mg/m^3 groups. There were no exposure-related hematological alterations. There were, however, several blood chemistry indications of hepatic functional alteration in rats exposed to 28.2 mg/m^3 including a significant decrease in glucose (73% of control for males and 78% of control for females), increased globulins (116% of control for males and 119% of control for females), increased total protein (116% of control for females), increased albumin (117% of control for females), an increase in cholesterol (133% of control for females), and an altered albumin/globulin ratio. An increase in liver weight was noted in the 5.8 mg/m^3 males and the 28.2 mg/m^3 males and females, as well as in surviving rats of the 154 and 413

mg/m^3 groups (no specifics given). Increased kidney and thyroid weight and decreased thymus weight were also exhibited in the 28.2 mg/m^3 group. Livers were enlarged in the 154 mg/m^3 males and discolored in the 154 and 413 mg/m^3 males (no incidence given). As in the livers of rats exposed in the 90-day study, centrilobular hepatocyte enlargement (hypertrophy) was observed in the livers of an unspecified number of animals exposed to 28 mg/m^3. Centrilobular hepatocyte enlargement, with or without vacuolation, was observed among rats exposed to 154 mg/m^3. In addition to hepatocellular hypertrophy and vacuolation, necrosis was also noted among rats exposed to 413 mg/m^3. Effects observed in other organs (no incidence given) included increased follicular epithelial cell height in thyroid tissue and increased incidence of cervical lymph node enlargement in the 154 mg/m^3 males, and epithelial degeneration in bronchi/bronchioles and cell debris in bronchi, bronchioles, and alveoli of the 413 mg/m^3 animals. A LOAEL of 28.2 mg/m^3 chlordane was determined based on the wide range of biochemical indicators indicating alteration of hepatic function at this concentration. The NOAEL was 5.8 mg/m^3.

Khasawinah, A.M., C.J. Hardy, and G.C. Clark. 1989c. Comparative inhalation toxicity of technical chlordane in rats and monkeys. J. Toxicol. Environ. Health 28: 327-347. (The 90-day monkey study.)

Cynomolgus monkeys (6/sex/group) were exposed to 0, 0.1, 1.0, or 10 mg/m^3 technical chlordane (adjusted to continuous 24 h exposures to 0.024, 0.24, or 2.4 mg/m^3, respectively) for 90 days, 8 h/day, 5 days/week. Similar parameters were examined in the monkeys as those reported for rats. The respiratory system (and pulmonary function as indicated by blood gas analysis, lung mechanics, and pulmonary ventilation) of the monkeys was unaffected by inhalation exposure to chlordane. Minor blood effects were noted between controls and some of the dose groups, including increases in mean corpuscular volume, decreases in WBC, alteration in prothrombin times and blood urea-nitrogen, and decreased platelet counts, although all values were within normal limits. The only effects were increased mean liver and thyroid weights for the 10-mg/m^3 group compared to controls; however, the weight changes were not statistically significant and did not correspond to any histopathological changes. Histopathological examination was the same as for rats save that only control and high dose groups were examined. There were no exposure-related histopathological findings observed in any animals. No adverse changes were noted and the NOAEL was 10 mg/m^3.

IRDC (International Research and Development Corporation). 1973. Eighteen-month oral carcinogenic study of chlordane in mice. Unpublished report to Velsicol Chemical Corporation. MRID No. 00067568. Available from U.S. Environmental Protection Agency.

U.S. EPA. 1986b. Carcinogenicity Assessment of Chlordane and Heptachlor/Heptachlor Epoxide. Washington, DC: Office of Health and Environmental Assessment. NTIS, PB87-208757.

Groups of 100 male and 100 female CD-1 mice were fed diets containing analytical grade chlordane at concentrations of 0, 5, 25 or 50 ppm (approximately 0, 0.71, 3.57, or 7.14 mg/kg-day) for 18 mo. Mice were 6 weeks of age when exposure began. A 6 mo interim sacrifice of 10 mice/sex/group did not reveal compound-related lesions. The terminal sacrifice was at 19.5 mo. No exposure-related changes in body weight gain or food consumption were observed.

Many mice were lost due to autolysis so that only about half of the mice were examined histologically. The number of mice for which tissue was available for histopathological analysis was less in the high dose group than in the other treated groups but comparable to that in controls. U.S. Environmental Protection Agency-sponsored examination of the liver histological slides found statistically significant increased incidence of hepatic carcinomas in males and females in the 25- and 50-ppm groups (see Table 2). No effects were observed other than cancer, even at the highest dose tested (50 ppm).

NCI (National Cancer Institute). 1977. Bioassay of chlordane for possible carcinogenicity. Technical Report Series No. 8. U.S. Department of Health, Education and Welfare; National Institutes of Health. PB 271 977.

Rat and mouse bioassays were conducted with analytical grade chlordane administered in the diet (71.7% *cis*-chlordane, 23.1% *trans*-chlordane, 0.3% heptachlor, 0.6% nonachlor, 1.1% hexachlorocyclopentadiene, and 0.25% chlordane isomers).

Groups of 50 male and 50 female Osborne-Mendel rats were fed low- or high-dose diets for 80 weeks, and then observed for 29 weeks. Chlordane dietary concentrations were reduced twice during the study because of obvious toxic effects. Time-weighted average concentrations were 203.5 and 407 ppm for males and 120.8 and 241.5 ppm for females. These concentrations correspond to approximate average doses of 10.2 and 20.4 mg/kg-day for male, and 6.0 and 12.1 mg/kg-day for female rats. Matched controls consisted of 10 male and 10 female rats.

Table 2. Incidence of liver lesions in CD-1 mice exposed to analytical chlordane in the diet for 18 mo.

	Males (ppm in diet)				Females (ppm in diet)			
Lesion	0	5	25	50	0	5	25	50
Hepatocyte hyperplasia	20/33	34/55	7/52	7/39	26/45	32/61	13/50	11/37
Hepatic nodules	1/33	6/55	3/52	0/39	0/45	1/61	4/50	0/37
Hepatic small carcinomas	0/33	3/55	9/52[a]	4/39	0/45	0/61	7/50[a]	2/37
Hepatic large carcinomas (≥5mm)	3/33	2/55	32/52[a]	28/39[a]	0/45	0/61	25/50[a]	24/37[a]
Total hepatic carcinomas	3/33	5/55	41/52[a]	32/39[a]	0/45	0/61	32/50[a]	26/37[a]

[a]Significantly increased relative to control incidence, one-tailed Fisher Exact test ($p < 0.01$); $p > 0.05$ for comparison between other elevated incidence and respective controls.

Source: U.S. EPA (1986b).

A pooled control group consisted of the matched-control rats and 50 male and 50 female untreated rats from similar bioassays conducted during periods that overlapped with the chlordane bioassay by at least a year. Surviving rats were sacrificed at 109 weeks. Major organs and tissues from sacrificed rats and from rats found dead were examined grossly and microscopically.

The average body weights of high-dose male and female rats were consistently lower (by about 10%) than that of control rats, throughout the study. Obvious clinical signs of toxicity (rough and discolored hair coats, palpable masses) occurred "frequently" in treated rats during the first year and increased in frequency during the second year. No statistically significant differences in survival were found for exposed male rats compared with control rats, but there was a statistically significant dose-related trend for decreased survival in exposed female rats. No information about the cause of death was given. No statistically significant increases of liver tumors or liver nonneoplastic lesions were found in exposed rats compared with incidence in control rats. Statistically significant increases of proliferative lesions of follicular cells of the thyroid and of malignant fibrous histiocytoma were found in exposed male rats compared with controls. The study authors concluded, however, that these differences were not biologically significant because the incidences were within the range of incidence for control rat populations. No other exposure-related neoplastic or nonneoplastic lesions were found in rats in this study. The NOAEL for noncancer effects is 12.1 mg/kg-day based on female rats.

Groups of 50 male and 50 female B6C3F1 mice were fed diets with low and high concentrations of analytical chlordane for 80 weeks, and then observed for 10 weeks. The low- and high-dose groups were tested at different calendar times, but each exposed group was tested with a concurrent control. Chlordane dietary concentrations were changed during the study. Time-weighted average concentrations were 29.9 and 56.2 ppm for male mice and 30.1 and 63.8 ppm for female mice. These concentrations correspond to approximate average doses of 4.3 and 8.0 mg/kg-day for male, and 4.3 and 9.1 mg/kg-day for female mice. Matched controls consisted of 10 male and 10 female untreated mice. A pooled control group consisted of the matched-control mice and 70 male and 80 female untreated mice from similar bioassays conducted during periods that overlapped with the chlordane bioassay by at least a year. Surviving mice were sacrificed at 90 to 91 weeks. Major organs and tissues from all mice were examined grossly and microscopically.

No significant exposure-related changes in average body weight were found in male or female mice. Survival of exposed female mice was essentially the same as control female mice, but survival of male mice in both exposed groups was significantly decreased relative to control males. Neurotoxicity (tremors) was observed in high-dose males and females after 20 weeks.

Exposure-related neoplastic or nonneoplastic lesions in mice were restricted to the liver. Hepatocytomegaly, nodular hyperplasia, and diffuse hyperplasia were found in exposed male and female mice, but statistically these incidences were not significantly different from controls (see Table 3). Statistically significant elevated incidences for hepatocellular carcinomas, relative to controls, were found in low- and high-dose male mice and in high-dose female mice (Table 3). Hepatocellular carcinomas were described as displaying a spectrum of histological features. At one end of the spectrum, nodules of hepatocytes with only moderate changes (from normal hepatocytes) in staining characteristics, size and shape of cells, and lobular architecture occurred. At the other end of the spectrum, tumors with clearly anaplastic cytologic characteristics and no resemblance to normal lobular architecture occurred. Metastasis to the lung occurred in two high-dose males and three high-dose females. No effects were observed other than cancer, even at the highest dose tested (50 ppm).

Table 3. Incidence of liver lesions in B6C3F1 mice exposed to analytical chlordane in the diet for 80 weeks

	Males (ppm in diet)				Females (ppm in diet)			
Lesion	Pooled Control	Matched Control	29.9	56.2	Pooled Control	Matched Control	30.1	63.8
Hepatocytomegaly	0/92	0/18	1/48	56.2	1/78	0/19	0/47	0/49
Diffuse hyperplasia	3/92	0/18	3/48	0/49	1/78	0/19	3/47	3/49
Nodular hyperplasia	3/92	2/18	7/48	0/49	2/78	1/19	3/47	3/49
Hepatocellular carcinoma	17/92	2/18	16/48[a]	43/49[b]	3/78	0/19	3/47	34/49[b]

[a]Different from pooled ($p = 0.04$) and matched ($p = 0.06$) controls; one-tailed Fisher Exact test.
[b]Different from pooled and matched controls ($p < 0.001$); one-tailed Fisher Exact test.

Source: NCI (1977).

Khasawinah, A.M. and J.F. Grutsch. 1989b. Chlordane: thirty-month tumorigenicity and chronic toxicity test in rats. Regul. Toxicol. Pharmacol. 10: 95-109.

Velsicol Chemical Corporation. 1983a. Thirty-month chronic toxicity and tumorigenicity test in rats by chlordane technical. Unpublished study by Research Institute for Animal Science in Biochemistry and Toxicology, Japan. MRID No. 00138591, 00144313. Available from U.S. Environmental Protection Agency.

ICF-Clement. 1987. Pathology peer review of chlordane in F344 rats. Pathology review participants: Goodman, D.G., A.W. Mackin, R.R. Maronpot, J.A. Popp, R.A. Squire, J.M. Ward, and M.R. Anver.

U.S. EPA. 1988b. Review of Pathology Working Group Slide Reevaluation of Livers of Rats in a 30-Month Oral Exposure to Chlordane. Office of Pesticides and Toxic Substances. Memorandum from Henry Spencer to George LaRocca, March 14.

In a 30-mo oral study, Fischer 344 rats (80/sex/group) were exposed to 0, 1, 5, or 25 ppm technical chlordane in the diet. Based on food consumption and body weight data, the study authors calculated the doses to be 0, 0.045, 0.229, and 1.175 mg/kg-day for males and 0.055, 0.273, and 1.409 mg/kg-day for females. Doses were based on a 4-week pilot study in which hepatic effects (increased liver cell volume and fatty degeneration) were seen at 50 ppm and higher. Hematology, biochemistry, urinalysis, organ weights, and pathology (of major tissues and organs) were assessed at weeks 26 and 52 (eight males and nine females/group), and on all survivors at week 130. Because the original 1983 pathology report was completed before the publication of the National Toxicology Program's diagnostic criteria for proliferative liver lesions, and because diagnostic discrepancies were found in the original pathology report, the sponsor of the study (Velsicol Chemical Corporation) convened a Pathology Working Group (PWG) of six U.S. pathologists to review the liver histopathological slides from this study. These slides were

evaluated on a coded “blind” basis without knowledge of dose group, age or sex. The PWG's liver pathology findings are reported herein. The assessment of chlordane in the U.S. EPA (1986b) report was based on the original pathology report (Velsicol Chemical Corporation, 1983a) and is superceded by the data presented here.

No treatment-related clinical signs, deaths, or biochemical and hematological parameters were observed. The original pathologist noted that exposure-related lesions were found only in the liver. Absolute liver weight was significantly increased ($p < 0.05$) in 5- and 25-ppm males (13.33 and 13.30 gm versus 10.44 gm in controls) at 130 weeks and in 25-ppm females at 26 weeks (5.02 gm versus 4.62 gm in controls) and 52 weeks (5.77 gm versus 5.27 gm in controls). These increases in liver weight among the 25-ppm but not the 5-ppm animals were accompanied by severe liver pathology. A high incidence of large granular lymphocyte leukemia was found in all groups of female and male rats, including controls (Table 4). Elevated incidence of leukemia in control F344 rats living beyond 104 weeks, relative to rats terminated at 104 weeks, has been reported as a common age-related occurrence that is accompanied by nonneoplastic liver lesions including necrosis, cytomegaly (i.e., swelling or hypertrophy) and fatty degeneration (Solleveld et al., 1984; Stromberg and Vogtsberger, 1983). Because age-related leukemia confounded exposure-related liver lesions, the PWG examined incidence for nonneoplastic liver lesions in rats without leukemia. Nonleukemic female rats in the 5 and 25 ppm groups had statistically significant increases in hepatocellular hypertrophy relative

Table 4. Incidence of nonneoplastic and neoplastic lesions in F344 rats[a] exposed to technical chlordane in the diet for 30 mo.

	Males (ppm in diet)				Females (ppm in diet)			
Lesion	0	1	5	25	0	1	5	25
Large granular lymphocyte leukemia	43/64	43/64	40/64	46/64	25/64	18/64	21/64	19/64
Hepatocellular adenoma	2/64	4/64	2/64	7/64[b]	0/64	1/64	0/64	0/64
Regional hepatocellular hypertrophy in nonleukemic livers	0/20	1/23	0/20	0/16	2/37	4/43	9/40[c]	15/44[c]
Foci of cellular alteration in nonleukemic livers	8/20	12/23	14/20	8/20	28/37	35/43	32/40	32/44

[a]Excludes the 16 interim-kill animals per group.
[b]One-tailed Fisher Exact test, $p = 0.08$.
[c]$p < 0.05$ for one-tailed Fisher Exact test; Cochrane-Armitage trend test for females was $p = 0.0003$.

Source: Khasawinah and Grutsch (1989b).

to controls, whereas nonleukemic females in the 1 ppm group, and nonleukemic males in the 1, 5, and 25 ppm groups, had no significant increases (Table 4). Overall incidence for hepatocellular adenomas in 25-ppm male rats (not including interim-kill rats) was increased by a marginal statistical significance relative to controls (7/64 versus 2/64; Table 4). Incidences for hepatic neoplasms in other exposed groups were not increased. According to the authors, no increased incidence of foci of cellular alteration in nonleukemic livers (the first stage of rat liver carcinogenesis) was found in exposed groups relative to controls.

Incidences for hepatocellular adenomas in the concurrent F344 rat controls (3.1% in males and 0% in females) were below historical values for control F344 rats in United States (range 0 to 17%) and Japanese laboratories (2.5% for males dying before 104 weeks and 9.6% for males in studies of longer duration). In consideration of this, and the marginal significance of the observed increased incidence of hepatocellular adenomas in 25-ppm males compared with controls ($p = 0.08$), the PWG concluded that chlordane treatment in this study did not produce a tumorigenic response in male or female F344 rats. The Toxicology Branch of EPA's Office of Pesticides and Toxic Substances concurred with this conclusion (U.S. EPA, 1988b). The PWG also concluded that regional hepatocellular hypertrophy was associated with administration of 5- or 25-ppm chlordane in the diet to female rats for 30 mo. Interpolation of the data on regional hypertrophy in female rats (Table 4) shows that at a dietary dose of 0.15 mg/kg-day (assuming 100% absorption of chlordane in the diet) the incidence is 10%. This information is used in Section 5.3.3 to compare with the inhalation dose giving the same toxic effect in the Khasawinah et al. (1989a) study. Thus, in female rats, 1 ppm (0.055 mg/kg-day) was the NOAEL and 5 ppm (0.273 mg/kg-day) the LOAEL for chlordane-induced nonneoplastic liver lesions.

Khasawinah, A.M. and J.F. Grutsch. 1989a. Chlordane: 24-month tumorigenicity and chronic toxicity test in mice. Regul. Toxicol. Pharmacol. 10: 244-254.

Velsicol Chemical Corporation. 1983b. Twenty-four month chronic toxicity and tumorigenicity test in mice by chlordane technical. Unpublished study by Research Institute for Animal Science in Biochemistry and Toxicology, Japan. MRID No. 00144312, 00132566. Available from U.S. Environmental Protection Agency.

ICR mice (80/sex/group) were given 0, 1, 5, or 12.5 ppm technical chlordane in the diet for 104 weeks. The study authors reported that average doses corresponded to 0, 0.15, 0.75, and 1.875 mg/kg-day, based on an assumed food consumption of 15% of the body weight per day. Dosages were chosen based on a 4-week pilot study in which a LOAEL of 25 ppm was determined for hepatic toxicity (increased hepatic cell volume). Hematology, biochemistry, urinalysis, organ weights, and pathology (of major tissues and organs) were assessed at week 52 (eight males and eight females/group) on all animals that died during the study, and on all survivors at week 104.

When male mice were analyzed without regard to the presence or absence of tumors, no consistent, exposure-related changes were found in clinical signs of toxicity, body weights, mortality, urinalysis parameters, or hematological parameters, except that 12.5-ppm male mice showed a statistically and probably biologically significant elevation in serum alanine aminotransferase activity (308 IU/L versus 102 IU/L in controls) at week 104. Statistically significant increased absolute and relative liver weights were seen in high-dose males (200 and 203%) and females (144 and 129%) compared to control values at the end of the study. Absolute (but not relative) liver weight was also significantly elevated ($p < 0.05$) in female mice exposed to 1 ppm (22%) and 5 ppm (14%). No consistent changes in other organ weights were found. When animals with adenocarcinomas were compared with those without adenomas or adenocarcinomas, however, the animals with malignant tumors had statistically significant elevations of all blood biochemistry measurements made (SGOT, SGPT, glucose, urea nitrogen, cholesterol, and protein). The authors concluded that the prevalence of the malignant tumors, and not the exposure to chlordane, altered the blood biochemistry parameters.

Exposure-related neoplastic and nonneoplastic lesions were restricted to the liver. Statistically significant increased incidences, relative to controls, were found for the following nonneoplastic lesions: hepatocellular swelling (hypertrophy) in male and female mice in the 5- and 12.5-ppm groups, hepatic fatty degeneration in 12.5-ppm males, and hepatic necrosis in 5- and 12.5-ppm male mice. The incidence for hepatic necrosis among male mice was 7/80 for the controls, 8/80 for the 1-ppm group, 25/80 for the 5-ppm group, and 27/80 for the 12.5-ppm group. These data are modeled in Appendix A. Male mice in the 12.5-ppm group showed statistically significant elevated incidence of hepatocellular adenomas (also called hepatocellular nodules by the study authors) and hepatic hemangiomas compared with controls (Table 5). The hepatic hemangiomas were described as benign tumors of capillary cells associated with the hepatocellular adenomas. The 1989 report mentions the occurrence of liver adenocarcinomas but does not present incidence data. However, the earlier report of this assay, Velsicol Chemical Corporation (1983a), cited by U.S. EPA (1986b), shows that the hepatocellular carcinoma

Table 5. Incidence of hepatocellular adenomas and hemangiomas in male ICR mice exposed to dietary chlordane for 24 mo.

Dietary Concentration	Incidence of Hepatocellular Adenomas	Incidence of Hemangiomas
0	12/79	4/79
1	14/79	1/79
5	14/80	8/80
12.5	27/80[a]	14/80[b]

[a]P < 0.01.
[b]P < 0.05.

Source: Khasawinah and Grutsch (1989a).

incidence in males in the 0-, 1-, 5-, and 12.5-ppm groups is 3/71, 3/71, 7/72, and 9/72, respectively. This data is used for the quantitative cancer risk estimates (Table 6d). One ppm chlordane (0.15 mg/kg-day) was the NOAEL, and 5 ppm chlordane (0.75 mg/kg-day) was the LOAEL for nonneoplastic liver lesions (hepatic necrosis) in male ICR mice.

Barrass, N., M. Stewart, S. Warburton, J. Aitchison, D. Jackson, P. Wadsworth, A. Marsden, and T. Orton. 1993. Cell proliferation in the liver and thyroid of C57B1/10J mice after dietary administration of chlordane. Environ. Health Perspect. 101(suppl. 5): 219-224.

Barrass et al. (1993) carried out two assays in which chlordane with a concentration of 50 ppm was fed to C57Bl/10J mice. The first was a 24-mo bioassay of chlordane in a group of 100 male mice, with histopathological analysis of liver sections. They found that in the animals surviving after 2 years of treatment the incidence of hepatocellular adenomas and carcinomas was 15/39 and 5/39, respectively. In the animals with unscheduled deaths during the assay, the incidence of adenomas and carcinomas was 1/40 and 2/40, respectively. The incidence of total liver tumors in a in concurrent colony control group was 8/400. Therefore, the incidence of adenomas and carcinomas combined was 23/79, which is statistically significant relative to controls. However, because the incidence of carcinomas alone in the control group was not given, the statistical significance of the carcinomas alone can not be calculated nor can the risk of carcinoma alone be determined.

The second assay was a 6-mo cell proliferation study with nine serial sacrifices in which replicating cells were labeled by infusing BrdU via subcutaneous mini-osmotic pumps 3 days before sacrifice. Thyroid and liver cell replication and histopathology were measured. In this assay, there were no morphological thyroid abnormalities; the thyroid labeling index reached a peak after 5 days and reached control levels after 99 days. Centrilobular hepatocellular hypertrophy was seen starting early and increasing throughout the experiment. The liver labeling index showed an initial peak phase lasting 4 weeks and a sustained elevated phase lasting for the entire 200-day period but gradually declining by day 200.

Malarkey, D.E., T.R. Devereux, G.E. Dinse, P.C. Mann, and R.R. Maronpot. 1995. Hepatocarcinogenicity of chlordane in B6C3F1 and B6D2F1 male mice: evidence for regression in B6C3F1 mice and carcinogenesis independent of ras proto-oncogene activation. Carcinogenesis 16: 2,617-2,625.

These authors reported the age-specific prevalence of hepatocellular adenomas, carcinomas and two measures of liver toxicity: centrilobular hypertrophy and "toxic change", which consists of cell necrosis, cell vacuolization and inflammation. These observations were made in males of two mouse strains, B6C3F1 (210 animals) and B6D2F1 (160 animals), fed diets containing 55 ppm of technical chlordane starting from 63 days of age and continuing until 568 days of age (72 weeks of treatment) and 668 days (86 weeks of treatment), respectively. This is the same dietary concentration used in the high-dose groups of the NCI (1977) study in B6C3F1 mice. The protocol included one group of B6C3F1 mice in which the chlordane was discontinued after 491 days of age (61 weeks of treatment). Control mice were not sacrificed until age 759 days (99 weeks of treatment) to obtain a sufficient number of spontaneous tumors for analysis of ras mutations. Mutations in K-ras and H-ras oncogenes were measured in spontaneous tumors from controls and chlordane treated animals.

They found that in both strains the prevalence of adenomas and carcinomas increased with time and that the B6C3F1 mice developed tumors about 100 days before the B6D2F1 mice. The prevalence was nearly 100% in both strains at terminal sacrifice. By week 86, the time of terminal sacrifice of the B6D2F1 mice, the prevalence of adenomas and carcinomas was 91%. After 72 weeks of treatment (age 568 days), which was about 8 weeks less than the duration of the NCI (1977) test, the prevalence of carcinomas in B6C3F1 mice was about 88%, as determined from the prevalence versus time graphs in the Malarkey et al. (1995) paper. This is the same as the incidence of tumors in the NCI bioassay, which was 43/49 (88%) after 80 weeks of treatment. Therefore, the incidence of carcinomas in the current study closely matches the experiment done under similar conditions 18 years earlier in mice of the same strain. Malarkey and co-workers also found that for the B6C3F1 mice the multiplicity of carcinomas was constant over time at an average of 1.5 tumors per tumor-bearing animal, whereas the multiplicity of adenomas increased from about two to four adenomas per tumor-bearing animal from 400 to 568 days. The multiplicity of both adenomas and carcinomas at the terminal sacrifice was higher in B6C3F1 (5.4 tumors per tumor-bearing animal) than in B6D2F1 mice (3.2 tumors per tumor-bearing animal). They found that the onset of chronic liver hyperplasia preceded the onset of tumors by over 100 days but that "toxic change" closely paralleled the prevalence of tumors. This indicates that liver toxicity is not a precursor of tumor development. The severity, but not the prevalence, of "toxic change" decreased after the prevalence of liver tumors reached 100% in the two strains.

In one group of B6C3F1 mice in which chlordane was withdrawn from the diet after 14 mo, the multiplicity of carcinomas alone remained at 1.5, but that of adenomas went down 22% from 3.2 to 2.5, and that of combined adenomas and carcinomas went down 30% (from 4.4 to 3.1). In these experiments, the prevalence of animals with carcinomas went down from 80 to 54%, and the prevalence of adenomas went down from 100 to 93%, whereas the prevalence of combined adenomas and carcinomas remained at 100%.

There were no mutations of H-ras or K-ras oncogenes in tumors of treated animals of either strain, whereas H-ras mutations occurred in 4/10 spontaneous tumors in control animals. They also observed that the proliferative lesions preceding tumor formation in treated animals consisted of large hepatocytes with acidophilic cytoplasm, whereas in control animals they were of normal size with basophilic staining. The authors concluded from the oncogene measurements and the histopathology of the liver lesions that the mechanism of chlordane-induced liver tumors was different than the mechanism of spontaneous tumors. This evidence, along with the evidence of regression of some chlordane-induced tumors, indicated to the authors that the tumorigenic pathways of chlordane-induced tumors are different than those associated with mouse genotoxic murine hepatocarcinogens. They cited a paper (Fox et al., 1990) that concluded that promoters or nongenotoxic carcinogens are not believed to directly induce point mutations in the ras gene. The authors postulate a progression of tumors from a dependent stage, where growth is dependent on the presence of chlordane, to an autonomous stage, which does not regress after its withdrawal.

4.3 Reproductive/Developmental Studies—Oral and Inhalation

No multigenerational reproductive studies, by any route, exist for technical chlordane. Several items within the current chlordane database suggest that reproductive effects could be a relevant endpoint for chlordane. The study of Cassidy et al. (1994) indicates alterations in reproductive related behaviors in male rats as a consequence of chlordane exposure that could affect actual reproductive performance. Data on tissue distribution of chlordane and its metabolites also indicate the potential for reproductive consequences. Taguchi and Yakushiji (1988) measured chlordane residues in the milk of 15 mothers who had lived in chlordane-treated residences for an average period of 1.8 years, all of which were elevated relative to those in the milk of unexposed mothers. The following residues, in order of their mean concentration, were detected: *trans*-nonachlor, heptachlor epoxide, oxychlordane, *cis*-nonachlor, gamma-chlordene, *trans*-chlordane, and *cis*-chlordane. In a subgroup of these women who had been exposed for approximately 2 years, the overall chlordane residues in milk (0.254 mg/kg milk fat) were similar to those of PCBs (0.389 mg/kg milk fat). Rani et al. (1992) reported accumulation of heptachlor (a component of technical chlordane) in the ovary, uterus, and adrenals in nonpregnant rats within 30 min after an oral dose of 120 mg/kg heptachlor. In pregnant rats, levels were markedly elevated in the uterus compared to nonpregnant rats; the higher accumulation is believed to be a result of a slower metabolic turnover of heptachlor. These results indicate that chlordane and its metabolites may have the potential to adversely affect reproductive processes through bioconcentration in reproductive organs and postnatal development through elimination via milk.

In contrast to the situation for reproductive effects, a number of studies have examined the potential of chlordane or its metabolites to affect developmental and various postnatal processes covering a variety of endpoints.

Fischer 344 rats (19 to 23/group) exposed to 0, 21, or 28 mg/kg-day chlordane on Gestational Days 6 through 19 had significant decreases in maternal weight gain (dose-related, food intake data not given), but no other clinical signs of maternal toxicity (Narotsky and Kavlock, 1995). A significant increase in percent loss of pups per litter was seen at both doses; possible explanations for these findings were functional deficits from prenatal exposure, direct

exposure to chemical through maternal milk, or inability of dam to lactate or care for the pups. The lowest dose, 21 mg/kg-day, is a LOAEL.

In a developmental screening toxicity study, CD-1 mice (25 females/group) were exposed to 0 or 50 mg/kg-day chlordane in corn oil via gavage during gestational days 8 through 12 and monitored through Postnatal Day 3 (Chernoff and Kavlock, 1982). No effects on viability and postnatal growth were observed in the offspring. No other information was presented.

Pregnant Sprague-Dawley rats were dosed orally with 0, 0.1, 0.5, or 5 mg/kg-day technical grade chlordane from Day 4 of gestation to Day 21 of lactation, and offspring were dosed from Postnatal Day 22 to 80 (Cassidy et al., 1994). The hypothesis tested in this study is that chlordane or its isomers/metabolites act to mimic sex steroids and/or change their concentrations to alter (in this case to masculinize) functions and behavior. To test this hypothesis, they made eight behavioral and other functional measurements on male and female offspring. Exposure during lactation was evidenced by a high concentration of *trans*-chlordane, oxychlordane, and heptachlor epoxide, as well as a smaller amount of heptachlor, in the milk of dams sampled on Lactation Day 8 which the authors claim is within the time period during which sexual dimorphic organization occurs. The plasma levels of these compounds in the offspring increased from Day 40 to Day 80 showing that the animals were exposed during this time. The measurements and results are listed below.

(1) Plasma chlordane and metabolites: females had greater levels than males at 60 days but there was no significant difference at 80 days.
(2) Plasma testosterone at 80 days: dose-dependent decrease in females, not significant for 0.1 mg/kg-day but significant at the two highest doses.
(3) Body weights from 25 through 80 days: 5 to 10% increase in weight of females compared to controls with a non-monotonic dose-response relationship.
(4) Performance in escaping and navigating a water maze in repeated trials on days 76 through 79: treated females had better performance than controls with no consistent pattern as a function of dose.
(Males did not differ from controls in any of the above tests.)
(5) Open field activity levels at 81 weeks: neither treated males or females differed significantly from controls.
(6) Auditory startle response: both treated males and females of the low-dose group had increased response in one of three measures of auditory startle response, but no significant effect occurred in the other measures or at other doses.
(7) Male mating behavior: low- and mid-dose males performed better than controls but the improvement of high-dose group males was not significant.
(8) Radiolabeled chloride ion uptake in brain of males: high dose males had reduced uptake of chloride ion but the results were not stated for the other two dose groups.

Although these results could indicate that chlordane treatment mimics the sex steroids, masculinizing both females and males, there are several difficulties with this interpretation. For example, the effects in the high dose group are generally less than the low- and mid-dose groups, indicating either no effect or a complex dose-response mechanism. Also, the measurements of testosterone levels show no change in males (even though mating behavior in males is improved), and smaller and non-significant reductions in testosterone in low-dose females (where the largest

effects occur) than in mid- and high-dose females where several effects are less or non-existent. These observations show that if testosterone or its receptors are somehow involved in the effects of chlordane, the dose-response model (or mechanism) for the effects must be extremely complex, and in need of further clarification.

Several investigators (Barnett et al., 1985a,b; Spyker-Cranmer et al., 1982) have assessed the effects of chlordane on the immunological system of offspring that were exposed during gestation, and found that chlordane may affect cell-mediated immunity. Pregnant BALB/c mice (6/group) were fed 0, 0.16, or 8 mg/kg-day chlordane during the 19-day gestation period (Spyker-Cranmer et al., 1982). At birth, pups were weighed and examined for viability; alterations in these parameters were not indicated in the study. Pups were randomized within treatment groups (45 pups/group) and fed normal diets. Offspring were weaned at 28 days, and immunological tests were performed at 101 days of age. A significant decrease in ear thickness (0.175 to 0.17 versus 0.065 to 0.05 mm) in response to a challenge with a sensitizing agent ($p < 0.01$) was exhibited in a cell-mediated immune assay in the 8 mg/kg-day group in both sexes. T-cell-dependent humoral immune response to sheep erythrocytes was not significantly different among groups. The lower dose of 0.16 mg/kg-day is a NOAEL for this endpoint.

Pregnant albino mice (6/group) were exposed orally by gavage to 0, 1, or 2.5 mg/kg-day technical grade chlordane dissolved in olive oil for 7 consecutive days during late gestation (i.e., gestational days 12 through 19 (Al-Hachim and Al-Baker, 1973). Each mouse received 5 to 7 doses. It was not indicated whether animals were nursed or foster reared, and thus whether exposure also occurred through the milk. Groups of 10 randomly selected offspring were tested for conditioned avoidance response (<37 days of age), electroshock threshold (38 days of age), and response in open-field tests (6 weeks of age). Exposed animals exhibited depressed acquisition of avoidance response (mean responses 13.1, 9.7, and 9.7 in controls, low- and high-dose), increased seizure threshold (mean responses 90.1, 108.6, and 134.9 in controls, low- and high-dose), and increased exploratory activity (mean responses 93.9, 88.4, and 137.7 in controls, low- and high-dose). A three-way ANOVA was performed and indicated significant dose-related differences between treatment groups ($p < 0.001$). The lowest dose, 1 mg/kg-day, is a LOAEL.

In an effort to understand the effects of prenatal chlordane exposure on adult bone marrow expansion potential, Barnett et al. (1990a) exposed female mice orally to chlordane at either 0, 4, or 8 mg/kg-day for 18 days during pregnancy. Offspring were nursed, which would provide some postnatal chlordane exposure. Bone marrow hematopoietic activity, as measured by the ability of bone marrow cells to undergo clonal expansion in response to stimulating factors, and spleen colony forming units (after irradiation) were both evaluated in offspring of these mice at 100 and 200 days of age. Results showed a significant dose-related depression ($p < 0.05$) of both measures at 100 and 200 days of age. The bone marrow of offspring exposed to 4 or 8 mg/kg chlordane had 63 to 75% of control numbers of granulocyte-macrophage colony forming units (CFU) at 100 days of age; at 200 days postexposure these values were still significantly decreased at 50 to 77% of control. The formation of splenic-CFU was similarly affected. Bone marrow of male and female offspring exposed to 4 mg/kg chlordane had only 78 to 87% of control splenic-CFU at 100 days of age and those exposed to 8 mg/kg had only 67 to 64%. At 200 days of age female mice exposed to 8 mg/kg still had only 72% of control splenic-CFU and male mice

exposed to 4 mg/kg only 84% of control splenic-CFUs. In a subsequent study in which these same measures were evaluated at 18 days of gestational age (and without chlordane exposure via milk) these results were confirmed indicating that damage to stem cells occurred during the fetal period (Barnett et al., 1990b). A LOAEL of 4 mg/kg-day is indicated by these results.

Barnett et al. (1985a) found that in utero exposure to 8 or 16 mg/kg-day chlordane (in peanut butter) in pregnant BALB/c mice during gestational days 1 through 18 both resulted in a significant decrease ($p < 0.05$) in virus-specific delayed type hypersensitivity response postinjection of an influenza type A virus (degree of footpad swelling) in the offspring, but only at 48 h after virus injection. At 48 h post-injection, the control infected animals had a change in swelling of 1.17 mm, the 8 mg/kg group 0.54 mm, and the 16 mg/kg group 0.46 mm. There was, however, no deficit in the chlordane treated animals in ability to resolve the infection. In another mouse study by Barnett et al. (1985b), delayed type hypersensitivity response in offspring, as well as depressed mixed lymphocyte reactivity of spleen cells in male offspring, occurred after in utero exposure to 4 or 16 mg/kg-day during gestational days 1 through 19. In the first study 8 mg/kg-day is an effect level and in the second study 4 mg/kg-day is an effect level. For both of these studies, offspring were also exposed to chlordane via milk.

Cranmer et al. (1984) administered chlordane (in peanut homogenate) at doses of 0, 0.16, or 8 mg/kg-day to dihybrid female mice throughout gestation to assess plasma corticosterone levels in adult offspring (45 pups/group). At birth, pups were weighed and examined for birth defects; malformed, moribund, or small newborns were removed from further study. Offspring were sacrificed at 101, 400, or 800 days of age. Due to the high number of postnatal deaths in the high-dose offspring (55% died within the first week), there were no male offspring left for sacrifice at 800 days. At 101 days of age, plasma concentration of corticosterone was elevated ($p < 0.01$) in 0.16 mg/kg-day males at 6.5 µg/dl plasma as compared to 2.6 to 3.2 µg/dl plasma in controls. At 400 days of age, corticosterone levels remained significantly elevated ($p < 0.05$) in 0.16- (6.8 versus 4.5 µg/dl plasma) and 8-mg/kg-day males (8.1 versus 4.5 µg/dl plasma) and 0.16-mg/kg-day females (10.8 versus 6.8 µg/dl plasma). The investigators hypothesized that elevated plasma corticosterone levels may reflect the diminished ability of the liver to metabolically reduce corticosterone, or that the elevated levels may possibly be due to perturbations in corticosteroid production by the hypothalamus or pituitary. Chlordane may be impairing normal development of the neuro-endocrinological feedback system, leading to transient or permanent malformations of the system in the adult animal. The investigators also suggested that the more marked effect on plasma corticosterone levels in male mice compared to female mice may be a result of chlordane's interference with sex hormone control. As the toxicological significance of these effects are unclear, no effect levels are assigned.

4.4 Other Toxicologically Relevant Studies

4.4.1 Mechanistic Studies

The findings of Malarkey et al. (1995) give indications of the mechanism of tumor formation. The authors exposed B6C3F1 and B6D2F1 mice in a discontinuous protocol (see Section 4.2), evaluating tumor characteristics and progression with continuous administration of chlordane and following the withdrawal of dietary administration. They found no H-ras or K-ras mutations in hepatocellular tumors from chlordane-exposed B6C3F1 or B6D2F1 mice, whereas H-ras mutations were found in 4/10 spontaneous hepatocellular tumors examined from control B6C3F1 and in 6/15 liver tumors from control B6D2F1 mice. This observation of lower H-ras mutation frequency in tumors from exposed mice than in spontaneous tumors from control mice has been observed for other nongenotoxic agents that cause liver tumors in mice, including dieldrin, Arochlor, phenobarbital, and chloroform. The majority of neoplastic hepatocytes and altered hepatocellular foci in exposed mice had acidophilic cytoplasm, whereas neoplastic hepatocytes and altered hepatocellular foci in controls had basophilic cytoplasm. These two findings suggest that chlordane-induced tumors arise from different cell phenotypes than spontaneous tumors. Also, the multiplicity of combined adenomas and carcinomas as well as that of adenomas decreased after discontinuation of treatment. These results provide evidence for a progression of neoplasms from a dependent to an autonomous stage of development such that only the latter persist after the cessation of exposure.

4.4.2 Genotoxicity

The U.S. EPA (1986b) previously reviewed and evaluated published genotoxicity studies on chlordane. Approximately two-thirds of 25 published studies were judged unacceptable or inconclusive due to study deficiencies or reporting deficiencies. Several conclusions, listed below, were drawn based on the remaining nine studies.

(1) Chlordane was not mutagenic in bacterial assays, with or without mammalian activation systems.
(2) Data obtained for chlordane-induced gene mutations in in vitro tests with mammalian cells were equivocal.
(3) Chlordane produced negative results in mouse dominant lethal assays, in an in vitro mammalian cell cytogenetics assay, and in vitro DNA repair assays using mouse, rat, and hamster hepatocytes.
(4) Chlordane induced sister chromatid exchanges in fish and human cells in culture, and gene conversion in yeast.

The U.S. EPA (1986b) concluded that the available genotoxicity data did not "provide a fully comprehensive data set upon which to definitively assess the mutagenic potential of chlordane."

In a recent review of chlordane genotoxicity studies, Jackson et al. (1993) concluded that three studies provided sufficient evidence to classify chlordane as having "limited evidence of mutagenicity." These studies found weakly positive results for gene mutation in V-79 cells (Ahmed et al., 1977), positive results in the mouse lymphoma assay (McGregor et al., 1988), and positive results for the induction of sister chromatid exchanges in cultured human lymphocytes (Sobti et al., 1983).

4.5 Synthesis and Evaluation of Major Noncancer Effects and Mode of Action—Oral and Inhalation

The overall hazard profile evaluation for chlordane is one of moderate toxicity. Chronic exposure, both oral and inhalation, to technical chlordane has been documented to produce hepatic effects in animals, neurotoxic effects in humans, and possibly to modify sex steroid-mediated behaviors in animals. Only the hepatic effects have both reliable dose-response information and a theoretical mode-of-action for which there is supportive data.

Subchronic and chronic studies in animals consistently demonstrate that exposure to technical chlordane elicits both adaptive and adverse hepatic effects. Technical chlordane and its metabolites are lipophilic and have been demonstrated to accumulate within fatty tissues in both animals and humans. A principal metabolite of technical chlordane, generated by enzyme systems localized largely in hepatic tissues, is the epoxide, oxychlordane. Although the tissue reactivity of this epoxide has not been thoroughly studied, it (like other epoxides) has the potential to covalently interact with cellular constituents and macromolecules, which may lead to more obvious and expressed toxic effects. The long residence of this epoxide in fatty tissues makes this species suspect in manifesting these effects. This mechanism (meaning the molecular sequence of events leading to toxic effects) could reasonably be explanatory of the observed liver toxicity in animals exposed chronically to chlordane. That this mechanism could apply to humans is supported by the detection of this epoxide in human tissues. However, the human studies available, although limited in their scope of information, give no further evidence that this mechanism is operative as no liver toxicity is apparent. Several of these studies looked specifically for this adversity.

In humans as well as animals neurotoxicity has been identified as the most likely endpoint in acute exposures to chlordane (Grutsch and Khasawinah, 1991) and for other chlorinated cyclodienes such as aldrin and dieldrin (Murphy, 1986). Some evidence also exists for blood (Fleming and Timmeny, 1993) and immunological (McConnachie and Zahalsky, 1992) effects as being relevant in acute exposures. Although the mechanistic bases for these effects are not known, biochemical perturbations have been implicated (Murphy, 1986). Effects in humans resulting from chronic exposure to chlordane are not clear. Occupational studies available on highly-exposed workers report no neurological symptomatologies although this conclusion was reached from limited examinations or questionnaires. In contrast to these reports, studies of individuals exposed from treatment of their residences document neurophysiological and neuropsychological deficits (Kilburn and Thornton, 1995) from exposure via dermal and inhalation routes. No dose-response information and little reliable exposure information is available to confirm and quantify these chronic effects in humans. The RfC proposed in this assessment (8E-5 mg/m^3, Section 5 below) is below that concentration (> 0.5 $\mu g/m^3$ or 5E-4 mg/m^3) associated with neurological effects in the Kilburn and Thornton (1995) study.

The preliminary results of Cassidy et al. (1994) indicate that chlordane may masculinize certain behaviors in female rats suggesting that chlordane may chemically mimic androgenic steroids. If confirmed, these findings would indicate the potential for reproductive effects. Alterations of some reproductive behaviors were noted among males in this study but no parallel tests were carried out with females.

From these observations, it is apparent that a multigenerational reproductive animal study could be considered a data gap in the chlordane database, as well as both subchronic and chronic

animal studies in which neurotoxicity is carefully evaluated. Follow-up studies on neuropsychological and neurophysiological characteristics of chlordane plant workers with known exposures would also serve to provide confirmatory and possibly quantitative dose-response information on human neurotoxicity from chronic exposure to technical chlordane. This would be possible only if former plant workers with known exposure levels and employment records could be found.

4.6 Weight of Evidence Evaluation and Cancer Classification

The overall weight of evidence for the carcinogenicity of chlordane to humans consists of information from humans supplemented by information from animal and in vitro systems designed to model particular aspects of human biology.

There are two main lines of evidence that chlordane is likely to be a human carcinogen. First, liver tumors occurred in five strains of mice given chlordane in the diet. They occurred in both males and females, and the incidence was closely replicated in an experiment repeated 18 years after a 1977 NCI bioassay. Tumors were not observed in liver of rats or other sites in mice, although rats had liver enlargement and cell swelling, which can be signs of preneoplastic toxicity. Five compounds structurally related to chlordane (aldrin, dieldrin, heptachlor, heptachlor epoxide, and chlorendic acid) have induced liver tumors in mice. Chlorendic acid has also produced liver tumors in rats. Although there has been controversy about the relevance of the chemically-induced mouse liver tumor as a model of human carcinogenicity, this evidence firmly establishes chlordane as a mouse liver tumor carcinogen.

The second line of evidence is in humans exposed to chlordane by direct dermal contact and/or inhalation at high concentrations over a prolonged time. This evidence is too weak to conclude that chlordane is causally related to human cancer, but it is suggestive that such exposure might be associated with excess risk to a broad range of hematopoietic diseases. This evidence consists of: (1) excess of NHL in Iowa and Minnesota farmers using chlordane as an insecticide for livestock and crops, and an elevated but not significant excess odds ratio for NHL in agricultural and forestry workers in Washington State; (2) several human case reports of blood dyscrasias (aplastic anemia and leukemia) in people exposed either by handling chlordane at home or by pesticide applicators; (3) a report of impairment of lymphocyte proliferation and suggestive autoimmunity in people exposed to chlordane as a home termiticide; and (4) data showing that people exposed at home have chlordane metabolites in blood. There is no evidence that exposure to chlordane in food or drinking water is associated with cancer.

There is limited evidence of genotoxicity of chlordane, consisting of positive mutagenicity findings in V-79 cells, a positive mouse lymphoma assay, and sister chromatid exchange induction in human peripheral lymphocytes, but no evidence in bacterial assays or in any in vivo assays.

Whereas the genotoxic potential of chlordane is not clearly understood, several toxicological properties have been described which may play roles in the expression of chlordane carcinogenicity in rodents, including: chlordane induction of hair follicle nuclear aberrations in CD-1 mice (Schop et al., 1990); irreversible binding of chlordane metabolites to intracellular macromolecules, including DNA and RNA (Brimfield and Street, 1981); chlordane inhibition of

intercellular communication (Telang et al., 1982; Ruch et al., 1990; Bessi et al., 1995); chlordane stimulation of protein kinase C activity (Moser and Smart, 1989); chlordane induction of in vitro hepatic lipid peroxidation and DNA single-strand breaks (Hassoun et al., 1993); and chlordane suppression of in vitro immune responses (Johnson et al., 1987; Chuang et al., 1992).

A search for H-ras and K-ras mutations in liver tumors induced by chlordane in two mouse strains showed no mutations, whereas spontaneous tumors in the same two strains did show mutations; this result indicates that chlordane induces tumors by different mechanisms than those responsible for spontaneous tumors. The absence of ras mutations has been observed in tumors induced by other non-genotoxic carcinogens. One recent experiment showed that a fraction of liver tumors induced in mice by chlordane regress (their multiplicity decreases) after withdrawal of chlordane from the diet; other tests at lower doses are needed to further explore the mechanism of this regression.

The mode of action for chlordane as a carcinogen is probably not solely as a direct genotoxin, although DNA damage has not been ruled out. Since it is persistent in body tissues as an epoxide (ocychlordane), it could affect carcinogenesis at many stages. There is evidence that it induces malignant liver tumors by a process independent of the induction of spontaneous tumors, and a suggestion that it might induce two populations of liver tumors, one of which is persistent after the withdrawal of continuous dosing and the other population regressing after cessation of continuous dosing. Since these experiments were done only at doses high enough to induce tumors in 100% of the animals, the low-dose mode of action is not known.

There have been no populations identified as unusually sensitive to the carcinogenic effects of chlordane. The people at most risk are the ones most highly exposed; these are farmers who have handled, mixed and applied chlordane to livestock and crops, and people exposed in their homes to improperly applied chlordane as a termiticide or in garden uses of chlordane not yet disposed since sales were discontinued in 1988. No effects have been observed in humans from the small concentrations of chlordane that commonly occur in food.

The main uncertainty about the current risk assessment is whether the effects observed in highly exposed people will occur from the low concentrations found in food or in water and air near waste sites where chlordane is found, and if it does occur, what is the probability of cancer due to that exposure. The estimate of this probability is derived from data on liver cancer in mice, although the relationship of mice liver tumors to cancers of an unknown site in humans is not known. There are no experimental data which provide information about the probability that cancer is induced by low chlordane concentrations; in the absence of such information the linear, no-threshold dose extrapolation model is the appropriate model for low-dose extrapolation. The persistence of chlordane in soils and fish and in fat and bone marrow after it has been ingested serves as a rationale for protective assumptions.

The mechanistic relationship between liver tumors in mice and hematopoietic toxicity and lymphoma in humans has not been established. However, in the absence of definite information, it is prudent to regard the liver cancer in mice as an indication that chlordane may be carcinogenic in humans.

This evidence matches closely the criteria of the 1986 Guidelines for Carcinogen Risk Assessment for a B2 carcinogen (probable) because of inadequate evidence of carcinogenicity in human studies, sufficient evidence of hepatocellular carcinomas in multiple mice strains, and the evidence of several toxicological responses believed to play a role in carcinogenesis. Using the 1996 Proposed Guidelines for Carcinogen Risk Assessment, chlordane would be characterized as a likely carcinogen in humans, and the above paragraphs would constitute the weight-of-evidence narrative.

4.7 Other Hazard Identification Issues

4.7.1 Possible Childhood Susceptibility

A number of factors may differentially affect the response of children to toxicants such as chlordane. These factors include diet and physical environment as well as maturation of physiological and biochemical processes (Roberts, 1992). Although some work has been attempted in this area for exposure to organochlorines (McConnell, 1992; Fenske, 1992), there is too little information to make any statements about how these factors may specifically affect the toxicological responses of chlordane in children, be they cancer or noncancer.

4.7.2 Possible Gender Differences

The extent to which men differ from women in susceptibility to chlordane toxicity is not known. Although gender differences in the effects of chlordane have been observed in the studies reviewed in this document (listed below), they are rare and show no consistent pattern.

(1) In the 90-day Wistar rat inhalation study (Khasawinah et al., 1989a), females but not males had transient changes in white cell populations that were not considered adverse or dose-related and disappeared 13 weeks after exposure.
(2) In the 30-mo Fischer rat study (Khasawinah and Grutsch, 1989b), hepatocellular hypertrophy was more prevalent in females than males.
(3) In the 24-mo ICR mice study (Khasawinah and Grutsch, 1989a), the background incidence and the dose-related trend of liver adenoma and hemangioma incidence was larger in males than females.
(4) In measurements of plasma corticosterone following in utero exposure, Cranmer et al. (1984) found greater elevation in males than females, but the significance of the finding is not clear.

Gender differences associated with other agents which cause the same toxic effects seen here with chlordane, namely liver damage and liver cancer, might offer some insights about gender differences expected with chlordane. The six observations summarized below (from Calabrese, 1996) may have some bearing on gender differences expected with chlordane, but the situation is not clear enough to make predictions in the absence of data on chlordane itself.

(1) Liver cancer incidence from aflatoxin B1 exposure in African populations is higher in men than women.
(2) In rats of several strains the liver metabolism of aflatoxin B1 to the active forms M1 and Q1 is greater in males than females, and the DNA binding of these metabolites is greater in males.
(3) In the United States and Europe liver cancer is about two-fold higher in men than women; whether this is due to differential exposure to liver carcinogens is not known.

(4) Levels of hepatic aryl hydrocarbon hydroxylase, specifically P4501A1, are higher in male than female rats. These are enzymes that can both activate and detoxify carcinogenic agents.
(5) Female rats are more susceptible than males to carbon tetrachloride-induced liver damage.
(6) Benzene-induced toxicity to the hematopoietic system is greater in female rats than males, presumably because benzene is distributed more readily into body fat and is retained in tissues of females for a longer time.

Listed below are three other findings that may have some relevance to gender differences.
(1) Chlordane acts synergistically with other pesticides in binding to estrogen receptors incorporated into a yeast system (Arnold et al., 1996), although synergism apparently does not occur with weakly estrogenic chemicals when tested in cells with a high concentration of estrogen receptors (Ramamoorthy et al., 1997). The Arnold et al. (1996) article was formally withdrawn by the authors in a 1997 letter to Science (McLachlan, 1997) because they could not reproduce their findings. Therefore this point is not valid.
(2) Male B6C3F1 mice have higher and more variable spontaneous liver tumor incidence than females (U.S. EPA, 1986a).
(3) There are pronounced gender differences in the clinical action of psychotropic (Yonkers et al., 1992) and other drugs (Harris et al., 1995). These differences are of interest because of the neurotoxic action of chlordane noted above.

5.0 DOSE RESPONSE ASSESSMENTS

5.1 Oral Reference Dose

5.1.1 Choice of Principal Study and Critical Effect—with Rationale and Justification

Chlordane has a particularly wide base of chronic studies which consistently demonstrate that the liver is the target organ. Most of these studies, however, have limitations. The IRDC (1973) study in mice was conducted for only 18 mo at high doses (up to 7.1 mg/kg-day) that resulted in excessive mortality. The NCI (1977) study was also carried out at high doses for around 20 mo, with the dose levels being adjusted during the exposures to avoid excessive toxicity. The Barrass et al. (1993) and Malarky et al. (1995) studies were carried out at a single dose. In contrast, the Kasawinah and Grutsch chronic rat study (1989b) was carried out over a wide range of exposure values which included dosing for an extended period (30 mo), an extensive and thorough examination of the target organ (liver), and considering a major confounding factor, leukemia. However, no clearly adverse effects, hepatic or otherwise, were noted. This situation is in contrast to the 24-mo mouse study (Khasawinah and Grutsch, 1989a) wherein hepatic necrosis is observed at a dose level free of any hepatic neoplasia. The occurrence of such an adverse lesion in mice but not in rats under approximately equal dose-rates indicates that mouse livers may be more sensitive to the effects of chlordane or its metabolites than are rat livers. The dose-related occurrence of an adverse effect in the livers of mice justifies the choice of the Khasawinah and Grutsch (1989a) chronic study in mice as the principal study.

The extensive animal data base consistently demonstrates that the liver is the target organ for chlordane. Hepatic effects also were noted in inhalation studies in rats (Khasawinah et al.,

1989a) indicating that the liver is a common target organ regardless of route of chlordane administration. As they show progression with dose, from nonadverse to clearly adverse, and as they are consistent throughout the data base for chlordane, hepatic effects are appropriate as the critical effect in this assessment. Hepatic necrosis is judged to be the most clearly adverse noncancerous lesion of those reported, which included fatty degeneration and increased liver cell volume (hypertrophy). The latter effect is commonly associated with ultrastructural cellular changes involving increased metabolic capacity due to the presence of the toxicant (Sipes and Gandolfi, 1991) and are more appropriately considered as nonadverse, adaptive changes.

5.1.2 Methods of Analysis—Benchmark Dose and No-Observed-Adverse-Effect Level/Lowest-Observed-Adverse-Effect Level

The NOAEL for hepatic necrosis in male mice in the principal study (Khasawinah and Grutsch, 1989a) is 0.15 mg/kg-day (1 ppm), the LOAEL is 0.75 mg/kg-day (5 ppm). This study was described in Section 4.2, page 24.

Benchmark dose (BMD) analyses were performed on the incidence of hepatic necroses in male mice. Specifics on models, data input and calculations are included in Appendix A as are results documenting that mouse liver necroses were not modeled acceptably by either benchmark model employed. The NOAEL/LOAEL approach is used for all subsequent quantitation in the RfD dose-response assessment.

5.1.3 Oral Reference Dose Derivation—Including Application of Uncertainty Factors and Modifying Factors

Uncertainty factors (UF) are applied to account for recognized uncertainties in extrapolation from experimental conditions to the assumed human scenario (i.e., chronic exposure over a lifetime). Historically, UFs are applied as values of 10 in a multiplicative fashion (Dourson and Stara, 1983). Recent EPA practice, however, also includes use of a partial UF of $10^{1/2}$ (3.333) (U.S. EPA, 1994b) on the assumption that the actual values for the UFs are lognormally distributed. Application of these factors in the assessments is that, when a single partial UF is applied, the factor is rounded to 3, such that the total factor for a UF of 3 and 10, for example, would be 30 (3 × 10). When two partial UFs are evoked, however, they are not rounded, such that a UF of 3, 3, and 10 would result in a total uncertainty of 100 (actually $10^{1/2} \times 10^{1/2} \times 10^{1}$).

The NOAEL for male mice chronically exposed to chlordane is 0.15 mg/kg-day. The following full UFs are applied to the NOAEL: 10 for extrapolation for human variability (intraspecies differences in response), 10 for consideration of animal to human (interspecies) extrapolation. A partial ($10^{1/2}$) UF is also applied for lack of any full scale multigenerational reproductive study. The total UF = $10 \times 10 \times 10^{1/2} = 300$. No modifying factor (MF) is considered necessary.

RfD = 0. 0.15 mg/kg-day ÷ 300 = 5E-4 mg/kg-day.

5.2 Inhalation Reference Concentration

5.2.1 Choice of Principal Study and Critical Effect—with Rationale and Justification

The subchronic study of Khasawinah et al. (1989a,b,c) is the only long-term repeated inhalation studies currently available in the database. The subchronic study included both monkeys and rats with rats being used for further analysis. Although monkeys could be more comparable to humans for exposure purposes, it is not at all clear if monkeys would be more or less sensitive than humans to the effects of chlordane and its isomers and metabolites. It is clear from this study that effects were shown in rats and not monkeys when both species were exposed simultaneously in the same chambers to the same concentrations of chlordane. To base the RfC on the monkey data would require that the highest concentration, 10 mg/m^3, be used as a "free-standing" NOAEL, a procedure that has not historically been a practice in the IRIS process. The incentive to use the monkey data as a direct surrogate for humans is ameliorated further due to the RfC methodology (U.S. EPA, 1994b) allowing estimation of a human equivalent concentration (HEC) from the rat data. It is, however, interesting to note that the HEC dosimetric adjustment (done below) implies that in inhaling the same external concentration, as was the case in this experiment, humans and presumably monkeys would receive less dose than would rodents. The anticipated result from this situation, that rats would respond to lower external concentrations than monkeys, is precisely what was seen in this study.

The animal data base consistently demonstrates that liver is the target organ for chlordane. Although some mild thyroid histopathology also was reported in the subchronic study, hepatic effects also were noted in chronic oral studies in rats (Khasawinah and Grutsch, 1989b) and mice (Khasawinah and Grutsch, 1989a) indicating that liver is a common target organ regardless of route of chlordane administration. As hepatic effects show progression with dose, from nonadverse to clearly adverse, and as they are consistent throughout the data base for chlordane, hepatic effects are appropriate as the critical effect in this assessment.

Hepatic-related alterations in blood chemistry are designated as the critical effect. These are alterations in cholesterol, albumin, globulins and total proteins. In the 28-day inhalation pilot study in rats (Khasawinah et al., 1989b) a progression of hepatic effects was seen. At the highest concentrations adverse hepatocellular pathologies (i.e., vacuolation and necrosis) were noted. At lower concentrations minor hepatic effects (centrilobular hepatocellular hypertrophy) and an entire pattern of hepatic-related alterations in blood chemistry were noted, these latter alterations being the critical effect and basis for the LOAEL in this short-term study. Although the concentration-range in the subchronic study (Khasawinah et al., 1989a) was apparently not high enough to elicit hepatic vacuolation or necrosis, a pattern of hepatic-related alterations in blood chemistry nearly identical to those observed in the 28-day study was noted at the highest concentration used in the subchronic study, 10 mg/m^3. The pattern of alterations in blood chemistry observed in the subchronic and 28-day pilot studies (Khasawinah et al., 1989a,b) such as cholesterol, albumin, globulins and total proteins are indicative of dysfunction of the liver, the major site of synthesis of plasma proteins (Kaneko, 1989). The other effect described in the subchronic study, reversible centrilobular hepatocyte hypertrophy, is commonly associated with ultrastructural cellular changes involving increased metabolic capacity due to the presence of the toxicant (Sipes and Gandolfi, 1991) and are more appropriately considered as nonadverse, adaptive changes.

5.2.2 Methods of Analysis—No-Observed-Adverse-Effect Level/Lowest-Observed-Adverse-Effect Level and Benchmark Concentration

The LOAEL for the rat portion of the principal study (Khasawinah et al., 1989a) was based on blood chemistry indicators of hepatic dysfunction that were restricted to rats exposed to the highest concentration of chlordane at 10 mg/m^3. The corresponding NOAEL was 1 mg/m^3.

Analyses for benchmark concentrations (BMC) were not attempted as the critical effect (biochemical indicators) was a collection of biochemical alterations that occurred only at a single concentration and therefore was not amenable to BMD analysis. The critical effect comprises hepatic-related alterations in blood chemistry including a decrease in glucose and increases in globulins, albumin, cholesterol, and total protein all of which occurred at the highest concentration (10 mg/m^3) only. Higher chlordane concentrations used in the 28-day pilot study demonstrate further progressive changes in hepatic function and histopathology and may be considered as evidence for a concentration-response relationship between chlordane and hepatic effects. The differences in the duration of these studies precludes combining them for a formal analysis such as a benchmark dose. Therefore, the NOAEL/LOAEL approach will be used for all further analyses.

The NOAEL of 1 mg/m^3 is duration adjusted to a continuous exposure = NOAEL(ADJ): 1 mg/m^3 × (8 h/24 h) × (5/7 days) = 0.24 mg/m^3.

5.2.3 Inhalation Reference Concentration Derivation—Including Application of Uncertainty Factors and Modifying Factors

The NOAEL for hepatic effects in rats in the principal study of Khasawinah et al. (1989a) is 1 mg/m^3. The NOAEL(ADJ) would be calculated based on an 8 h daily exposure for 5 of 7 days per week = 1 mg/m^3 × 8/24 × 5/7 = 0.24 mg/m^3.

The methodology for development of reference concentrations (U.S. EPA, 1994b) allows for dosimetric adjustment of particulate aerosols between animals and man to arrive at an HEC. For this particulate exposure of chlordane, specific information on particulate size and distribution is requisite to this adjustment. Particle sizing information included in an unpublished version of this study (Velsicol Chemical Corporation, 1984) was used to estimate a MMAD of 1.8 μm and a corresponding particle distribution estimate (sigma g) of 3.1. These parameters were obtained by plotting the given size cut-offs against the cumulative mass; 51.4% of the mass at 2 μm and 82.9% of the mass at 5.5 μm. Although the data available to estimate these particle parameters were limited to 2 data points, it should be noted that one point (51.4% of total mass at <μm) was, by definition, inclusive of the MMAD. These estimates of the particle characteristics were thus judged reasonable estimates as the RDDR for an extrarespiratory effect (ER), the RDDR(ER), of 2.7 obtained from these particle parameters (U.S. EPA, 1994b).

NOAEL(HEC) = NOAEL(ADJ) × RDDR

NOAEL(HEC) = 0.24 mg/m^3 × 2.7 = 0.65 mg/m^3

Uncertainty factors are applied to account for recognized uncertainties in extrapolation from experimental conditions to the assumed human scenario (i.e., chronic exposure over a lifetime). Historically, UFs are applied as values of 10 in a multiplicative fashion (Dourson and Stara, 1983). Recent EPA practice, however, also includes use of a partial UF of $10^{1/2}$ (3.333) (U.S. EPA, 1994b) on the assumption that the actual values for the UFs are lognormally distributed. Application of these factors in the assessments is that, when a single partial UF is applied, the factor is rounded to 3, such that the total factor for a UF of 3 and 10, for example, would be 30 (3 × 10). When two partial UFs are evoked, however, they are not rounded, such that a UF of 3, 3, and 10 would result in a total uncertainty of 100 (actually $10^{1/2} \times 10^{1/2} \times 10^{1}$).

The following full UFs are applied to this effect level: 10 for subchronic to chronic extrapolation, 10 for consideration of intraspecies variation (human variability), a partial UF is applied for interspecies extrapolation as an HEC has been calculated, and another partial UF is applied for lack of any reproductive studies. The total UF = $10 \times 10 \times 10^{½} \times 10^{1/2} = 1000$.

No MF is proposed for this assessment.

RfC = NOAEL(HEC) / UF = 0.65 mg/m^3 / 1000 = 7E-4 mg/m^3

5.3 Cancer Assessment

No reliable human dose information is available in the case reports of blood dyscrasias or in the case-control studies of farmers using chlordane. Therefore, quantitative estimates based on human data are inappropriate.

For animals there are incidence data for hepatocellular carcinoma in mice in three studies constituting five data sets (males and females in two of the studies). In one of these studies adenomas were reported in addition to carcinomas; these were not counted in order to be consistent with the other data sets and because carcinomas by themselves constitute a potential human health hazard. Because of this omission of adenomas, the risk of chlordane may be slightly underestimated in comparison to other mouse liver carcinogens where typically adenomas are combined with carcinomas to obtain a risk estimate.

5.3.1 Choice of Study/Data with Rationale and Justification

The slope estimates from the five dietary mouse liver tumor data sets (IRDC [1973] males and females, NCI [1977] males and females, and Khasawinah and Grutsch [1989a]) were combined by taking the geometric mean. These slope estimates are derived in Appendix A. The justification for the mean is that there is no a priori reason that any one data set is more appropriate than the others for human risk estimation. The two data sets from the IRDC 1973 were more difficult to fit to a multistage model than the other three, but omitting them from the average produces little change.

5.3.2 Dose-Response Data

The dose-response data are given in Tables 2, 3, and 5 of this document and the details on the multistage model fit to the data are given in Appendix A.

5.3.3 Dose Conversion

The two steps in estimating the human equivalent dose from the animal dose are: (1) converting the dietary concentrations to milligrams per kilograms per day in the animals; and (2) converting the animal dose to the equivalent human dose. The first step had been done in the IRDC and Khasawanah and Grutsch studies and the assumption of a default value of 0.13 × concentration was made for the NCI data. The second step used the "3/4 power" assumption where the human equivalent dose, in units of milligrams per kilograms body weight per day, is equal to the animal dose × (w/70)^(1/4), and the animal weights, w, were taken from the NCI report for that data set and the default assumption of 0.030 kg was used for the other two data sets in absence of information.

In estimating the inhalation unit risks, the assumption is made that effects in animals depend on the inhaled dose (milligrams per kilograms per day) and that there is 100% absorption. This assumption was checked by comparing the dose at which a 10% incidence of liver cell hypertrophy was observed in the 13-week rat inhalation study of Khasawinah et al. (1989a) with the dose at which the same effect was observed in the 30-mo rat dietary study of Khasawinah and Grutsch (1989b). In section 4.2 it was shown that these values were 0.64 mg/m^3 for the inhalation air concentration and 0.15 mg/kg-day for the dietary study. The dose absorbed by rats breathing an air particulate concentration of 0.64 mg/m^3 with a MMAD of 1.8 μm and a sigma g of 3.1 is the product of concentration, minute volume, and fraction of particulates deposited divided by the body weight. The minute volume (I) was estimated from the formula I = 0.8 × (W)^(0.821) = 0.227 m^3/day for a 0.216 kg rat (U.S. EPA, 1988a). The fraction of particles deposited as calculated from the standard Agency model (U.S. EPA, 1994b) is 0.45. Therefore, the deposited dose is

$$(0.64\ \text{mg/m}^3 \times 0.227\ \text{m}^3\text{/day} \times 8/24 \times 5/7 \times 0.45 \times 1)/(0.216\ \text{kg}) = 0.073\ \text{mg/kg-day}.$$

This is about one-half the dose at which rats in the chronic dietary study had liver cell hypertrophy. Although one would expect that a slightly smaller dose would be sufficient to cause equivalent effects in a chronic study, the similarity of the two dose estimates (0.15 mg/kg-day for the oral study and 0.073 mg/kg-day from the inhalation study) is noteworthy and warrants the conclusion that liver cell hypertrophy occurs at approximately the same absorbed dose regardless of the route of administration.

Based on this analysis, the inhalation unit risk for humans is derived from the oral slope factor by estimating the intake in humans assuming 100% absorption and a breathing rate of 20 m^3/day in the normal fashion.

5.3.4 Extrapolation Methods

The linearized multistage model, Global 86, was used to calculate the upper limit on Q(1) and the lower confidence limit on the dose corresponding to an incidence of 0.1. The parameters

used were the extra risk option and the degree of polynomial determined by the algorithm of Global 86. Details are presented in Appendix A.

5.3.5 Oral Slope Factor and Inhalation Unit Risk

The oral slope factor, 3.5E-1 per mg/kg-day, is the geometric mean of the following five slope factors from the individual data sets: CD-1 males, 0.858; CD-1 females, 0.217; B6C3F1 males, 0.345; B6C3F1 females, 0.114; and ICR males, 0.710. If the calculation were to be done according to the proposed cancer guidelines, which uses a straight-line extrapolation from the LED10 point, the corresponding geometric mean would be 5.7E-1 per mg/(kg-day). This slope is slightly higher than the extrapolation to small doses, reflecting the positive curvature ("sub-linearity") of the dose-response data. Both estimates are included here pending the finalization of the currently-proposed guidelines, but the former is used here because the 1986 guidelines have not been replaced.

The inhalation unit risk is 1E-4 per ($\mu g/m^3$), derived from the oral slope factor and assuming a human air intake of 20 $\mu g/m^3$ per day, 100% absorption of inhaled chlordane, and a human body weight of 70 kg. The justification for the conversion from oral to inhalation dose is given above in Section 5.3.3.

6.0 MAJOR CONCLUSIONS IN CHARACTERIZATION OF HAZARD AND DOSE-RESPONSE

6.1 Hazard Identification

Technical chlordane is a mixture of over 140 different compounds with a carbon skeleton consisting of joined 5- and 7-carbon rings and having varying degrees of chlorination. It was first produced in 1947 as an insecticide for agricultural crops and livestock, for lawns and gardens, and also for underground treatment of building foundations. The U.S. EPA canceled above-ground uses in 1978 because of concern about cancer risks and canceled all uses after 1988.

These compounds are insoluble in water, soluble in fat, and they bioconcentrate in fat tissue. The most persistent compounds in biological tissue are oxychlordane (an epoxide) and heptachlor epoxide, which are of primary toxicologic significance. It persists in rats for at least 56 days after withdrawal of chlordane from the diet. There is some evidence that steady-state levels in human fat are not reached before 3 to 12 mo of exposure. This information is discussed in Section 3.0.

People have not been harmed by working in factories where chlordane is manufactured, but there are several reports of adverse effects in people having direct contact with chlordane, such as farmers, pesticide applicators, and people excessively exposed in improperly treated buildings. These effects include NHL in farmers using chlordane and other farm chemicals, and neurological and immunological impairments, blood abnormalities, and other chronic health problems in people living in treated residences.

In animal experiments chlordane has induced liver tumors in all five strains of mice that have been tested and liver and thyroid toxicity in mice and rats. A recent study of cancer mechanisms in mice concluded that chlordane-induced liver tumors arise from different cell types than spontaneous tumors, and that some, but not all, of the induced tumors regress after chlordane dosing is stopped.

Administration of chlordane to rodents during fetal development has resulted in early mortality after birth, decreases in immunological and bone marrow functioning, and possibly impairment of normal sex hormone function in young adult animals.

Because of the repeated findings of liver carcinogenicity in mice, chlordane is probably a carcinogen in humans. Under the 1986 Carcinogen Risk Assessment Guidelines, chlordane is classed as a probable human carcinogen (B2), and under the proposed 1996 guidelines it would be characterized as a likely carcinogen.

An area of scientific uncertainty in this assessment concerns the roles of neurotoxic, immunotoxic, and hematotoxic effects in the chronic toxicity of chlordane in humans. In acute chlordane exposures, neurotoxicity has been established as a common endpoint with similar symptomatologies in both humans and animals (Grutsch and Khasawinah, 1991). Chronic studies with chlordane in animals have considered and found the liver to be the primary target organ. However, occupational studies of workers with chronic chlordane exposure have established no liver toxicity, although hematotoxicity has been strongly associated with the high levels of occupational exposure (Fleming and Timmeny, 1993). Recent epidemiology results from a

non-occupationally exposed cohort, however, do show strong evidence of neurotoxicity (Kilburn and Thornton, 1995) and some indications of immunotoxicity (McConnachie and Zahalsky, 1992). These findings could mean that in rats the liver is more sensitive to chlordane effects than is the nervous or immune system. Some data do exist for rats (transient tremors in rats observed in NCI [1977]) indicating neurotoxicity occurring in chronic studies at levels higher than those eliciting liver effects. This possibility cannot be confirmed, however, as no long-term, repeated-dose animal study has been conducted in which neurotoxicity has been specifically evaluated. These findings could also be a result of study bias as occupational studies were designed to elucidate hepatic effects; both Alvarez and Hyman (1953) and Fishbein et al. (1964) employed a number of liver function tests and only addressed neurological symptoms through a general physical exam and did not address immunological effects at all. The possibility exists that this assessment is based on studies flawed in design and endpoint assessment. Due to this circumstance, any future alteration of the RfD (or RfC) based on an improved database (such as a chronic study) would have to be considered in light of what is known about the neurotoxic and immunotoxic potential of chlordane in both animals and humans.

Another area of scientific uncertainty in this assessment concerns the toxicological significance of endocrine mimicry effects of chlordane. Toxicity data for this chemical includes a study demonstrating biochemical and behavioral alterations consistent with technical chlordane (or its metabolites) mimicking male sex-steroids (Cassidy et al., 1994). That these effects could include reproductive behaviors is suggested in this study. Tissue distribution data also offer evidence that developmental/reproductive toxicity could be a potential endpoint as technical chlordane (or its metabolites) appears to have an increased affinity toward reproductive organs during pregnancy. Evidence that these altered behaviors and circumstances would have functional consequences that could alter reproduction is lacking, however, because no multigenerational reproductive studies exist.

Because chlordane shares the qualities of persistence and bioaccumulation in soils and tissues with other pesticides still in use, it is likely to be present with these other agents in breast milk, soils, fish residues, and animal feeds, and is likely to contribute to the etiology of diseases or conditions (such as NHL) that are associated with exposure to these sources. Therefore, when the total exposure to chlorinated substances is being assessed, contributions from chlordane and other pesticides no longer in use should not be neglected.

6.2 Dose-Response Assessment

Quantitative estimates of human risk as a result of low-level chronic chlordane exposure are based on animal experiments since no adequate human exposure data are available in studies where adverse effects have been observed in people. Liver toxicity in rats is the effect observed at the lowest tested doses in both feeding and inhalation studies.

The human chronic dose of ingested chlordane considered to be without adverse effect (the RfD) is 5E-4 mg/kg-day. This value was based on the occurrence of hepatic necrosis observed in a lifetime mouse feeding study by first determining the NOAEL (0.15 mg/kg-day) and then dividing this dose by a composite UF. An uncertainty factor of 300 was used: 10 for extrapolation from rats to humans; 10 for variations between humans; and 3 because the entire

data base is lacking a multigeneration reproductive study, which is considered necessary for a full characterization of toxicity.

The UF for the RfD is 300 and the overall confidence is medium. The components of this overall rating consist of the confidence in the principal study, rated medium, and confidence in the entire data base, which is medium. The principal study is a mouse chronic oral study performed with relatively large group sizes, where histopathological analyses on the known animal target tissue, the liver, was thoroughly performed. The critical effect of hepatic toxicity was consistent across routes of exposure as the inhalation RfC is also based on hepatotoxicity. However, recent evidence indicates that neurotoxicity, a known human endpoint in acute exposures, may be a relevant endpoint in chronic human exposures, and no chronic animals studies have examined neurotoxicity. Studies on pre- and postnatal animals indicating chlordane mimicry of sex-steroids raise reproductive concerns and no multigenerational reproductive studies, by any route, currently exist. Therefore, there is some concern that the appropriate endpoints have not been adequately examined in the existing data base. Despite this concern, the wide array of special studies (which may raise similar controversy for other chemicals having less data than does chlordane) and numerous chronic studies having consistent results are judged adequate information to rate the overall confidence in the database as medium.

The human chronic air concentration (RfC) considered to be without adverse effect is 8E-5 mg/m^3. This value was based on the occurrence of blood-indicators of hepatic dysfunction derived from a 3-mo rat inhalation study by first designating a NOAEL (10 mg/m^3) and then dividing this concentration by a composite UF. An uncertainty factor of 3000 was used: 10 for extrapolation from the 3-mo study to a lifetime study; 10 for extrapolation from rats to humans; 10 for variability between humans; and 3 for data base deficiencies, principally the lack of any multigeneration reproductive study. It is assumed that these RfD and RfC values are low enough to protect humans from all forms of toxicity.

The overall confidence in this RfC value is low. The components of this overall rating consist of the confidence in the principal study, rated medium, and the confidence in the entire data base, which is low. The principal study is a rat subchronic inhalation study performed with relatively large group sizes, in which histopathological analyses on the known animal target tissue, the liver, was thoroughly performed. The confidence in the database for technical chlordane is rated low. In animal studies, the critical effect of hepatic toxicity was consistent across routes of exposure as oral studies also indicate hepatotoxicity as the critical endpoint of toxicity (Khasawinah and Grutsch, 1989a). The same limitations, uncertainties and controversies surrounding the oral RfD assessment also apply to this assessment (above) and the possibility exists that this assessment, too, is based on studies flawed in design and endpoint assessment. Due to this circumstance, any future alteration of the RfC based on improved database (such as a chronic study) would have to be considered in light of what is made known about the neurotoxic and immunotoxic potential of chlordane in both animals and humans.

To estimate the human cancer risk to chlordane, the Agency has assumed that, in general, the risk is proportional to the dose and the quantitative risk of cancer which might appear in humans is the same as the risk of liver cancer in mice. The liver cancer risk in five different mouse data sets (males and females in three mouse strains), when converted to an equivalent human

dose, averages 0.35 per mg/(kg-day) and the total range is a factor of 8. The slope factor, which is the lifetime risk from a lifetime average ingestion exposure of 1 µg/(kg-day), is 3.5E-4. If chlordane exposure came only from drinking water, the risk for a drinking water concentration of 1 µg/L would be about 1E-5. A chlordane concentration of 1 µg/m^3 would induce a risk of about 1E-4.

The relation between the quantitative cancer and noncancer risk estimates given above can be seen if we were to calculate the cancer risk to people hypothetically exposed continuously via ingestion to the RfD dose or via inhalation to the RfC concentration. These lifetime cancer risks would be approximately 1.75E-4 for ingestion and 7E-5 for inhalation, which fall into the range of E-6 to E-4.

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8.0 APPENDIXES

Appendix A: Benchmark Dose Analyses Cancer Risk Estimates.

(1) Computational Models—Discontinuous (Quantal) Data

The polynomial mean response regression model (THRESH) (ICF Kaiser, 1990a) and the Weibull power mean response regression model (THRESHW) (ICF Kaiser, 1990b) were used to fit data by the maximum likelihood method.

THRESH $P(d) = q_0 + (1- q_0) * (1-\exp(-q_1 (d-d_0) - ... -q_k (d-d_0)^k)$

THRESHW $P(d) = 1 - e^{-\alpha-\beta(d-d0)\gamma}$

where

d = dose
P(d) = probability of a response (health effect) at dose d
q_0 , q_1 , q_2 , α, β, γ, k = estimated parameters

For data input to THRESH, the degree of the polynomial was set to the number of dose groups minus one, the response type was extra [P(d) - P(0) / 1- P(0)]. The risk estimated was 10%. For THRESHW, data inputs were the same and the lower limit of γ was set at 1. For both THRESH and THRESHW calculations were performed with and without the threshold parameter (d_0).

(2) Data

Incidence of hepatic necrosis in male mice from the study of Khasawinah and Grutsch (1989a) was modeled.

(3) Model Fit

Model fit was judged by the p-values generated with the χ^2 goodness-of-fit generated by THRESH or THRESHW.

(4) Results

Organ Data Modeled	Dose (ppm)	Observed Incidence	Predicted Incidence THRESH and THRESHW (P= 0.1)
Male mice, hepatic necrosis	0	7/80	7.5/80
	1	8/80	9.7/80
	5	25/80	18/80
	12.5	27/80	31/80
BMC_{10} (MLE) for THRESH and THRESHW	2.4 (3.3)		

Table 1. Benchmark concentration modeling at the 10% response level (BMC_{10}) of hepatic necrosis observed in male mice (Khasawinah and Grutsch, 1989a). The actual data from the study (Observed Incidence) is compared against the results obtained from applying both the THRESH and THRESHW models (Predicted Incidence). For THRESH and THRESHW, P = 0.1. Both the maximum likelihood estimate (MLE) and the BMC at 10% response level (BMC_{10}) are shown.

(5) Discussion

Both models (the polynomial THRESH and Weibull THRESHW) without a threshold parameter were able to marginally fit the observed data (i.e., the p value for the goodness-of-fit statistic was > 0.05 at 0.1). With a threshold term estimated, the model fits were $p < 0.05$, indicating no fit. With a background term estimated for both models, the model outputs underpredicted observed incidence at 5 ppm (around 0.3, 25/80) by about 8% and overpredicted observed incidence at 12.5 ppm by about 5%. The 8% margin of underprediction around the frequency of 0.3 is nearly equal to the designated response level of 10%. This degree of error in the critical area where the 10% response level would be derived is deemed unacceptable. At the response level chosen, 10%, the BMC determined by the models, although not reliable for the reasons given above, was intermediate between the NOAEL of 1 ppm and the LOAEL of 5 ppm at 2.4 ppm.

Due to this unacceptable degree of error in the modeled output, the NOAEL/LOAEL method will be used rather than results from this benchmark analysis.

Derivation of Cancer slope factors from bioassay data:

The following tables summarize the derivation of the slope factors for the five data sets.

Table 6a. Dose-response modeling of liver tumor data for mice chronically exposed to chlordane in the diet.

Dietary Concentration (ppm)	Estimated Mouse Dose (mg/kg-day)	Human Equivalent Dose[1] (mg/kg-day)	Tumor Incidence No. responding/No. examined
Source: IRDC (1973): Male CD-1 mice, analytical chlordane in diet for 18 mo, hepatic carcinomas. [1]Human Equivalent Dose = Mouse Dose × (mouse BW/human BW)$^{1/4}$ = mouse dose × 0.143882			
5	0	0	3/33
25	0.71	0.1022	5/55
5	3.57	0.5137	41/52
0	7.14	1.0273	32/39

Model: GLOBAL86 computer algorithm, linearized multistage procedure, extra risk;
Polynomial model form: $P(D) = 1\text{-}\exp(-Q0 - Q1xD - Q2xD^2 - Q3xD^3)$.

Results:
All Data: MLEs of dose coefficients: Q0 = 5.502E-2; Q1 = 1.837; Q2 = 0.152; Q3 = 0.
Chi-square fit statistic = 11.843, p =.579E-3. 95% upper limit on low-dose slope = 4.542E-2 per mg/kg-day.

MLE of dose (mg/kg-day) at:
0.1 extra risk = 0.0571, 95% CLL = LED10 = 0.0439;
1E-4 extra risk = 5.443E-5, 95%CLL = 4.163E-5;
1E-5 extra risk = 5.443E-6, 95%CLL = 4.163E-6;
1E-6 extra risk = 5.443E-7, 95%CLL = 4.163E-7.

High-dose data dropped due to poor fit: $P(D) = 1\text{-}\exp(-Q0 - Q1xD - Q2xD^2)$; MLEs of dose coefficients:
Q0 = 6.860E-2; Q1 = 0; Q2 = 5.493.
Chi-square fit statistic = 0.738, p = 0.390. 95% upper limit on low-dose slope = 0.857 per mg/kg-day.

MLE of dose (mg/kg-day) at:
0.1 extra risk = 0.139, 95% CLL = LED10 = 0.088;
1E-4 extra risk = 4.267E-3, 95%CLL = 1.165E-4;
1E-5 extra risk = 1.349E-3, 95%CLL = 1.166E-5;
1E-6 extra risk = 4.267E-4, 95%CLL = 1.165E-6.

Table 6b. Dose-response modeling of liver tumor data for mice chronically exposed to chlordane in the diet.

Dietary Concentration (ppm)	Estimated Mouse Dose (mg/kg-day)	Human Equivalent Dose[1] (mg/kg-day)	Tumor Incidence No. responding/No. examined
Source: IRDC (1973): Female CD-1 mice, analytical chlordane in diet for 18 mo, hepatic carcinomas. [1]Human Equivalent Dose = Mouse Dose x (mouse BW/human BW)$^{1/4}$ = mouse dose x 0.143882.			
0	0	0	0/45
5	0.71	0.1022	0/61
25	3.57	0.5137	32/50
50	7.14	1.027	26/37

Model: GLOBAL86 computer algorithm, linearized multistage procedure, extra risk;
Polynomial model form: $P(D) = 1\text{-}exp(-Q0 - Q1xD - Q2xD^2 - Q3xD^3)$.

Results:
All Data: MLEs of dose coefficients: Q0 = 0; Q1 = 0.6814; Q2 = 0.9067; Q3 = 0.
Chi-square fit statistic = 15.41, p = 0.865E-4. 95% upper limit on low-dose slope = 1.515 per mg/kg-day.

MLE of dose (mg/kg-day) at:
0.1 extra risk = 0.132, 95% CLL = LED10 = 0.0696;
1E-4 extra risk = 1.467E-4, 95%CLL = 6.602E-5;
1E-5 extra risk = 1.468E-5, 95%CLL = 6.602E-6;
1E-6 extra risk = 1.468E-6, 95%CLL = 6.602E-7.

High-dose data dropped due to poor fit: $P(D) = 1\text{-}exp(-Q0 - Q1xD - Q2xD^2)$; MLEs of dose coefficients:
Q0 = 0; Q1 = 0; Q2 = 3.573.
Chi-square fit statistic = 2.502, p = 0.114. 95% upper limit on low-dose slope = 0.270 per mg/kg-day.

MLE of dose (mg/kg-day) at:
0.1 extra risk = 0.172, 95% CLL = LED10 = 0.143;
1E-4 extra risk = 5.290E-3, 95%CLL = 3.677E-4;
1E-5 extra risk = 1.673E-3, 95%CLL = 3.690E-5;
1E-6 extra risk = 5.290E-4, 95%CLL = 3.691E-6.

Table 6c. Dose-response modeling of liver tumor data for mice chronically exposed to chlordane in the diet.

Dietary Concentration (ppm)	Estimated Mouse Dose (mg/kg-day)	Human Equivalent Dose[1] (mg/kg-day)	Tumor Incidence No. responding/No. examined
Source: NCI (1977): Male B6C3F1 mice, analytical chlordane in diet for 80 weeks (+ 10 weeks observation), hepatic carcinomas.			
[1]Human Equivalent Dose = Mouse Dose x (mouse BW/human BW)$^{1/4}$ = mouse dose x 0.143882.			
0 (pooled control)	0	0	17/92
0 (matched control)	0	0	2/18
29.9	4.3	0.6187	16/48
56.2	8.0	1.1511	43/49

Model: GLOBAL86 computer algorithm, linearized multistage procedure, extra risk;
Polynomial model form: $P(D) = 1\text{-}exp(-Q0 - Q1xD - Q2xD^2)$.

Results (using pooled control data):
All Data: MLEs of dose coefficients: Q0 = 0.183; Q1 = 0; Q2 = 1.131.
Chi-square fit statistic = 4.61, p = 3.180E-2. 95% upper limit on low-dose slope = 0.324 per mg/kg-day.

MLE of dose (mg/kg-day) at:
0.1 extra risk = 0.305, 95% CLL = LED10 = 0.211;
1E-4 extra risk = 9.402E-2, 95%CLL = 3.083E-4
1E-5 extra risk = 2.973E-3, 95%CLL = 3.085E-5;
1E-6 extra risk = 9.402E-4, 95%CLL = 3.086E-6.

Source: NCI (1977): Female B6C3F1 mice, analytical chlordane in diet for 80 weeks (+ 10 weeks observation), hepatic carcinomas.			
[1]Human Equivalent Dose = Mouse Dose x (mouse BW/human BW)$^{1/4}$ = mouse dose x 0.143882.			
0 (pooled control)	0	0	3/78
0 (matched control)	0	0	0/19
29.9	4.3	0.6187	3/47
56.2	8.1	1.3093	34/49

Model: GLOBAL86 computer algorithm, linearized multistage procedure, extra risk;
Polynomial model form: $P(D) = 1\text{-}exp(-Q0 - Q1xD - Q2xD^2)$.

Results (using pooled control data):
All Data: MLEs of dose coefficients: Q0 = 2.95E-5; Q1 = 0; Q2 = 0.5242.
Chi-square fit statistic = 7.663, p = 5.638E-3. 95% upper limit on low-dose slope = 0.125 per mg/kg-day.

MLE of dose (mg/kg-day) at:
0.1 extra risk = 0.448, 95% CLL = LED10 = 0.365;
1E-4 extra risk = 1.381E-2, 95%CLL = 8.026E-4;
1E-5 extra risk = 4.368E-3, 95%CLL = 8.045E-5;
1E-6 extra risk = 1.381E-3, 95%CLL = 8.047E-6.

Table 6d. Dose-response modeling of liver tumor data for mice chronically exposed to chlordane in the diet.

Dietary Concentration (ppm)	Estimated Mouse Dose (mg/kg-day)	Human Equivalent Dose[1] (mg/kg-day)	Tumor Incidence No. responding/No. examined
[1]Human Equivalent Dose = Mouse Dose x (mouse BW/human BW)$^{1/4}$ = mouse dose x 0.143882.			
0	0	0	3/71
1	0.15	0.0216	3/71
5	0.75	0.1079	7/72
12.5	1.875	0.2698	9/72

Model: GLOBAL86 computer algorithm, linearized multistage procedure, extra risk;
Polynomial model form: $P(D) = 1\text{-}\exp(\text{-}Q0 \text{ -}Q1xD \text{ -}Q2xD^2)$.

Results:
All Data: MLEs of dose coefficients: Q0 = 4.28E-2; Q1 = 0.378; Q2 = 0.00
Chi-square fit statistic = 0.43, p = 0.73. 95% upper limit on low-dose slope = 0.710 per mg/kg-day.

MLE of dose (mg/kg-day) at:
0.1 extra risk = 2.79, 95% CLL = LED10 = 0..15;
1E-4 extra risk = 2.64E-4, 95%CLL = 1.41E-4;
1E-5 extra risk = 2.64E-5, 95%CLL = 1.41E-5;
1E-6 extra risk = 2.64E-6, 95%CLL = 1.41E-6.

Source: Khasawinah and Grutsch (1989a); U.S. EPA (1986b): Male ICR mice, technical chlordane in diet for 24 mo, hepatocellular adenocarcinomas alone.

Table 6e. Dose-response modeling of liver tumor data for mice chronically exposed to chlordane in the diet.

Dietary Concentration (ppm)	Estimated Mouse Dose (mg/kg-day)	Human Equivalent Dose[1] (mg/kg-day)	Tumor Incidence No. responding/No. examined
[1]Human Equivalent Dose = Mouse Dose x (mouse BW/human BW)$^{1/4}$ = mouse dose x 0.143882.			
0	0	0	16/71
1	0.15	0.0216	16/71
5	0.75	0.1079	22/72
12.5	1.875	0.2698	37/72

Model: GLOBAL86 computer algorithm, linearized multistage procedure, extra risk;
Polynomial model form: $P(D) = 1\text{-}exp(\text{-}Q0 \text{ -}Q1xD \text{ -}Q2xD^2)$.

Results:
All Data: MLEs of dose coefficients: Q0 = 2.50E-1; Q1 = 5.53E-1; Q2 = 4.45
Chi-square fit statistic = 2.67E-2, p = 0.945, 95% upper limit on low-dose slope = 2.34 per mg/kg-day.

MLE of dose (mg/kg-day) at:
0.1 extra risk = 0.104, 95% CLL = LED10 = 4.50E-2;
1E-4 extra risk = 1.81E-4, 95%CLL = 4.27E-5;
1E-5 extra risk = 1.81E-5, 95%CLL = 4.27E-6;
1E-6 extra risk = 1.81E-6, 95%CLL = 4.27E-7.

Source: Khasawinah and Grutsch, 1989a; U.S. EPA (1986b): Male ICR mice, technical chlordane in diet for 24 mo, hepatocellular adenocarcinomas alone.

The final quantitative estimate is based on the geometric mean of the five data sets, all hepatocellular carcinomas in mice. The slope factors (units of risk per milligrams per kilograms per day) for the individual data sets are: CD-1 males, 0.858; CD-1 females, 0.217; B6C3F1 males, 0.345; B6C3F1 females, 0.114; and ICR males, 0.710. The geometric mean is 0.349 per mg/kg-day. In the calculation of human equivalent dose from dietary concentrations, data in the publications were used (where given) to arrive at the animal dose in milligrams per kilograms per day units and the human equivalent dose was calculated from the animal dose using the equation: human dose = animal dose × (w/70)E1/4, where w is the adult body weight of the mice. The animal weights were assumed to be 30 grams in the absence of data in the publications; however, the NCI study stated that males weighed 35 grams and females weighed 30 grams, and the actual weights were used rather than the 30 gram default value. In the NCI study, the animal dose was assumed to be 0.13 × the concentration in the feed as the default assumption in the absence of animal dose values in the report. Also in the NCI study, the pooled control tumor incidence data were used rather than the matched control (not given above) data. None of the studies mentioned above except Khasawinah and Grutsch reported hepatocellular adenomas, and the above data are for carcinomas alone. The slope factor for combined adenomas and carcinomas from the latter study is 2.34 per mg/kg-day, which is about three times the slope factor for the carcinomas alone in that study. Analysis (not shown here) of the Barrass et al. (1993) study, which had data at a single dose for only combined adenomas and carcinomas, yielded a slope value of 0.47 per mg/kg-day, which is comparable to the above data for carcinomas alone. Taken together, the data for combined benign and malignant tumors is not considered extensive or reliable enough for risk estimation. Neither the male nor the female IRDC data could be fit to the multistage model, and the procedure of deleting the high dose group was used to get an adequate fit. In the case of the females, the best fit was obtained by a polynomial with all terms zero except Q(6), the sixth degree in powers of dose. Since the low-dose slope determination for the IRDC data was judged to be difficult, the effect of deleting the IRDC data from the geometric mean was evaluated. The resulting geometric mean was 0.303 per mg/kg-day, which was close enough to the geometric mean of all five data sets (0.35 per mg/kg-day). Therefore, this difficult data affected the result to only an insignificant extent. The earlier IRIS summary of the IRDC data sets used a version of the multistage model where the degree of the polynomial was fixed at 3; since that model did not adequately fit the data, it was not used here.

The 1996 proposed cancer guidelines recommend an alternate method of low-dose extrapolation in which a linear extrapolation to small doses is done based on the lower 95% confidence limit of the dose giving a 10% incidence of tumors. Using this method, the five data sets would have the following slope values: CD-1 males, 1.136; CD-1 females, 0.699; B6C3F1 males, 0.502; B6C3F1 females, 0.218; and ICR males, 0.676. The geometric mean of these values is 0.567, compared with 0.349, the mean of the slopes from the linearized multistage model. The higher slope values at the 10% incidence point of the dose-response curve compared with the low-dose slope estimates is a reflection of the positive curvature (concave upwards) of all the animal dose-response curves except the ICR mice data set.

Appendix B: Summary of and Response to External Reviews and Comments

The Toxicological Review for Chlordane (except for Section 6, which was written after the document was sent to the external peer reviewers) and the summaries for RfD, RfC, and cancer have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists chosen by EPA in accordance with the Agency's Policy for Peer Review and Peer Involvement (U.S. EPA, 1994c). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. Public comments also were read and considered. Three external peer reviewers provided written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A fourth peer reviewer from the National Cancer Institute was asked to give his comments, without a specified format, on just the cancer summary document. A summary of comments made by the external reviewers and EPA's response to these comments follows. All three external peer reviewers (see Contributors and Reviewers) recommended that this document and the accompanying assessments were acceptable with minor revisions. The brief comments of the fourth reviewer dealt with details of the cancer epidemiology studies and his comments were incorporated into the document.

(1) General Questions

The three external reviewers offered editorial comments and many valuable, but minor, suggestions, all of which have been incorporated into the text to the extent feasible. Specific questions to the reviewers, comments by the peer reviewers and responses by EPA are addressed below.

Question 1: Are you aware of relevant data/studies that were not discussed in the document?

Comments: A total of 13 additional references were mentioned by two of the four reviewers.

Response to Comments: The readily available and appropriate references were obtained and evaluated for relevance to the file. The additional studies cited by one of the reviewers on prenatal chlordane exposure represent additional information on myeloid cell development and are therefore incorporated into the file. None of the additional articles materially changed the conclusions of the document.

Question 2: For the RfD and RfC, were the appropriate critical effects chosen? For the cancer assessment were the tumors observed biologically significant?

Comments: One reviewer states that the critical effect of the RfD should be other than hepatocellular hypertrophy from Khasawinah and Grutsch (1989b). This reviewer gives the specific recommendation to substitute the liver effects observed in the chronic mouse study (Khasawinah and Grutsch, 1989a). Reasons cited for making this comment include the questionable adverse nature of hepatocellular hypertrophy and the association of this effect with age-dependent leukemia, a major confounder in this study.

One reviewer wanted to know the significance of the finding that H-ras and K-ras oncogenes were absent in tumors induced by chlordane.

Response to Comments: We agree with the reviewer that the hepatocellular hypertrophy reported in rats in Khasawinah and Grutsch (1989b) is probably of no toxicological significance. The hypertrophy evidenced no pathological progression in this study even at the 130 week time point and was correlated with increased levels of metabolizing enzymes (cytochromes P-450); it is, therefore, judged as adaptive. The recommendation of the reviewer to substitute the mouse liver study and effects was instituted.

As a consequence of this action, the critical effect of the RfC (also hepatocellular hypertrophy) was considered to be invalidated. The toxicology of the inhalation data base was reconsidered by viewing concurrently the hepatic effects that occurred in the 28-day pilot inhalation study along with those few effects observed in the subchronic study. This approach allowed an overall progression of hepatic effects to be elucidated, from necrosis to blood chemistry indicators of hepatic dysfunction. As these latter effects clearly relate to organ dysfunction, as they were in the progression of adverse hepatic alterations that took place at higher concentrations, and as they were seen in both the 28-day pilot study and in the subchronic study (at the highest concentration only), these indicators were collectively called out as the new critical effect.

A statement was inserted that promoters and nongenotoxic carcinogens are not believed to directly induce ras genes.

Question 3: Were the most appropriate studies used for the various assessments?

Comments: One reviewer reiterated the comment about regional hepatocellular hypertrophy made in item 2 above. The other two reviewers thought that better information might be available: one would like epidemiology studies looking for infectious diseases and cancer in people exposed in utero or as children, the other would like neurotoxicology studies to be done.

Response to Comments: We agree that the data base is not adequate in these respects and we have already flagged neurotoxicity as a data gap in the Risk Characterization section of the Toxicological Review and in the Confidence sections of the RfD and RfC. For the RfD, the chronic mouse study of Khasawinah and Grutsch (1989a) is now being used. For the RfC, the 28-day pilot study (Khasawinah et al., 1989b) is now being used in conjunction with the subchronic study (Khasawinah et al., 1989a) to provide some form of a concentration-response continuum.

Question 4: Should other studies be added under the “supporting/additional” category?

Comments: One reviewer thought that information about the dose of chlordane used in the genotoxic assays and about the response of these assays to structural analogs of chlordane would

improve the description of genotoxicity. The two other reviewers had no suggestions for the improvement of the supporting studies section.

Response to Comments: In this document no attempt was made to reanalyze the genotoxicity information. Instead, the results of the genotoxicity review done in the EPA Carcinogenicity Assessment of Chlordane document (U.S. EPA, 1986b) were incorporated unchanged. No additional genotoxicity studies appeared in the literature search and none were cited by the reviewers. Since there is little evidence for genotoxicity and whatever evidence there is based on assays no longer used, we felt that trying to refine the picture by citing structure-activity relationships and doses relative to standard mutagen would be counterproductive.

Question 5: For noncancer assessments are there other data that should be considered in developing UFs?

Comments: One reviewer cited two additional papers as possibly being relevant to the UF for the RfC.

A second reviewer brought up the controversial issue of synergism between weakly estrogenic pesticides by citing a 1997 letter to Science in which the work of Arnold, et al. (1996) could not be repeated.

Response to Comments: One of the papers, Nye and Dorough (1976), was actually cited in the document but it deals with the distribution of chlordane in the tissues of rats after intratracheal administration, and therefore has little bearing on the uncertainty factor for the RfC. A comment was inserted in section 4.7.2 of the Toxicological Review document discussing the controversy. This paper was formerly withdrawn by the original authors in a letter to Science (McLachlan, 1997).

Question 6: Are the confidence and weight-of-evidence paragraphs clear and accurate?

Comments: One reviewer thought they were clear and comprehensive.

A second reviewer wanted the document to mention that reversal of neurotoxicity in humans after withdrawing chlordane exposure had not been studied.

A third reviewer suggested that the confidence statements for both the RfD an RfC should be recast to present a more balanced view on comments made on the significance of possible effects that may occur in humans such as neurotoxic, hematologic, and reproductive effects. This reviewer suggested mentioning that occupational studies revealed no evidence of hematotoxicity despite the fact that hematologic examinations are a commonly performed health surveillance procedure; that despite nearly 50 years of potential exposure to chlordane there are no indications of reproductive effects in humans and that in the existing animal studies there were no histopathological changes in either the nervous system, blood-forming organs, or reproductive systems that could be attributed to chlordane and suggested adding to the RfC confidence statement information about the relevance of the monkey study. This same reviewer made specific mention to not claim that there is a common mechanism between liver cancer in mice and

cancers of the hematopoietic system in humans. This reviewer also mentioned the possibility of investigating and adding discussions on trends in incidence of liver cancer and NHL in humans after the introduction of chlordane in 1947.

Response to Comments: Concerning the third reviewer's comments, a statement was not added about reproductive effects as another reviewer pointed out that this is a significant data gap. Also, the document already states that there is no evidence of adverse effects in people engaged in manufacturing operations, but that there are case reports of adverse hematopoietic effects in pest applicators and evidence of NHL in farmers. Therefore such a statement here would be misleading. We agree that the link is not established by studies that can be quoted. The document stated only that there was a possible link, and the wording was changed to indicate that this link is speculative. Time trends were not added to the discussion because they are inherently ambiguous when attempting to trace cause and effect. There are too many factors influencing time trends to be able to conclude anything about chlordane as a cause.

(2) Comments on Chemical-Specific Questions

Question 1: Are case studies of hematopoietic disease in people exposed at home and exposed as pesticide applicators supportive of the NHL observed in farmers in terms of confirming the carcinogenicity of chlordane in humans and therefore do they furnish a rationale for upgrading the classification of human evidence from inadequate to limited?

Comments: Two of the three reviewers said the case studies were not supportive of human carcinogenicity and the third reviewer said they were, maintaining that the Agency should err on the side of human safety.

Response to Comments: No change was made in the classification of human evidence of carcinogenicity. It is still classified as inadequate because scientific judgment is a primary consideration in EPA assessments and there is no new evidence submitted by the reviewers that would change the rationale.

Question 2: Is there an underlying mechanism that would explain both the effects observed in animals (liver toxicity and carcinogenicity in adult exposures and multiple effects after in utero exposures) and effects in humans (toxicity in the nervous and immune systems)?

Comments: Two of the reviewers stated there was no common mechanism and the third speculated that both liver and brain could be producing epoxies that could explain the effects.

Response to Comments: No change will be made in the discussion, since the EPA authors did not find evidence of such mechanisms.

Question 3: Is the Agency correct in its judgment that neurotoxicity is a more appropriate endpoint for human chronic exposure than liver toxicity?

Comments: One reviewer said that neurotoxicity may be more important, but there is no dose-response information on which a firm conclusion can be based. A second reviewer said that

other end points (besides the liver) are important, but that the end points are likely to be different for adult exposures than for in utero exposures. A third reviewer said that both neurotoxicity and liver toxicity are important considerations.

Response to Comments: No changes are necessary in view of these comments.

Additional comment 1: One reviewer concluded his remarks with a list of nine recommendations, most of which were covered in the above questions. However, two additional recommendations were: (1) include information, if available, on the activity of known compounds in the various studies cited in the RfD document in the Additional Studies section as examples of the ability of chlordane to affect developmental processes; and (2) expand comments on the UF for calculating RfC to clarify how calculation of HEC addresses interspecies variation.

Response: Both of these requests will be accomplished to the extent feasible, although the revised RfC does not calculate HEC for reasons elaborated upon in the RfC section.

Additional comment 2: One reviewer suggested a number of editorial clarifications in the Toxicological Review document and the three summary documents.

Response: Most of these suggestions have been incorporated, but the few that were not incorporated were rejected for one of three reasons: (1) they asked for details in experimental protocols that, in our judgment were irrelevant to the outcome; (2) they wanted basic facts about epidemiologic methods covered elsewhere by textbooks; and (3) they suggested alternative wording that was equivalent to the existing text.

Additional comment 3: The reviewer from the National Cancer Institute agreed with the classification of the human evidence of carcinogenicity as less than sufficient, and that the proper classification is B2, probable human carcinogen. With respect to the Cantor et al. (1992) study conclusions, he advised against saying that "[the multiple comparisons made could have] biased the results upwards" because they actually could also increase the chance that relative risk estimates too low will occur. He pointed out that in the Woods et al. (1987) study the odds ratio for chlordane exposure was 1.61, not 1.46 as stated in the EPA report.

He also pointed out a paper by Brown et al. (1990) on leukemia in farmers exposed to pesticides and one by Pesatori et al. (1994) about pest control operators in Florida.

Response: The wording of the Cantor et al. (1992) conclusions was changed to more closely agree with that in the paper.

In the Woods et al. (1987) paper the 1.61 odds ratio was the overall odds ratio for men of all occupations exposed to chlordane, whereas the 1.46 ratio quoted was for farmers only. The former value was added to the text.

The Brown et al. (1990) and the Pesatori et al. (1994) papers were added to the text.