004704

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

7/11/1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Submission of the Toxicology Branch Chapter of the Registration Standard for Lindane

Tox Chem No. 527

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Enclosed is the Toxicology Branch (TB) chapter for the Registration Standard for lindane including the following three parts.

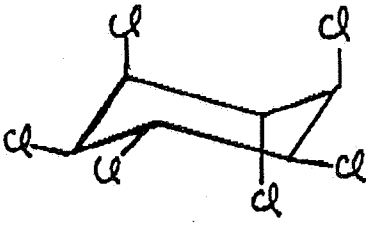
I. Lindane Policy Discussion(s)
II. Table A. Generic Data Requirements for Lindane
III. Summary of Evaluated Data -"one-liners" and review of selected studies. Additional "one-liners" and study reviews will be submitted at a later date.

1 753

I. Lindane Policy Discussions

A. Introduction

Lindane is the common name for preparations of the gamma isomer of 1,2,3,4,5,6- hexachlorocyclohexane (see structure below). Technical preparations of lindane must contain 99.5% or more of the gamma isomer. Other names used for the chemical lindane are gamma BHC and gamma HCH (BHC = benzene hexachloride, HCH = hexachlorocyclohexane). It should be noted that use of the term BHC in describing lindane is misleading because lindane does not contain an unsaturated benzene ring. Lindane must be distinguished from hexachlorobenzene a fungicide, which has separate pesticidal uses and its own toxicity data base. HCH exists in several isomeric forms because of the spatial orientation of the chlorine atoms on the cyclohexane ring. Thus preparations of technical HCH contain mixtures of chiefly alpha, beta, gamma, delta, epsilon and minor quantities of other isomers (up to eight). Technical HCH was once used as an insecticide and earlier toxicity data was generated using the mixture of the isomers. Later studies have indicated that the different isomers have different toxicity profiles Because of lindane's close relationship to the benzene derivative and because there are several isomers of hexachlorocyclohexane any discussion of the toxicity of lindane must carefully assess the exact composition of the test material used in the study. The chemical structure of lindane is as follows:



Other commercial names for lindane include: Exagama, Forlin, Gallogama, Gamaphex, Gammex, Inexit, Isotox, Lindafor, Lindgam, Lindagrain, Lindagranox, Lindalo, Lindamul, Lindpoudre, Lindaterra, Novigam, Silvanol.

Lindane has been used as an insecticide since the 1940's. There are currently numerous tolerances for residues of lindane in/on various agricultural commodities (see Tolerance Reasssessment). The following table outlines the distribution of the usage of lindane in 1978 (this information was obtained from "Preliminary Benefit Analysis of Lindane" prepared by the U.S. EPA and USDA in June 1978):

Estimated Pounds of Gamma Isomer Used by Site

	lbs ai (gamma)	% of total[a/]
hardwood logs, lumber	200,000	23.0
seed treatment	426,000	48.0
forestry	4,000	0.5
livestock	140,000	16.0
pineapples	19,000	2.0
ornamentals	17,000	2.0
Christmas trees	unknown	
pecans	27,000	3.0
pets	30,000	3.0
structures	1,000	0.1
household	12,000	1.0
cucurbits	11,000	1.0
Total	887,000	100.0

a/ May not add to 100% due to rounding error.

According to Mark I. Dow, Ph.D., BUD/EPA, the net poundage and use distribution for the use of lindane probably has not changed significantly from the 1978 estimates.

As indicated in the above table there are a large variety of usages as well as exposure conditions for lindane. In addition to the uses indicated in the table, lindane is also used on humans to control lice and scabies. The use of lindane directly on humans is regulated by the Food and Drug Administration and will not be further evaluated in this Registration Standard.

The regulatory history of lindane at EPA includes a full Rebuttable Presumption Against Registrataion (RPAR) review. Position Documents were published in 1977 (PD1), in 1980 (PD2/3) and in 1983 (PD4). PD4 addressed several toxicity issues of immediate concern, but did not provide a comprehensive evaluation of the full toxicity data base supporting the registration of lindane. Although there have been numerous publications in the scientific literature, there has been only a limited amount of new data submitted to the Agency since completion of the PD4. This Registration Standard reassesses the toxicity data base for lindane and specifies additional studies which are required to support the registration(s) of lindane. The discussions presented in PD4 for the most part will not be reiterated in this Registration Standard.

B. Data Summary

1. Table A - Generic Data Requirements for Lindane - See Part II.

2. Summary of Evaluated Data ("one liners" and review of selected studies)- See Part III.

3. Data Gaps and Special Toxicity Problems

a. Data Gaps

The following toxicity studies are data gaps which should be filled to support the continued registration of various uses of lindane.

i. acute inhalation LC_{50} - rats
ii. dermal sensitization
iii. 21-day dermal-rabbits
iv. 90-day inhalation
v. chronic feeding/oncogenicity-rats
vi. general metabolism (including pharmacokinetics)
vii. special testing - aplastic anemia
viii. mouse oncogenicity-this requirement is being reevaluated (see part c ii below)

b. Explanation for Data Gaps.

i. Acute inhalation LC_{50}-rats. There is no available study which adequately determines the LC_{50} of technical lindane.

ii. Dermal sensitization. A dermal sensitization study is required because lindane products are likely to result in repeated human skin contact under conditions of use.

iii. 21-day dermal-rabbits. Registered uses of lindane include livestock and pet treatment products. These and certain other products may require frequent handling by the user/applicator. Thus, a 21-day dermal toxicity study with rabbits is required to support these uses.

iv. 90-day inhalation. Registered uses of lindane include products intended for repeated indoor uses in the form of aerosols, sprays, etc. A 90 day inhalation study is required to support these and certain other uses such as soil treatments and subterranian termite control (refer to the Data-Call-In notice dated Feb. 23, 1984).

v. Chronic Feeding/Oncogenicity-Rats. A chronic feeding/oncogenicity study in rats is required to support uses on agricultural commodities and certain other uses involving repeated long-term exposure to lindane. The chronic feeding aspects of this study must include an emphasis on kidney and liver function and pathology. Pathology of the testes should be included. Critical assessments of all blood elements and the spleen and bone marrow should also

be included in addition to the usual parameters of a chronic feeding study. Extra slides of the kidney, testes, liver, spleen, and bone marrow should be prepared and examined. The oncogenicity aspects of this study should particularly include critical assessment of the liver and the hematopoietic system.

vi. General metabolism. A general metabolism study is required to support uses on agricultural commodities and certain other uses involving repeated long-term exposure to lindane (e.g. soil treatment and subterranian termite control (refer to the Data-Call-In notice dated Feb. 23, 1984).

vii. Special testing-aplastic anemia.

Lindane has been associated with possible induction of aplastic anemia (a more specific term for blood dyscrasias) in humans. The PD4 concluded that there were insufficient data to establish a cause-effect relationship between lindane and blood dyscrasias. The possibility that lindane may induce aplastic anemia in certain susceptible humans still remains. For this reason a special study is requested to assess whether lindane can induce or increase the frequency of anemia in test animals. A suggested protocol for this study is briefly outlined below.

It is suggested that the test animals (preferably rats or mice) first be exposed to known inducers of aplastic anemia such as radiation or certain chemicals. A standard curve for induction of anemia by the agent(s) should be constructed. Then additional animals should be dosed with both lindane and the inducing agent. An enhancement of aplastic anemia by lindane would be the criterion for a positive result. This experiment was suggested by Dr. Louis Kasza, Pathologist, Toxicology Branch.

The registrant is strongly encouraged to discuss this study in advance with the Agency and then design the study and submit the protocol to the Agency for review. Discussions on chemical and radiation induced aplastic anemia can be found in the following references (the registrant is welcome to use other appropriate references):

(a) Hematology, 3rd Edition, (1983), W.J. Williams, E. Beutler, A.J. Erslev, and M.A. Lichtman, McGraw-Hill Book Company, pages 151-170.

(b) Veterinary Pathology, 5th Edition, (1983), T.C. Jones and R.D. Hunt, Lea-Febiger, pages 886-894.

(c) Pathology of Laboratory Animals, Volume I, (1978), K. Benirschke, F.M. Garner and T.C. Jones (editors), Springer-Verlag, pages 890-1050.

c. Special Toxicity Problems

i. Teratology and reproduction studies

[The following statements were provided by William L. Burnam, Deputy Branch Chief, Toxicology Branch.]

The potential of lindane to induce teratogenic and fetotoxic effects has been adequately characterized by studies in the rat, rabbit and mouse in which the routes of administration were by gavage and subcutaneous injection. In these studies which were reviewed previously by the Agency and referenced in Lindane Position Document 4, no teratogenic effects were noted and adverse fetal effects were seen only at doses which also caused maternal toxicity.

Oral (gavage) intubation in rats indicated a NOEL of 5 mg/kg/day for maternal effects and a NOEL of 10 mg/kg/day for fetal effects. Similar NOELs were noted in a gavage study in rabbits. In a gavage study in mice, both the maternal and fetal NOEL was 30 mg/kg/day. No further teratogenicity testing is required with lindane.

Doses up to 100 ppm produced no adverse reproductive effects in rats fed lindane for three generations. No further reproduction testing is required with lindane.

ii. Oncogenicity in mice

The Carcinogenesis Assessment Group (CAG) of the Office of Research and Development (ORD) of EPA has reviewed the issue of lindane oncogenicity in mice and has determined that lindane has been demonstrated to be associated with increased incidences of liver tumors in at least two studies. These studies are the NCI study (1977) and the Thorpe and Walker (1973) study conducted at the Shell Research Laboratories in England. For a more detailed discussion of this determination, see PD4. The conclusion that lindane causes increased liver tumors in mice was also made by the International Agency for Research on Cancer (IARC, 1979). Other studies have indicated that the alpha isomer of hexachlorocyclohexane consistently increases liver tumors in both rats and mice and a metabolite of lindane in humans (2,4,6-trichlorophenol) is associated with leukemia in rats and with liver tumors in mice.

For the preparation of this Registration Standard, Toxicology Branch has taken into consideration seven studies designed by the investigators to assess the oncogenic potential of lindane in mice. For various reasons none of these studies could be classified as CORE MINIMUM or better in terms of current review criteria. The principal reasons that these studies could not be

classified as CORE MINIMUM is that only summary tables were presented and an independent analysis of the data by Toxicology Branch could not be made. Other reasons include that some of the studies were of too short a duration of dosing and still others used too few mice at initiation of the studies and the studies were run concurrently with other test materials.

On June 28, 1985, Hazard Evaluation Division (HED) staff met with the CAG to discuss the issue of lindane oncogenicity in mice. As a result of this meeting it was agreed that CAG would reconsider the issue of lindane oncogenicity in mice and on July 3, 1985, HED forwarded a request to CAG to provide specific responses to the following items (refer to Anne Barton memo to Dr. Elizabeth Anderson dated July 3, 1985):

"1. A rat chronic feeding/oncogenicity study with lindane has been identified as a data gap for this insecticide. Are additional mouse studies with lindane necessary to assess for oncogenic effects in this species.

2. Do the available oncogenicity studies provide a satisfactory assessment for other organs/tissues being possible target organs/tissues for an oncogenic effect of lindaner in mice.

3. If the available data are appropriate, classify lindane per proposed Agency Guidelines according to the weight of available evidence. The attached summary of mutagenicity experiments with lindane prepared by Dr. I. Mauer should be incorporated into the classification.

4. Confirm that the appropriate Q_1^* for the risk assessments for lindane is 1.33 $(mg/kg/day)^{-1}$."

An additional oncogenicity study with mice may or may not be a requirement (data gag) for lindane pending receipt of responses to be provided by CAG.

iii. Mutagenicity

[The following summary was provided by Dr. I. Mauer, Geneticist, Toxicology Branch.]

As summarized in Appendix IV of PD4, lindane has demonstrated little if any mutagenic or genotoxic activity. Except for two Salmonella studies by Rohrborn (1975, 1976), lindane has been reported to be negative for gene mutation in bacterial Ames assays (Shirasu et al., 1976; Lawler et al., 1975; Purchase et al., 1978;

van Dick and van der Voorde, 1976), in a Drosophila sex-linked recessive lethal assay (Benes and Sram, 1969), in yeast cells (Schuber, 1969; Shahin, 1977) as well as in host-mediated assays (Buselmaier et al., 1972). Although a few in vitro studies with human lymphocytes (Zimonjic et al., 1981; Tzoneva-Maneva et al., 1971) or Chinese hamster lung cells (Ishidate and Odashima, 1977) have suggested lindane may induce chromosome breakage at toxic concentrations, in vivo cytogenetic studies were negative (Rohrborn, 1976, 1977; Dikshith et al., Reno, 1976; Gencik, 1977; Buselmaier et al., 1972; Epstein et al., 1972; Jenssen and Ramel, 1980), or equivocal (Nigam et al., 1981; Shtannov et al., 1980, Cerey et al., 1975). The beta-isomer of HCH has been reported to cause chromosome breakage in rat bone marrow cells (Shimazu et al., 1976). Except for one "weakly" positive in vitro study for unscheduled DNA synthesis in rat thymocytes and human lymphocytes by Rocchi et al., (1980), lindane has been reported as negative in other in vitro assays for DNA damage/repair in bacteria (Lawler et al., 1979; Shirasu et al., 1972, van Dijck et al., 1976), in transformed human (VA-4) cells (Ahmed et al., 1977), as well as in rat and mouse hepatocytes (Probst et al., 1981). Finally, mammalian cell transformation assays were negative employing human WI-38, Chang liver and hamster BHK21 cells (Purchase et al., 1978).

Some studies have reported spindle inhibition with lindane, but these have not been validated by EPA. That lindane may act as a spindle inhibitor is suggested by cytological activity in plant cells, in which it produces c-mitosis and polyploidy (Jeanne, 1979; Das and Singh, 1978; Kar and Singh, 1979; Nyborn, 1947; Anderegg et al., 1977; Baqar et al., 1971) and in rat liver cells, where it increaes mitotic indices and tetraploidy (Hitachi et al., 1975). However, other studies reported negative results (e.g., deBrabander, 1976).

In response to the Special Data Call-In Notice (February 23, 1984), more recent mutagenicity testing submitted by registrants through the Centre International d'Etudes du Lindane (CIEL) have confirmed that lindane has little or no genetic activity, even when employing more sensitive tests for detecting DNA events. For example, negative results were reported in bacterial Ames testing in the presence of a microsomal metabolic activation system (S-9) from the tumor-susceptible mouse strain, CF-1. Negative results were also obtained in the presence of inactivators of potential nucleophilic reacting products, such as the epoxide hydrase inhibitor and glutathione depletor, 1,1,1-trichloropropene (TCPO), as well as following preincubation with nor-harman, a DNA intercalator and co-carcinogen, which is a known inhibitor of DNA synthesis and repair. Negative results have also have reported in adequate mammalian assays for gene mutation at the HGPRT locus of Chinese hamster lung (V79) cells, and for sister chromatid exchanges in CF-1 mice administered lindane by both the oral and parenteral (ip) routes.

Hence, the weight-of-evidence based on conventional genotoxicity testing indicates that lindane does not interact directly with DNA or interfere with genetic mechanisms. Moreover, further standard mutagenicity assays are unlikely to alter this assessment.

No further mutagenicity studies are required at this time.

[Note: Refer to PD4 for the list of references.]

C. Risk Assessment/Tolerance Reassessment

1. Risk Assessment

HED has requested the CAG to reevaluate the oncogenicity of lindane in mice and to confirm the appropriate Q_1^* for use in risk assessments (see section c ii-Oncogenicity in mice - above). Until the Q_1^* value is confirmed by the CAG, oncogenic risk assessments for lindane cannot be provided.

2. Tolerance Reassessment

Tolerances ranging from 0.01 ppm to 7.00 ppm for residues of lindane in/on various agricultural commodities have been established (40 CFR 180.133).

The best available study for determining the ADI for lindane is the recently submitted subchronic feeding study in rats (1983) which has a NOEL of 4 ppm. Based on dietary analysis, food intake and body weight data from this particular study, the NOEL of 4 ppm is equivalent to 0.3 mg/kg/day. Using this latter value and a safety factor of 2000, the provisional Acceptable Daily Intake (ADI) is 0.00015 mg/kg/day and the Maximum Permissible Intake (MPI) for a 60 kg person is 0.0090 mg/day. The Theoretical Maximum Residue Contribution (TMRC) for lindane based on established tolerances is 1.4189 mg/day/1.5 kg of diet. The percent of the MPI used up is thus 15765.64%. See the attached computer printout.

It should be recognized that even if the safety factor was 100 the percent MPI used up would be 788.28%.

Thus, the sum of the TMRCs from existing tolerances greatly exceed the MPI.

Note: Although the TMRC from existing tolerances greatly exceeds the MPI, the actual residues of lindane in foodstuffs may be lower. Information on the actual residue levels of lindane has been sought (refer to the Residue Chemistry Branch Chapter for the lindane registration standard).

CFR 180.133 Lindane 7/11/85 004704

Unverified Printout

ACCEPTABLE DAILY INTAKE DATA

ADI,Uncer NOEL mg/kg	SF		ADI mg/kg/day	MPI mg/day/60kg
0.300	[redacted]	[illegible]	[illegible] (0.00015)	[illegible]

NOEL Change not needed [illegible]

Published Tolerances

Crop	Tolerance	Food Factor	mg/day/1.5kg
Cattle(26)	7.000	7.18	0.75437
Goats(62)	7.000	0.03	0.00315
Sheep(145)	7.000	0.19	0.02039
Hogs(69)	4.000	3.43	0.20003
Cucumbers,inc pickl(40)	3.000	0. 3	0. 3205
Lettuce(84)	3.000	1.31	0.0[illegible]7
Melons(92)	3.000	2.00	0.0[illegible]14
Mushrooms(97)	3.000	0.03	0.00135
Pumpkin,inc squash(131)	3. 00	0.11	0.00506
Summer Squash(153)	3.000	0.03	0.00135
Tomatoes(165)	[illegible]	[illegible] 7	0.12[illegible]37
Apples(2)	1.000	[illegible].53	0. [illegible]795
Apricots(3)	1.000	0. 1	0.00165
Asparagus(5)	1.000	0.14	0. [illegible]15
Avocados(6)	1.000	0. 3	0.[illegible]
Broccoli(19)	1.000	0.10	0.00155
Brussel Sprouts(20)	1.000	0.03	0.00045
Cabbage,sauerkraut(22)	1. 00	[illegible].74	0.0110[illegible]
Cauliflower(27)	1.000	[illegible].07	0.[illegible]1[illegible]7
Celery(2[illegible])	1.000	0. 9	0. [illegible]42[illegible]
Cherries(30)	1. 00	0.[illegible]	0.[illegible]155
Collards(37)	1. 00	0.[illegible]5	0.00125
Eggplant(53)	1.000	0.03	0.00045
Grapes, inc raisins(64)	1.000	0.49	0.[illegible]734
Guava(184)	1.000	0.03	0.00045
Kale(75)	1.000	0.03	0.00045
Kohlrabi(76)	1.000	0.03	0.[illegible]045
Mangoes(86)	1.000	0.03	0.00045
Mustard Greens(99)	1.000	0.06	0.00092
Nectarines(100)	1.000	0. 3	0.00045
Okra(103)	1. 00	0.07	0.00107
Onion(dry bulb)(108)	1.000	0.72	0.01073
Peaches(114)	1. 00	[illegible] 0	0. 1349
Pears(116)	1.000	0.26	0.00383
Peppers(120)	1.000	0.12	[illegible]
Pineapple(123)	1.000	0.30	0.[illegible]445
Plums,inc prunes(125)	1.000	0.13	0. [illegible]195
Quinces(132)	1.000	0.03	0.00045
Spinach(150)	1.000	0.03	0.00077
Strawberries(152)	1.000	[illegible].18	0. 0270
Swiss Chard(158)	1.000	0. 3	0.00045
Pecans(118)	0. 1[illegible]	0. 3	0. [illegible]000

MPI	TMRC	% ADI
0.0090 mg/day/60kg	1.4169 mg/day/1.5kg	15765.64

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Commodity	Parts per million
Tomatoes	10

(Sec. 408(d)(2), 68 Stat. 514 (21 U.S.C. 346(d)(2))

[46 FR 27938, May 22, 1981, as amended at 48 FR 32015, July 13, 1983]

§ 180.130 Hydrogen cyanide; tolerances for residues.

Tolerances for residues of the insecticide hydrogen cyanide from postharvest fumigation are established as follows:

250 parts per million in or on the following spices: Allspice, anise, basil, bay, black pepper, caraway, cassia, celery seed, chili, cinnamon, cloves, coriander, cumin, dill, ginger, mace, marjoram, nutmeg, oregano, paprika, poppy, red pepper, rosemary, sage, savory, thyme, tumeric, white pepper.

75 parts per million in or on barley, buckwheat, corn (including popcorn), milo (grain sorghum), oats, rice, rye, wheat.

50 parts per million in or on citrus fruits.

25 parts per million in or on almonds, beans (dried), cashews, cocoa beans, peanuts, peas (dried), pecans, sesame, walnuts.

§ 180.131 Endrin; tolerances for residues.

Tolerances are established for residues of the insecticide endrin (hexachloroepoxyoctahydro-endo, endo-dimethanonaphthalene) in or on the following raw agricultural commodities:

Commodity	Parts per million
Beets, sugar	0
Beets, sugar, tops	0
Broccoli	0
Brussels sprouts	0
Cabbage	0
Cauliflower	0
Collards	0
Cucumbers	0
Eggplant	0
Peppers	0
Potatoes	0
Squash, summer	0
Tomatoes	0

[42 FR 9178, Feb. 15, 1977]

§ 180.132 Thiram; tolerances for residues.

Tolerances for residues of the fungicide thiram (tetramethyl thiuram disulfide) in or on raw agricultural commodities are established as follows:

7 parts per million in or on apples, celery, peaches, strawberries, tomatoes.

7 parts per million in or on bananas, (from preharvest and postharvest application) of which not more than 1 part per million shall be in the pulp after peel is removed and discarded.

0.5 part per million in or on onions (dry bulb).

[36 FR 22540, Nov. 25, 1971, as amended at 37 FR 3182, Feb. 12, 1972]

§ 180.133 Lindane; tolerances for residues.

Tolerances are established for residues of the insecticide lindane (gamma isomer of benzene hexachloride) in or on raw agricultural commodities as follows:

7 parts per million in or on the fat of meat from cattle, goats, horses, and sheep.

4 parts per million in or on the fat of meat from hogs.

3 parts per million in or on cucumbers, lettuce, melons, mushrooms, pumpkins, squash, summer squash, and tomatoes.

1 part per million in or on apples, apricots, asparagus, avocados, broccoli, brussels sprouts, cabbage, cauliflower, celery, cherries, collards, eggplants, grapes, guavas, kale, kohlrabi, mangoes, mustard greens, nectarines, okra, onions (dry bulb only), peaches, pears, peppers, pineapples, plums (fresh prunes), quinces, spinach, strawberries, and Swiss chard.

0.01 part per million (negligible residue) in or on pecans.

[36 FR 22540, Nov. 25, 1971, as amended at 39 FR 13776, Apr. 17, 1974]

§ 180.134 Ovex; tolerances for residues.

Tolerances for residues of the insecticide ovex (*p*-chlorophenyl-*p*-chlorobenzenesulfonate) are established as follows:

5 parts per million in or on grapefruit, lemons, oranges, tangerines.

3 parts per million in or on apples, peaches, pears, plums (fresh prunes).

§ 180.135 Aldrin; toler

Tolerances for to
insecticide aldrin a
product dieldrin, res
cation of aldrin, in
tural commodities
follows:

0.1 part per millio
gus, broccoli, brus
bage, cantaloups, c
cherries, cranberrie
plant, grapes, lettu
melons, nectarines,
pimentos, pineapp
prunes), potatoes, p
ries, summer squas
tomatoes, watermel

Zero in or on al
cots, beans, black-e
clover, collards, c
grain, cowpeas, co
(escarole), garden
tops, garlic, grain s
ghum forage, horse
rabi, leeks, lespede
onions, parsnips, pe
pears, peas, pea has
radishes, rutabagas
fy roots, shallots,
hay, spinach, sug
tops, Swiss chard, t

Additional tolera
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interim basis pendi
all tolerances for t
new toxicity and re
available on or bef
0.1 part per millio
barley, oats, rice,
0.05 part per milli
fruit, lemons, lin
grain, and tangerin
million in or on th
oats, rye, and wheat

§ 180.136 Basic cop
ance for residues.

The tolerance f
fungicide basic cop
on pears from post
chemical is 3 parts
bined copper.

§ 180.137 Dieldrin; t

Tolerances for re
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al commodities are
lows:

TABLE A
GENERIC DATA REQUIREMENTS FOR LINDANE

Data Requirement		Composition	Use Patterns	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	MRID NO.
§158.135 Toxicology					
ACUTE TESTING:					
81-1	Oral LD_{50}-Rat	TGAI	All	Partial	00049330 and 00109
81-2	Dermal LD_{50}	TGAI	All	Partial	00049330 and 00109
81-3	Inhalation LC_{50}-Rat	TGAI	All	Parcial	—
81-6	Dermal Sensitization	TGAI	All	No	—
81-7	Acute Delayed Neurotoxicity-Hen	N/A			
SUBCHRONIC TESTING:					
82-1	90-Day Feeding-rodent - rat	TGAI	All	Yes	00128356
	nonrodent-dog	TGAI	All	No	—
82-2	21-Day Dermal	TGAI	All	No	—
82-3	90-Day Dermal	TGAI	—	No	—
82-4	90-Day Inhalation	TGAI	E,P,I	No	—
82-5	90-Day Neurotoxicity -hen	N/A			

TABLE A
GENERIC DATA REQUIREMENTS FOR LINDANE

Data Requirement	Composition	Use Patterns	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	MRID NO.
§158.135 Toxicology (continued)				
CHRONIC TESTING:				
83-1 Chronic Toxicity-				
rat	TGAI	A,E	No	—
dog	TGAI	A,E	Yes	—
83-2 Oncogenicity -				
rat	TGAI	A,E	No	—
mouse	TGAI	A,E	Partial	—
83-3 Teratology -				
1st species (rat)	TGAI	All	Yes	—
2nd species (hamster)	TGAI	All	Yes	—
83-4 Reproduction - 2 generations	TGAI	A,E	Yes	—
MUTAGENICITY TESTING				
84-2 Gene Mutation	TGAI	All	Yes	—
84-3 Chromosomal Aberration	TGAI	All	Yes	—
84-2 Other Mechanisms of Mutagenesis	TGAI	All	Yes	—

TABLE A
GENERIC DATA REQUIREMENTS FOR LINDANE

Data Requirement	Composition	Use Patterns	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	MRID NO.
§158.135 Toxicology (continued)				
SPECIAL TESTING:				
85-1 General Metabolism -	PAI or PAIRA	A,E	Partial	—
Aplastic Anemia -	TGAI	All	No	—
Dermal Absorption	PAIRA or PAI	All	Yes	—

Composition: TGAI = technical grade of the active ingredient. PAI=pure active ingredient. PAIRA radiolabelled. The use patterns are coded as follows: A = terrestrial, food crop, B = Terrestri food crop, D = Aquatic, nonfood, E = Greenhouse, food crop, F = Greenhouse, nonfood, H = domesti Data must be submitted no later than _____.

1. The need for an additional oncogenicity study in mice has not yet been determined. See discu in the Policy Discussion section.

2. Recently acquired data are currently in review. Additional metabolism data may not be requi of these submissions.

3. See discussion of this requirement in the Policy Discussion section.

Tox Chem No. 527 (Lindane) File Last Updated — Current

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Ca
Acute oral LD_{50} - rats [as published in Toxicol. and Applied Pharmacology, 14:515-534 (1969), T.B. Gaines (author)]; MRID # 00049330	Lindane (gamma isomer of hexa-chlorocyclo-hexane, HCH)	—	LD_{50} = 88 mg/kg males. LD_{50} = 91 mg/kg females. Onset, duration and description of symptoms not reported. No necropsy.	
Acute oral LD_{50} - rats [as published in Midwest American Aerovap, Inc., 1951]; MRID # 00109141	Lindane (gamma-isomer of HCH)	—	LD_{50} = 200 mg/kg. Responses include increased respiration, restlessness, intermittent muscle spasms, and chronic convulsions. Death results from dysfunction of the respiratory center. Onset of symptoms is from 2 to 24 hours.	
Acute dermal LD_{50} - rats [as published in Toxicol. and Applied Pharmacology, 14:515-534 (1969) T.B. Gaines (author)]; MRID # 00049330	Lindane	—	LD_{50} = 1000 mg/kg males. LD_{50} = 900 mg/kg females.	[
Acute dermal LD_{50} - rabbits [as published in Midwest American Aerovap, Inc., 1951]; MRID # 00109141	Lindane	—	LD_{50} = 300 mg/kg.	
Acute Inhalation LC_{50} - rats; Fraunhofer Institut; no study number; March 23, 1981 MRID # Not available	Lindane	—	LC_{50} > 0.603 mg/L - no deaths. Signs of irritation and nerve stimulation including restlessness, increased motor activity and rhinitis.	

Tox Chem No. 527 (Lindane) File Last Updated — Current

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	C
Subacute oral- rats (30 days) [study not identified] (see L.B. Dale review dated 4/12/68 in TB files.) MRID # not available	Lindane	—	NOEL = 30 mg/kg.	
✓ 12-Week feeding - rat; Research and Consulting Company Ltd.; #005220; February 3, 1983 MRID # 00128356	Lindane (99.85% pure)	250340 250341 250342	NOEL: 4 ppm (This dosage level is equivalent to 0.3 mg/kg/day based on diet analyses, food intake and body weight data.) LEL: 20 ppm (Permanent or long-lasting kidney damage occurred at this level). Levels of lindane fed in diet: 0, 0.2, 0.8, 4, 20, and 100 ppm. Protocol: Wistar KFM-Han (outbred) SPF rats, 20M + 20F/level, were fed lindane for 12 weeks, at which time 15M + 15F rats from each group were sacrificed. The remaining 5M + 5F rats from each group were then fed a control diet for 6 weeks (recovery period) and were subsequently sacrificed. [The above one liner was prepared by Dr. K.K. Locke]	
104 week chronic feeding - dogs; Huntingdon Research Center; #3422/70/234, June 10, 1970; #4187/71/345, September 2, 1971; and #3720/70/532, January 7, 1971 MRID # not available	Lindane	—	NOEL = 50 ppm. LEL = 100 ppm. Changes in the macroscopic appearance of the liver (no microscopic changes) and changes in the EEG. These are more pronounced at 200 ppm. Elevated SAP at 200 ppm.	

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C)

Tox Chem No. 527 (Lindane) File Last Updated --- Current

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Ca
Lifetime-Oncogenicity - rats; FDA Laboratory [as published in J. Pharmacol. Exptl. Ther.,100:59 (1950) O.G. Fitzhugh, A.A. Nelson, and J.P. Frawley (authors)] MRID #:not available	alpha, beta and gamma isomers of hexachloro-cyclohexane	---	Report's Conclusion NOEL = 50 ppm Liver weight increases at 100 ppm and above. No tumors in liver but signs of hyperplastic change noted. Dosage levels: 0, 5, 10, 50, 100 and 400 ppm.	
8 month-Oncogenicity - rats; U.S. Government (USDA) [as published in A.M.A. Archives of Pathology,64:614 (1957) P. Ortega, W.J. Hayes and W.F. Durham (authors)] MRID #:not available	Lindane	---	Report's Conclusion No frank liver tumors evident. Some signs of non-neoplastic liver change (hyperplasia) noted. Dosage levels: 0, 50 and 100 ppm.	I
24-72 week-Oncogenicity - rats Nagoya City University of Nagoya, Japan [as published in JNCI 54:801-805 (1975) N. Ito, H. Nagasaki, H. Aoe, S. Sugihara, Y. Miyata, M. Arai, and T. Shirai (authors)] MRID #:not available.	Lindane and other isomers of hexachloro-cyclohexane	---	Report's Conclusion alpha-isomer resulted in development of hepatocellular carcinoma and nodular hyperplasia (at 1000 ppm and above). gamma-isomer resulted in no frank liver tumors. Signs of body weight and hyperplastic liver changes at 500 ppm (only dose tested).	I
80-week-Oncogenicity - rats; NCI; #CG-TR-14;1977 MRID #: not available	Lindane	---	Report's Conclusion: No frank neoplastic effect noted at up to and including 236 ppm for males and 270 ppm for females (time weighted average).	I

Tox Chem No. 527 (Lindane) File Last Updated ---- Current

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Ca
80 week-Oncogenicity - mice; NCI; #CG-TR-14; 1977 MRID #: not available	Lindane	---	The mid-dose (80 ppm) group had the highest incidences of liver tumors, but the high-dose group was favorably comparable to the control. Thus, no dose response for an oncogenic effect was evident. Note: CAG/ORD has determined that this study is positive. Dosage levels: 0, 80 and 160 ppm (time weighted average)	
26 month-Oncogenicity - mice; Nara Medical University, Nara Japan [as published in Proceed. Of the 2nd International Symposium of the Princess Tokamatsu Cancer Research Fund H. Nagasaki, S. Tomii, T. Mega, M. Marugami, and N. Ito (authors)] 1972 MRID #: not available	Technical Hexachloro cyclohexane (alpha-66%, gamma 15% and other isomers)	---	All mice dosed with 660 ppm of the technical material developed liver tumors (hepatoma). Dosage levels: 0, 6.6, 66 and 660 ppm.	
32 week-Oncogenicity - mouse Osaka University; "Hanada Study" GANN 64:511 (1973) MRID #: not available	alpha, beta gamma and crude hexachloro-cyclohexane studied separately.	---	gamma-isomer - Positive for "hepatomas" at 600 ppm in males 3/4 survivors had tumors, but none in controls. In females 1/3 had hepatoma. No hepatomas at 100 or 300 ppm. alpha isomer - Both males and females were positive for hepatoma at 300 and 600 ppm; (7/7 males and	

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Ca
continued from preceeding page			6/8 females affected at 600 ppm; 7/7 males and 2/8 females affected at 300 ppm and 1/8 males at 100 ppm affected). beta-isomer - No hepatomas in either males or females at 100, 300, or 600 ppm.	
80 week-Oncogenicity - mice; Boehringer Sohn Ingelheim and Rhein # L 139 E/75 February 25, 1975 (translated into English April 1975) MRID #: not available	Lindane 99.5%	---	No oncogenic effects noted up to and including 50 ppm (HDT). Dosage levels: 0, 12.5, 25 and 50.	
24 week-Oncogenicity - mice; Nara Medical University [as published in JNCI 51:817-826 (1973) N. Ito, H. Nagasaki, M. Arai, S. Sugihara, and S. Makiura (authors)] MRID #:not availble.	alpha, beta gamma and episilon isomers of HCH and combinations of the isomers		alpha-isomer alone at 250 and 500 ppm and in combination with the other isomers resulted in "nodular hyperplasia and "hepatocellular carcinoma." None of the other isomers resulted in a neoplastic response when dosed alone. Dosage levels: 0, 100,300,and 600ppm	
38 week-Oncogenicity - mice; Gakashin University, Tokyo [as published in Chemosphere 1(6):279-282; M. Goto, M. Hottori, T. Miyagawo, and M. Enamoto (authors)] MRID #: not available	Technical (mixture of isomers, alpha, beta, delta and gamma isomers		Technical and alpha isomer resulted in most mice developing liver neoplasms (600 ppm). 5 of 10 mice dosed with 600 ppm lindane (gamma-isomer) developed liver tumors. Positive response for lindane. Dosage levels: 0, 600 ppm.	

Tox Chem No. 527 (Lindane) File Last Updated --- Current

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Ca
26 month-Oncogenicity - mice; Shell Research Laboratories [as published in Fd. Cosmet. Toxicol. 11:433-442 (1973) J. Thorpe and A.I.T. Walker (authors). MRID # not available	gamma-BHC 99.5% beta-BHC also tested	---	Ninety-three percent of male mice and 69% of female mice dosed with 400 ppm of gamma isomer developed either type a or type b liver neoplasms. The control frequency was only 23 or 24%. [NOTE: male mice dosed with 200 ppm beta-isomer also had significant increase in liver tumors]. Dosage levels: 0, 400 ppm (gamma) and 200 ppm (beta)	
Metabolism - mice; Institute of Toxicology, ETH and University of Zurich, Switzerland; no study number; April 15, 1983 MRID #: not available	^{3}H-hexa-chloro-cyclohexane (beta and epsilon isomers and ^{14}C thymidine	250339	The objective of this study was to determine whether or not the tumor formation by hexachloro-cyclohexane (HCH) required covalent binding to DNA. (Gamma isomer of HCH is lindane). It was concluded that beta and gamma isomers of HCH did not bind to DNA. In this study, young male NMRI, CF1 and C6B3F1 mice were orally given different ^{3}H-labeled isomers of HCH with or without ^{14}C-thymidine. Single doses of HCH were used. The mice, 2/treatment, were then sacrificed at different time intervals and DNA was isolated from the livers and studied by a great variety of procedures.	

Tox Chem No. 527 (Lindane) File Last Updated --- Curren

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL
Mutagenicity - in vitro gene mutation in mammalian cells; Institute of Toxicology, University of Mainz; #SP-540-VT21; May 28, 1984 MRID #: not available	Lindane Technical (99.8% ai)	253997	Negative for forward mutation at the HGPRT locus with and without metabolic activation system derived from CF-1 mice. Dose levels tested were up to levels of toxicity and limits of solubility.
Mutagenicicity - in vivo SCE in mice; Research & Consulting Co.; #RCC-025705; June 20, 1984 MRID #: not available	Lindane Technical (99.8% ai)	254504	Negative for bone marrow SCE in CF-1 mice treated orally at single doses (0, 2, 10, and 50 mg/kg) up to 1/3 of the LD_{50}.
Mutagenicity - Sister Chromatid Exchange in Mice (ip in vivo SCE) Research & Consulting Co. AG (Schweiz) RCC Project No. 025716 July 17, 1984	Lindane Technical (99.8%)	254504	Doses tested 0, 1.3, 6.4, 32.1 mg/kg (acute ip). Slight but significant increases in treated females (but not males) at all dose levels (due to lower values in control females) are considered of little or no biological significance.

Tox Chem No. 527 (Lindane) File Last Updated _____ Current

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Cat
Teratology-rat (gavage) Huntingdon Research Labs #4307/71/6163 Dec. 3, 1971 MRID # not available	Technical lindane	——	Teratogenic NOEL >20mg/kg/day (HDT) Maternal LEL = 10mg/kg/day Maternal NOEL = 5mg/kg/day Fetotoxic LEL = 20mg/kg/day Fetotoxic NOEL = 10mg/kg/day	I
Teratology-rabbit (gavage) Huncingdon Research Labs #4308/71/464 Dec. 2, 1971 MRID # not available	Technical lindane	——	Teratogenic NOEL > 20mg/kg/day (HDT) Maternal LEL = 10 mg/kg/day Maternal NOEL = 5 mg/kg/day Fetotoxic LEL = 20 mg/kg/day Fetotoxic NOEL = 10 mg/kg/day	
3 Generation Reproduction-rats (dietary) Huntingdon Research Labs #4289/71/445 Feb. 18, 1972 MRID # not available	Technical lindane	——	Doses up to 100 ppm (HDT) in diet caused no adverse reproductive effects during 3 generations.	I
Dermal Penetration-humans As published in Toxicology and Applied Pharmacology, 27: (1974). H. Maibach and R.J. Feldman (authors).	^{14}Lindane	——	Absorption of ^{14}C Lindane dissolved in acetone and applied to the forearm of humans is 9.3+/-3.7% based on urinary excretion of ^{14}C and comparision with intravenous application.	I

Study Type: Acute Inhalation LC_{50} [Inhalation Study With Lindane (gamma-hexachlorocyclohexane to determine LC_{50})]

Accession No.:

MRID No.:

Sponsor:

Contracting Lab: Frananhofer-Institut (Germany)

Date: March 23, 1981

The following was taken directly from a review written by Stanley B. Gross of Tox Branch dated Dec. 8, 1981. No changes were made and the hard copy of the study was not examined for the preparation of the Registration Standard.

Study Methods

Rats of the Wistar HAN/Boe strain, 5 or 10 animals/sex/level were exposed for 4-hour periods to lindane vapor deposited on respirable NaCl particles at concentrations of 273 and 603 mg/cum. These concentrations are equivalent to 0.273 and 0.603 milligrams per liter. The lindane/NaCl aerosol was produced by allowing vapor heated lindane (bath at 160 °C) to mix with NaCl aerosols made by nebulizig 0.5 percent aqueous solution of salt. This mixture was passed through a condensation zone before being mixed with conditioned room air as it was carried into the exposure chamber. There was a NaCl control group as well as nonexposed chamber control.

The chamber (250 cu cm) was not typical of those used in this country, however, it was appropriately baffled to ensure good distribution of the aerosol within the chamber. Chamber temperature was given as 20 °C and humidity as 63.4 percent. At 80 l/min input flow, the oxygen concentrations were calculated to be greater than 20 percent. The animal volume amounted to 1.6 percent of the chamber, and the animals were group caged.

Chamber samples were taken by collecting the aerosol particles on filters and analyzing xylene extracts of the deposited particles by gas chromatography. Analysis of the vapor phase indicated that this phase of the lindane was considered to be of no importance.

Aerosol particle size was determined by Active Scattering Aerosol Spectrometer System (Particle Measuring Systems, Boulder, Colorado) to have a geometric mean of 0.154 um (SD = 1.419) by count distribution which was calculated to be 0.417 um (SD = 2.601) by mass distribution assuming a density of 1 gm/cu. cm.

The animals were observed and weighed daily for 14 days after their exposure and necropsied.

Study Results:

None of the animals died due to the tests. They responded during the exposures with restlesness, increased motor activity, eye-lid closure, and rhinitis during exposure. Post exposure weight changes among the four groups (control, NaCl, 273 and 603 mg/cu.m. groups) were not remarkable. Necropsies were not remarkabl

Comments:

These were well run and well documented studies, however, the levels used were too low to determine an inhalation LC_{50} estimate. The current cut-off concentration limit is 5 mg/L = 5000 mg/cum. If the highest level physically possible is 603 mg/cum the Agency would accept this experiment, however, the investigators made no claim for this.

Summary and Conclusion:

This study cannot be used as a measure of an LC_{50} for lindane.

Note: As per conversation with Dr. S. B. Gross on June 12, 1985, this study should be classified as SUPPLEMENTARY.

R-3

Study Type: Subchronic (90-day)-rats [3-Month Toxicity Study in Rats with Lindane

Accession No.: 250340-250342

MRID No.: 00128356

Sponsor: Centre International d'Etudes du Lindane (C.I.E.L.)

Testing Laboratory: RCC, Research Consulting Ltd., 4452 Itingen, Switzerland

Date: February 3, 1983.
[Note: This study was reviewed extensively by Dr. K.K. Locke of Toxicology Branch (date June 17, 1983). Dr. Locke's review is some 28 pages in length and the following comments on this study were excerpted from that review and unchanged. The study was not rereviewed for preparation of the Registration Standard].

Conclusions:

Wistar KFM-Han (outbred) SPF rats, 20 males and females/level, were fed diets containing the following levels of lindane: 0, 0.2, 0.8, 4, 20 and 100 ppm. The feeding was started after one week of an acclimation period and was continued for 12 weeks, at which time 15 rats from each group were sacrified. The remaining 5 male and 5 female rats from the lindane-fed groups were placed on a lindane-free (control) diet for 6 weeks (recovery period) and then were sacrificed. The remaining 5 male and 5 female rats from the control groups were also continued on their diet for the additional 6 weeks before they were sacrified.

The following parameters were studied during the acclimation, dosing and recovery periods: observations for toxic signs and mortality, body weights, food consumption, hematology, clinical biochemistry, urinalysis, organ weights, organ to body weight and organ to brain weight ratios, gross necropsy, histopathology and levels of lindane in plasma, liver, kidneys, renal fat and brain.

The following results were obtained:

1. Lindane, at all levels tested, had no effect on the mortality, food consumption, hematology and urinalysis, and no toxic signs were observed in any group.

2. Male and female rats fed 100 ppm of lindane (highest level tested) gained, respectively, 8.4 and 14.9% less weight during the dosing period than did the controls.

3. Male rats fed 0.2-20 ppm and 100 ppm of lindane gained, respectively, 8.9-12.5% and 28.6% less weight during the recovery period, when compared with the controls. Lindane, at all levels tested, had no effect on the body weight gain of the female rats during the recovery period.

4. There were increases in the liver cytochrome P-450 levels at the termination of dosing, especially in the female rats. Females fed 0.8, 4, 20 and 100 ppm of lindane, had, respectively, 26, 42, 35 and 73% more of the hepatic cytochrome P-450 than did the controls. In the case of the males, a 17% increase in the liver cytochrome P-450 content was observed only in the rats dosed with 100 ppm of lindane. All of the increased hepatic cytochrome P-450 levels returned to the control values during the recovery period. The augmentation in the hepatic cytochrome P-450 content during dosing is regarded as an induction of the microsomal detoxyfying enzymes, and not as a toxic symptom.

5. There was a dose-unrelated increase in the liver carboxylase activity of the female rats at the termination of dosing, but not at the termination of the recovery period. Specifically, the increases were 8, 14, 28, 17 and 26% in the groups fed 0.2, 0.8, 4, 20 and 100 ppm of lindane, respectively. The enzyme was unaffected by lindane in the male rats.

6. There were small dose-related increases in the absolute weights of liver and kidneys (8-13%), in the liver and/or kidney to body weight ratios (7-14%), and in the liver and/or kidney to brain weight ratios (7-15%), all in the male and female rats fed 20 or 100 ppm of lindane.

7. Gross necropsy had revealed that lindane, at the 20 and 100 ppm levels, affected both kidneys of all male rats (or 15 rats/group), examined at the termination of dosing. These kidneys were covered with diffuse gray foci. However, this abnormality was reversible because the kidneys of all male rats (or 5 rats/group) which were sacrified at the termination of the recovery period did not have foci. Gross necrospy revealed nothing remarkable in the female rats at the termination of the dosing and the recovery period.

8. Histopathology had revealed that lindane caused changes in the kidneys and liver of the male and female rats. These changes occurred chiefly in the groups fed 20 or 100 ppm of lindane. The changes were frequent, dose-related and severe in the males, and infrequent, dose-unrelated and generally mild in the females. The following renal changes were observed: tubular degeneration, hyaline droplets, tubular casts, tubular distension, interstitial nephritis and basophilic tubules. Hypertrophy was the only dose-related liver change in both males and females.

Most of the renal changes, but not tubular degeneration, were observed also at the termination of the recovery period. Tubular degeneration was not observed at that time and neither was liver hypertrophy, meaning that liver hypertrophy was, apparently, a consequence of enzyme induction.

9. There was a dose-related increase in the plasma level of lindane, in the male and female rats, at the termination of dosing, but there was no lindane in plasma (beyond the control levels) at the termination of the recovery period. Similar findings were observed for liver, kidney, renal fat and brain. The highest levels of lindane were found in the kidneys of the male rats and in the renal fat of the female rats.

10. NOEL: 4 ppm, for both male and female rats. This dosage level is equivalent to 0.3 mg/kg body weight/day - based on diet analyses, food intake and body weight data (see page 9 of this review).

Although some renal changes occurred at this level, they were generally mild and were generally single occurrences. Other changes observed at the 4 ppm level, such as liver hypertrophy and increased levels of hepatic cytochrome P-450, were not toxic manifestations.

LEL: 20 pm (next highest level tested), for both male and female rats. Permanent kidney damage was observed at this level.

Core Classification: Guideline

R-6

A. Study Type: Chronic Feeding - Dogs [Lindane Toxicity Studies in Beagle Dogs]

Accession No.:

MRID No.:

Sponsor: Merck A.G., Frankfurt, West Germany

Contracting Lab.: Huntingdon Research Center

Dates: Part 1 June 10, 1970 (Initial studies and intake up to 50 weeks); Part 2 January 7, 1971 (report on feeding 200 ppm for 32 weeks); Part 3 September 2, 1971 (dietary intake for 104 weeks). Note: see study review below for explanation for three separate dates.

B. The test material used was described only as lindane, the supplier, and lot number(s) were not provided. No statement was made verifying the purity of the material used.

The dose levels for this study were: Initial study (range finding), five groups of two dogs (one male and one female) were dosed with either 0, 25, 50, 100, or 200 ppm for 7 weeks. Main study, four groups of eight dogs (four males and four females) were dosed with either 0, 25, 50, or 100 ppm for 104 weeks; satellite group (included and started after the main study indicated no overt signs of toxic responses to lindane), which consisted of one group of eight dogs (4 males and 4 females) dosed with 200 ppm of lindane. All diets were prepared by making a premix with lindane dissolved in acetone and evaporating the acetone by exposing the lindane fortified diet to infrared light for 1 week.

Samples of the test diets were sent to the sponsor for analyses for lindane content. Actual analytical data were not included in the report but the report indicated that the control group (0 ppm) had 0 to 0.6 ppm lindane; the low-dose group (25 ppm) had 23.2 to 28 ppm lindane; the mid-dose groups (50 ppm) had 51.9 to 55 ppm; the high-dose group (100 ppm) had 92.4 to 112 ppm, and the satellite group (200 ppm) had 179 or 186 (two analyses) for an average of 183 ppm.

C. The test animals used were purebred beagle dogs. The identity of the supplier of these dogs was not provided. They were innoculated prior to dosing against distemper, hepatitis, and leptospirosis as well as treated with an anthelmintic. The approximate age of the dogs at commencement of dosing was 18 to 20 weeks.

D. Survival

A single dog receiving 200 ppm died. It could not be firmly established if this death was a result of lindane poisoning because of the lack of overt symptoms throughout dosing (the dog died after 92 days of feeding). This dog, however showed some apparent initial (days 2-5) reactions to the test material ("unsteadiness of gait, lethargic and apparently unaware of its surroundings"). A single dog dosed with 25 ppm also died. This dog had exhibited two separate convulsive episodes and possibly a third at the time of its death (which occurred overnight) on day 613. None of the control dogs died. No definite test chemical induced deaths were noted.

E. Behavioral and Clinical Signs

The report asserts that although occasionally some convulsive episodes were noted among the dogs in control and other groups, there were no changes induced by lindane.

In the absence of data to the contrary, TB accepts the testers conclusions.

F. Body Weight

The tester asserts that there were no adverse effects on body weight, food consumption, or water consumption.

TB concurs with the tester's conclusion. It should be noted that since dogs vary in their weight and they are large animals, differences in these three parameters are meaningful only when large differences and dose responses are clearly evident.

Based on body weight and food consumption together with the lindane content of the diet, the dogs received lindane in the following daily dose level (in mg/kg/day).

	Males		Females	
Dose Level	0-50	50-104	0-50	50-104
25 ppm	0.84 ± .25	0.90	0.95 ± .22	0.75
50 ppm	1.71 ± .31	1.62	2.07 ± 0.32	1.58
100 ppm	3.51 ± .58	3.02	4.03 ± 0.88	.2.81
200 ppm	8.3 ± 1.9*	--	8.70 ± 2.6*	--

*Weeks 0-32 average

[Note: For sections G, H, and I below analyses were made two times before dosing, at 1, 3, 6, 12, and 24 months on test for the main experiment and once before dosing and after 4, 10, and 25 weeks for the satellite group.]

G. Hematology

The tester asserts that there were no compound-related changes in the various blood elements analyzed.

Evidence in the data tables presented indicated that analyses were made for: prothrombin index, platelets, erythrocyte sedimentation rate, packed cell volume, Hb, RBC, Retics, MCHC, MCV, WBC (total and differential).

Inspection of the data presented allows TB to concur with the tester that no compound-related effects were evident.

[Note: Since lindane has been implicated in possibly causing aplastic anemia in humans (refer to Position Document 4 prepared by EPA.) It is especially important to note that in this dog study lindane was not shown to be related to any obvious changes in red blood cells.]

H. Clinical Chemistries

The tester asserts that there were no changes in any of the clinical biochemistries indicative of lindane toxicity at dose levels up to and including 100 parts per million. However, at 200 ppm, lindane was associated with increases in serum alkaline phosphatase (SAP) activity.

Data were presented which indicate that besides SAP levels the following parameters were investigated: urea, glucose, albumin, globulin (and ratio) SGPT, bilirubin, SGOT Na^+ and K^+ and serum proteins. There were no obvious dose-related changes in these parameters.

SAP levels in the group receiving 200 ppm were elevated at weeks 4 (144%), 11 (121%), and 25 (217%).

In the group receiving 100 ppm, the SAP levels were at weeks 25 (+6%), 50 (+50%), and 102 (-1P%). Thus there was no persistent effect on lindane on this parameter at 100 parts per million.

The NOEL for clinical chemistries is 100 parts per million.

I. Urinalysis

The tester asserts that there were no effects of the test material on the various urinalysis parameters investigated.

Data were presented to show that the pH, volume, specific gravity, proteins, total reducing substances, glucose ketones, bile pigments, bile salts, urobilinogen and blood pigments, and microscopy were performed. There were no consistent dose-related effects on these parameters noted. The NOEL of this aspect of the study is 200 ppm.

J. Organ Weight

The tester asserts that except for the spleen in the main study and the liver in the satellite study the organ weights did not exhibit changes relative to the control dogs.

The weights (absolute and relative) of the following organs were reported: brain, pituitary, spinal cord, heart, lungs, liver, spleen, pancreas, thymus, uterus/prostate, kidney, thyroid, adrenal, and gonads.

The average spleen weight for the control group was 43.2 gms and for the low dose group it was 71.0 gm (+65%), mid 67.9 (+57%), and high 66.0 gm (+53%) and in the group receiving 200 ppm it was 66.8 gms (+25% versus its own control group). No dose response relationship is evident. Since the spleen is a vascular organ, its weight depends upon the blood content and may vary depending on the dissection. Changes in spleen weight are considered critical by this reviewer only when they show clearly defined dose responses, and have supporting pathology (macroand/or microscopic, see below). With these factors taken into consideration, there is no evidence that the apparent changes in spleen weight are related to ingestion of lindane. In terms of lindane toxicity, the spleen must be carefully regarded because lindane has been implicated in blood dyscrasia and the spleen is involved in conditioning the blood.

The liver. No changes in absolute or relative liver weight were evident in the main phase of the study. In the satellite group the dogs receiving 200 ppm had average liver weights of 409 gms or 13 percent higher than their control group. When expressed in relative liver weight, the difference was 38 percent greater. It should be noted that the satellite group was indirectly compared with a control group that was not specifically started and dosed for the same 32 weeks. TB must accept that lindane caused this increase which coincides with the increase in SAP.

The NOEL for organ weight changes is 100 parts per million. At 200 ppm there are liver weight increases.

K. Gross Pathology

No statement on lindane induced effects was made by the tester.

The macroscopic observations for each dog were listed. TB notes that six of the eight dogs receiving 100 ppm had macroscopic findings in the liver ("darker than normal," "friable," "slightly enlarged," "granular"). Only a single dog in the group receiving 50 ppm was reported as having a "slight enlargement of the liver." None of the dogs in either the low dose or control group had gross abnormalities of the liver evident.

The same types of macroscopic pathology was also evident in all of the dogs receiving 200 ppm lindane for 32 weeks.

The NOEL for this aspect of the study is 50 ppm. At 100 ppm and above there are changes in the appearance of the liver. The single dog apparently affected with "slight enlargement" of the liver is not sufficient to require that the LEL is 50 ppm.

L. Histopathology

The tester asserts that "no morphological change or variation from normal was considered to be related to the compound under test."

i. The liver showed evidence of macroscopic change and slight increases in weight, and the blood level of SAP was elevated all of which suggest possible liver pathology. The livers of all of the dogs receiving 200 ppm (for 32 weeks) were reported as normal following microscopic examination. Only three treated dogs in the main study (two in the high-dose and one in the mid-dose group) showed some evidence of possible effect. Their livers were described as having "some enlargement of centrilobular and midzonal hepatocytes, occasionally accompanied by ballooning of a few individual hepatocytes." At best this is a vague and indefinite response to the test material.

ii. The spleen showed possible evidence of increased weight. There was, however, no evidence of test material related microscopic lesions presented.

[Note: Some 40 or more tissue types were reported as being examined for each dog by the conventional haematoxylin and eosin stain. In addition sections of the liver and kidney were also stained for fat with Oil Red O, and sections of the liver were stained with PAS for glycogen.]

M. Other Assessments

i. Ophthalmoscopy. (pretest, and after 1, 3, 6, 12, and 24 months). - No effects in the eye were noted.

ii. EEG (electroencephalography) was evaluated using four controls and four high-dose group (100 ppm) dogs from the main study and four controls and the seven surviving dogs in the satellite study.

Of the four lindane-treated dogs in the main study, two were described as having "completely normal EEG's" while the other two had "EEG's with some slight abnormalities" which were said not to be statistically significant. More specifically there were slight increases in the 2 cycles/second activities associated with the dogs receiving 100 ppm lindane observed while the dogs were sleeping.

The dogs dosed with 200 ppm (for 32 weeks) also exhibited changes in EEG recordings which were particularly evident while the dogs were asleep. These were described as "slow waves superimposed on normal sleep pattern." Four of the seven dogs in this group were described as being either "nervous" or "excitable."

The NOEL for this aspect of the study is 50 parts per million. At 100 and 200 ppm there are evident signs of EEG recording changes. It should be noted that no dogs at dose levels lower than 100 ppm were actually tested, but since the response at 100 ppm was marginally different from the control, it is assumed that 50 ppm is a reasonable NOEL.

iii. Tissue levels of lindane. Two dogs from each test group were assessed for lindane concentration in the fat, liver, and brain. The residues in the fat showed a good correlation with the dietary concentration and the fat content of lindane was 64.6 to 73.3 ppm in the high-dose test group. In the liver, although the high-dose group had the highest lindane content (2.28 to 3.68 ppm), the low-dose group (0.3 to 1.14 ppm) had higher levels than the mid-dose group (0.50 to 0.77 ppm). The brain tissue from the dogs in the high-dose test group was also highest in lindane content (0.98 to 1.55 ppm), but the low and mid-dose groups were in the range of 0.21 to 0.54 ppm of lindane. Thus, lindane content of the tissues correlated with dietary lindane in the fat only.

N. Conclusion

The study is classified as CORE MINIMUM. The high-dose test group (200 ppm) received their diets for only 32 weeks, thus preventing a more meaningful description of the lesions which might be produced at 200 ppm of lindane over time. The NOEL as assigned by TB is 50 ppm. At 100 ppm there are indications of liver changes (which may be adaptive rather than actually a toxic response) as evidenced by the appearance of the liver, the effect is better defined at 200 parts per million. At 100 ppm there is also evidence of EEG changes.

No evidence of an oncogenic effect in this dog study was evident.

R-12

Study Type: Oncogenicity-Rats. (The chronic toxicities of technical benzene hexachloride and its alpha, beta and gamma isomers).

Accession No.:

MRID No.:

Sponsor: U.S. Government, FDA

Contracting Lab: Department of Pharmacology, FDA

Date: As published in J. Pharmacol. Expt. Therapeutics. 100: 59 (1950). (Authors, O.G. Fitzhugh, A.A. Nelson, and J.P. Frawley).

Summary of Study and Results

Groups of 10 male and 10 female weaning rats (Wistar strain) were dosed with diets containing either 0, 5, 10, 50, 100, 400, 800, or 1600 ppm of either the α-, β-, or γ-isomers of HCH for their lifespans.

Liver weight increases were noted for the rats dosed with 100 ppm γ-isomer and above (when the test material was added to the diet in corn oil). At 1600 ppm the livers were 142 percent larger in weight. Liver weight increases were also noted for each of the other isomers and for the technical preparation. The weights of the other organs were not reported to be affected. The liver and kidney were reported to have noticeable macroscopic patholological changes and the testes of the rats dosed with the technical preparation were also described as being smaller. Microscopically the liver was described as having a response typical of rats dosed with an organochlorine insecticide. There were no frank liver tumors described as being induced by the γ isomer. The microscopic pathology of the kidney revealed five types of lesions which included "focal nephritis," brown nonferrous pigment and hyaline granular degeneration as being chiefly a response to ingestion of the test material. Some microscopic evidence of testicular atrophy was also evident in the testes.

Conclusion:

This study is INVALID. There were too few animals at the initiation of the study to make meaningful assessments of the results. The data are in summary tables only. The data provide indications that the liver, testes, and kidney are candidate target organs for hexachlorocyclohexane toxicity.

R-13

Study Type: Oncogenicity - Rats [Pathclogic changes in the liver of rats after feeding low levels of various insecticides.]

Accession No.:

MRID No.:

Sponsor: U.S. Government

Contracting Lab: Savannah, GA

Date: As published in A.M.A Archives of Pathology 64:614 (1957) (Authors P. Ortega, W.J. Hayes, and W.F. Durham)

Summary of Study and Results

Lindane was fed to rats (six males and six females) for a total of 8 months with interim sacrifices at 2, 4, and 6, months at dose levels of 50 and 100 parts per million. Single rats (one male and one female) were sacrificed at the interim kills and the remaining three were sacrificed at 8 months. Two male rats (one receiving 50 ppm and the other receiving 100 ppm) and a single female rat receiving 100 ppm developed abnormal liver pathology (centrilobular-cell hypertrophy, peripheral migration of basophillic cytoplasmic granulations, and cytoplasmic inclusion bodies).

Owing to the few numbers of animals tested, this study is SUPPLEMENTARY. No firm evidence indicating a neoplastic response for lindane was established.

R-14

Study Type: Oncogenicity - Rats (Brief Communication: Development of Hepatocellular Carcinomas in Rats Treated with Benzene Hexachloride)

Accession No.:

MRID No.:

Sponsor:

Contracting Lab: None (Study conducted at Nagoya City Univ. Medical School, Nagoya, Japan)

Date: As published 1975. (As published in Journal of the National Cancer Institute 54: 801-805, 1975). Authors N. Ito, H. Nagasaki, H. Aoe, S. Sugihara, Y. Nigata, M. Arei, and T. Shirai

Summary of Study and Results:

Nine groups of male W rats (Japanese strain) were dosed as controls (8 rats), with episilon-BHC at 500 (13 rats) or 1000 (13 rats) ppm; gamma-BHC (lindane, 14 rats) 500 ppm; beta-BHC at 500 (13 rats) or 1000 (6 rats) ppm; or alpha-BHC at 500 (11 rats), 1000 (36 rats) or 1500 (13 rats) ppm. The feeding period ranged from 24 to 72 weeks. In particular, lindane was fed to the rats for 24 (6 rats) and 48 weeks (8 rats) and the alpha isomer of BHC for 24, 48, and 72 weeks.

There were few investigational endpoints for this study which was designed mostly to assess liver pathology. Responses to lindane in the diet resulted in loss of body weight and increases in liver-to-body-weight ratio. Pathological examination of the liver revealed lesions mostly in the rats dosed with α BHC. The only reported pathological observation noted in rats dosed with beta, gamma or episilon BHC was liver "cell hypertrophy." The rats dosed with alpha-BHC developed "oval cells," "bile duct proliferation," "cell hypertrophy," "nodular hyperplasia," and "hepatocellular carcinoma." Twenty-seven out of forty-one rats dosed with 1000 ppm and above developed the nodular hyperplasias and were in the groups dosed for 48 or 72 weeks. Four out of twentynine rats dosed for 72 weeks at either 1000 or 1500 ppm of alpha-BHC developed hepatocellular carcinoma.

Conclusion:

This study is SUPPLEMENTARY. This paper reports an important observation but the data cannot be validated or the classification of the lesion "nodular hyperplasia" be clarified on the basis of the information provided. This term is often used to

describe adenomas in the liver and the hyperplasias often progress into adenomas and carcinomas in the liver. There were too few animals available per group and the data are in summary form only.

Based on the information provided in this paper the alpha-isomer of BHC is oncogenic. The gamma-isomer of BHC results in less liver weight gain without associated pathology except for liver cell hypertrophy of a minimal degree.

A. Study Type: Oncogenicity - Rat [Bioassay of Lindane for Possible Carcinogenicity]

Accession No.:

MRID No.:

Sponsor: National Cancer Institute (NCI-RG-TR-14)

Contracting Lab: Gulf South Research Institute, New Iberia, LA

Date: Published - 1977

B. The test material was described as lindane the gamma isomer of 1,2,3,4,5,6- hexachlorocyclohexane. It was obtained from two sources. City Chemical Co., NY and Diamond Shamrock Co. Both sources asserted that the material was 100 percent pure.

C. The test animals used were Osborne Mendel strain rats obtained from the Battelle Memorial Institute, Columbus, OH. The experiment design consisted of three groups of rats: a control group (10 rats of each sex) and a mid (50 rats of each sex) and a high-(50 rats of each sex) dose test groups.

For the first 38 weeks of the study the low-dose group received 320 ppm and the high-dose group received 640 parts per million. For the next 42 weeks, the low-dose received 160 ppm and the high-dose group received 320 parts per million. After 80 weeks of dosing, the rats were fed control diet (without lindane) for an additional 30 weeks before sacrifice at weeks 108 to 109. The time weighted averages for the low-dose group was 236 ppm and for the high dose group 472 ppm for the 80 weeks of dosing.

The females were originally dosed at 320 ppm (low-dose group) and 640 ppm (high-dose group) for 2 weeks and then at 160 and 320 ppm for the next 49 weeks and still later at 80 and 160 ppm for 29 weeks) and were then fed control diets (without lindane) for 30 additional weeks before sacrifice. The time weighted average for dosing of the females was thus 135 ppm for the low dose and 270 ppm for the high-dose group for the time period in which they received diets containing lindane.

As indicated above, there were only 10 control rats of each sex which were actually matched with the rats receiving lindane in their diets. A pooled control group was made up from rats which were parts of other studies being conducted with the same strain of rat at approximately the same time as the lindane study. Thus, a pooled control group of 55 rats of each sex was used for comparison with the rats treated with lindane.

The rats were 35 days of age when placed on the study, but not all of the control rats were the same age as the lindane test rats and may have differed in age by one year.

D. Survival:

Forty-eight to fifty percent of the males in the low- and high-dose groups and six of the ten controls survived. Among the females, four of ten controls, but at least 60 percent of the low and high dose females survived to the end of the study.

E. Behavioral and Clinical Signs:

No tabulated data were presented. During the second year of the study some signs of "rough" and "discolored" hair coats (primarily among the males), pale mucous membranes, dermatitis, and vaginal bleeding were noted. It was not clear from the report if these symptoms were dose related.

F. Body Weight:

No significant body weight differences were reported to indicate a test chemical effect. No data on food or water consumption were reported.

[Note: No data on hematology (G), clinical biochemistries (H), urinalysis (I), organ weights (J), or gross pathology (K) were tabulated.]

L. Histopathology:

[Note: No individual animal data are presented; the data are in summary tables only.]

The tester asserts that there was no oncogenic effect of lindane and that there were no dose-related increases in non-neoplastic effects evident. Selected organs/tissues are discussed as follows.

1) The spleen. There were three incidences of hemangioma in the high-dose male group only and none in the females. In males this represents 7 percent of the available rats. There were no rats affected with leukemia or other tumors of the hematopoietic system. TB is concerned with this data concerning hemangioma possibly being associated with lindane primarily because the metabolite of lindane 2,4,6-trichlorophenol has been shown to cause leukemia in the rat (NCI-CG-TR-155) and because lindane has been implicated in blood disorders in humans. The small increase (7% of the 44 rats examined) was not statistically

significant (P 0.599, Fisher's One Tail P Statistic, TB computer program). The occurrence of the hemangiomas in the highdose group only is noted by TB, but there is insufficient quantitative response to conclude that the hemangiomas are a result of lindane in the diet.

2) The Liver - The liver has been implicated as an oncogenic target organ for lindane and/or its isomers in the rat and mouse. In this study, there were 0 in the controls, 3(7%) in the low-dose group, 2(4%) in the high-dose group incidences of neoplastic nodules among the males. Similarly, there were 0 in the controls, 4(8%) in the low-dose group and 2(4%) in the high-dose group incidences of neoplastic nodules. Although it is disturbing that the neoplastic nodules were found only in the test animals, there is insufficient basis to conclude that they were related to ingestion of lindane. Neoplastic nodules vary from 0 to 12 percent in this strain of rats (see Goodman reference indicated above). The possibility of lindane inducing liver neoplasms in rats will be assessed again in a requested rat oncogenicity study.

A non-neoplastic lesion described as "metamorphosis fatty" was not reported in the control males but was in 7 percent of the low dose and 11 percent of the high-dose males. In the females, it was also present only in the test animals and not in the controls.

3. The Kidney - The results of a 90-day feeding study with lindane indicated the presence of kidney lesions (see summary of review in this Registration Standard).

In this study the predominant lesion in the kidney noted at histopathology was "inflammation." In the males 50 percent of the controls and 48 percent (low-dose) and 61 percent (high-dose) group males were affected. Only females receiving lindane in the test diets had lesions described as "inflamation." There were 3 low dose (7%) and 5 high dose (10%) female rats affected.

N. Conclusion:

This study is SUPPLEMENTARY. The use of only 10 rats in the control group is not scientifically sound. No individual animal data were presented. Definite decisions on the oncogenic potential of lindane in rats cannot be made from this study.

A. Study Type: Oncogenicity - Mice [Bioassay of lindane for possible carcinogenicity.]

Accession No.:

MRID No.:

Sponsor: National Cancer Institute [NCI-CG-TR-14]

Contracting Lab.: Gulf South Research Institute, New Iberia, LA
Date: Published 1977

B. The test material was described as lindane, the gamma isomer of 1,2,3,4,5,6- hexachlorocyclohexane. It was obtained from two sources. City Chemical Co., NY and Diamond Shamrock Co. Both sources asserted that the material was of 100 percent purity.

C. The test animals used were mice of the BGC3F1 hybrid strain obtained from the Charles River Breeding Laboratories, Inc., Wilmington, MA. The experimental design consisted of three groups. The control group of 10 males and females each, and low- and high-dose groups of 50 males and females per group which were dosed with 80 or 160 ppm of lindane for 80 weeks. The mice dosed with lindane were allowed 10 additional weeks of feeding control diet prior to sacrifice. The mice were reported to be 35 days of age when placed on the study.

As indicated above, there were only 10 mice/sex in the matched control group. In order to try to bring the control group to a number comparable with the test groups, a pooled control group was formed consisting of 40 other mice from other studies that were then being conducted at the New Iberia facility. These other mice were not exactly the same age as the mice in the lindane study, but were taken from other studies which overlapped the lindane study by "at least one year."

D. Survival:

There was no obvious effect of the test material on survival. Eighty-eight percent of the males and 80 percent of the females were reported to have survived to the end of the study (90 weeks or about 20 months).

E. Behavioral and Clinical Signs:
(No tabulations included.)

The summary states that during the second year, the treated females appeared excitable when handled and the males were observed to be fighting. Other possible signs of reaction to lindane included rough hair coats, alopecia, and abdominal distention and it was stated that the treated mice were in "poor physical condition" during the last 6 weeks of the study. It was not

specified if the 6 weeks were during the dosing period or the "recovery" 10-week period.

F. Body Weight

Body Weight was reported not to be affected by the test material. No data on food or water consumption were reported. [Note: No data on hematology (G), clinical biochemistries (H), urinalysis (I), organ weights (J), or gross pathology (K) were reported.]

L. Histopathology

[Note: No individual animal data are presented, the data are in summary tables only.]

The tester asserts that there were no lesions either neoplastic or non-neoplastic that were attributable to the presence of lindane in the diet.

Some of the findings noted in the histopathology report are as follows:

1. The Liver: The following table reports the neoplastic response in the liver:

	Males			Females		
	Neoplastic		Hepatocellular	Neoplastic		Hepatocellular
	n	Nodule	Carcinoma	n	Nodule	Carcinoma
Control	10	1 (10%)	2 (20%)	10	1 (10%)	--
Low Dose	49	--	19 (39%)	47	2 (4%)	2(4%)
High Dose	46	1 (2%)	9 (20%)	46	--	3(7%)

With regard to hepatocellular carcinoma, the mid-dose group males have a unusually high proportion and the high-dose group females have three incidences and the control females, none. A recent summary (Goodman, Boorman, and Strandberg, "Selection and Use of the BGC3F1 Mouse and F344 Rat in Long-Term Bioassays for Carcinogenicity" in press) has indicated that the control range for hepatocellular carcinomas in this strain of mouse is 8 to 36 percent in males and 0 to 15 percent in females (p. 291 of the paper). The response as indicated above is just over the range for the mid-dose group males. Although a possible effect is noted in the mid-dose group, the high-dose group developed this tumor type in the same frequency as the control group.

These data by themselves do not require the conclusion that lindane produced an oncogenic response in mice liver.

2. Other organs affected with primary tumors included the lung, skin, mammary gland, uterus, adrenal gland, and pituitary but there were only a few incidences and no obvious dose response or truly rare tumors. Two mice (one male high-dose group) and one female (a control) developed lymphoma.

N. Conclusion:

This study is SUPPLEMENTARY. The use of only 10 mice in the control group is not scientifically sound. No individual animal data were presented. A decision on the oncogenic potential of lindane in mice cannot be made from this study.

A. Study Type: Oncogenicity - Mouse (The Toxicology of Dieldrin (HEOD). II. Comparative Long-Term Oral Toxicity Studies in Mice with Dieldrin, DDT, Phenobarbitone, beta-BHC and gamma-BHC.)

Accession No.:

MRID No.:

Sponsor: Shell Research Ltd.

Contracting Lab.: Shell Research Ltd., Tunstall Lab. Sittingbourne, Kent, England

Date: As published in Fd. Cosmet Toxicol. 11:433-442, 1973. (authors Thorpe and Walker)

B. Test material was described as gamma-BHC (99.5% purity) and was provided by Koch-Light Laboratories Colinbrook, Bucks. No information on the lot number or analysis of the diets was provided.

C. The test animals used were CF1 strain mice. Apparently they were provided and maintained by the tester's laboratory. The experimental design consisted of 45 males and 45 females as the control group and 30 males and 30 females in the gamma-BHC treated group. Also included in the study and assessed concurrently were groups of 30 male and 30 female mice dosed with dieldrin (10 ppm), DDT (100 ppm), beta-BHC (200 ppm), sodium phenobarbitone (500 ppm). These dose levels were selected based on a preliminary experiment. Dosing was scheduled for 105 to 109 weeks (up to 26 months).

The following will emphasize the repsonses of the mice to beta- and gamma-BHC.

D. Survival

Twenty of the control males (44%) and 14 of the control females (31%) survived to termination. Only five males (17%) and one female (3%) from the gamma-BHC groups and four (13%) males and five (17%) females from the beta-BHC group survived until termination. There were many deaths in the first 17 months and ataxia was reported to preceed death. Survival was also poor for the groups receiving other insecticides.

The presence of the test material is considered responsible for the poor survival of the mice dosed with beta-BHC and gamma-BHC.

E. No data were provided related to behavioral responses except for the statement that ataxia preceeded death for some of the mice.

F. No data were provided on body weight, food or water consumption.

G., H., I. and J. No data were provided for hematology, clinical biochemistries, urinalyses, or organ weights.

K. Gross Pathology

Only comments on the liver were provided. Liver enlargement was noticed by week 50 (apparently in the living mice) for the mice dosed with gamma-BHC and by week 60 for the mice dosed with beta-BHC. The appearance of the liver at necropsy was described as "enlarged, with irregular nodular surface." The individual nodules ranged from a few "millimeters "to several centimiters" in diameter. Some nodules were described as being of normal color while others had paler foci and yellow necrotic areas were also evident. No tabulation showing the incidence rate of these lesions were presented.

L. Histopathology

The following table summarizes the neoplastic response in the liver for all chemicals used in the study.

	Males Tumor Type				Females Tumor Type			
Chemical (ppm)	N	A	B	Total	N	A	B	Total
Control	45	20	4	24	44	23	0	23
Dieldrin (10)	30	47	53	100**	30	40	47	87**
DDT (100)	30	47	30	77**	30	47	40	87**
Phenobarbitone (500)	30	53	27	80**	28	43	32	75**
beta-BHC (200)	30	40	33	73**	30	30	13	43
gamma-BHC (400)	29	38	55	93**	29	34	34	69**

Data are in percent of mice with tumor type A or B or total.
** $P < 0.01$ different from the control (tester calculation).

On this basis, all five of the test chemicals were considered positive in both sexes (except for beta-BHC in females) for increasing the spontaneous production of liver tumors in mice. The tumor types produced were either type A or type B with the type B being an invasive variety. When a mouse had both type A and B, it was counted as having type B only.

As for other tissues/organs, the overall incidences of neoplasms tended to be reduced relative to the control. Thus, no other candidate target organ for a neoplastic effect of lindane was indicated by this study.

N. Conclusion

This study is SUPPLEMENTARY. Individual animal data were not presented. The data are in summary tables only. Only a single dose level of test material was used.

The data presented in this study, indicate that the gamma-isomer of hexachlorocyclohexane (lindane) causes neoplastic changes in mouse liver.

R-25

A. Study Type: Oncogenicity - Mouse [Testing of the substance Lindane for cancerogenic effects in mice using oral administration - duration 80 weeks]

Accession No.:

MRID No.:

Sponsor:

Contracting Lab.: C.H. Boehringer Sohn Ingelheim am Rhein

Date: February 25, 1975 (translated to English April 1975)

B. Test Material

The test substance was described only as lindane and as being of 99.5% purity from lot 22111-1.

C. The test animals were described as SPF mice (Chbb-NMRI). The supplier's name was not provided. The experiment consisted of four groups: the controls (100 males and 100 females) and three dosed groups of 50 males and 50 females each per group which were given diets consisting of 12.5, 25, or 50 ppm of the test material. No information on the results of testing the diet for lindane concentration was provided. The mice were scheduled to receive their test diets for 80 weeks. The mice were approximately 34 days old (23 gm, males and 21 gm females) at initiation of feeding of the test material

D. Survival

There were 14 (14%), 4 (8%), 13 (26%), and 9 (18%) deaths among the males and 14 (14%), 10 (20%), 5 (10%), and 10 (20%) deaths among the females. Thus, survival for the 80-week experiment is considered good in that there was always 37 or more mice per group which received the test diet for 80 weeks. There was no test-chemical-related effect on the mortality. NOEL > 50 parts per million.

E. Reactions

No comments were provided in the test report, the frequency of observation for test material reactions was not provided.

F. Body Weight

No test-chemical-related changes in either body weight or food consumption were evident. Note: summary tables only, no individual animal data were presented. NOEL > 50 parts per million.

The mean effective intake of compound per day was calculated (by the tester) to be 2.1, 4.1, and 8.2 mg/kg/day for males and 2.0, 3.9, and 7.8 mg/kg/day for females. These data were not supported by actual dietary analysis but were based on food consumption and body weight data.

G. Hematology

No tests on these parameters were conducted.

H. Clinical Biochemistry

No tests on these parameters were conducted.

I. Urinalysis

No tests on these parameters were conducted.

J. Organ Weights

No evidence was presented that organ weights were determined.

K. Gross Pathology

According to the protocol, gross necropsy was to be performed on all mice and all lesions and the results were supposed to have been recorded. The test report lists those findings "if they are of any relevance for the assessment of a cancerogenic effect of the compound." As a result of this limited reporting, probably less than half of the mice from each group are listed as having necropsy findings. There were no obvious increases in necropsy findings associated with increases of lindane in the diet.

L. Histopathology

The principle target organs which the protocol listed for histopathological assessment were the brain, heart, lung, liver, (with gall bladder), spleen, kidneys, adrenals, gonads, and urinary bladder as well as "all macroscopically perceptible tumors."

No comprehensive summary of the histopathological findings was presented. There were 26 control, 9 low-, 6 mid-, and 11 high-dose male mice and 22 control, 16 low-, 6 mid- and 11 high-dose test group female mice for which there were histologically identifiable lesions described as being neoplastic reported. Non-neoplastic lesions were not reported.

In the liver there were a total of 10 neoplastic incidences reported. Of these 9 were liver cell adenomas of which 5 (4 males, 1 female) were in the control group, 2 (a male and a

female) were in the low-dose group and 2 (both males) were in the high-dose group. The other neoplasm type was a "malignant hemangioendothelioma (a low-dose group male). No evidence was presented of dose related increases in liver neoplasms.

There were a total of 50 lung tumors reported consisting of primary lung tumor "type A", "type B", and "type I" (the test report describes the difference in their classification for these tumors). Of these 50, 21 were in the control group (13 males and 8 females) 11 were in the low-dose group (10 males and 1 female), 8 were in the mid-dose group (5 males, 3 females) and 10 were in the highdose group (6 males and 4 females). No evidence of dose-related increase in lung tumors was evident.

Some 35 incidences of "lymphatic leucoses lymphosarcomas were reported. There were 17 in the control (5 males and 12 females), 7 in the low-dose group (all females), 4 in the mid-dose group (1 male and 3 females), and 7 in the high-dose group (2 males and 5 females). No evidence of dose-related increases in this type of neoplasm was evident.

The high-dose groups had three types of neoplasms which were not found in other test groups. There were two incidences of "polymorphonuclear sarcoma" (one male and one female were affected) and one incidence of "spindle cell sarcoma" in a male mouse. Although it is noted that these are only in the high-dose groups there is insufficient evidence that they are compound related. They are not considered to be rare tumors for this species.

N. Conclusions

This study is SUPPLEMENTARY. There is insufficient raw data to support the conclusions. The individual animal pathology sheets for the test mice must be provided and include all macroscopic and microscopic observations. Tables summarizing all findings must be presented.

It would be difficult to upgrade this study higher than CORE SUPPLEMENTARY even if the individual animal pathology data were provided because the high-dose test group did not show any pharmacological response to the test material.

R-28

Study Type: Oncogenicity - Mice [Pathologic and ultrastructural studies in the hepatocarcinogenicity of benzene hexachloride in mice]

Accession No.:

MRID No.:

Sponsor: Government of Japan

Contracting Lab.: Nara Medical University, Nara, Japan

Date: As published in J. National Cancer Institute 51:817-826 (1973). [authors N. Ito, H. Nagasaki, M. Arai, S. Sugihara, and S. Makiura.]

Summary of Study and Results

Nineteen groups of mice (20 to 40 per group) were dosed with either pure alpha, beta, gamma or episilon isomers of BHC or with combinations of alpha plus the beta, gamma or episilon isomers for 24 weeks. At the end of the feeding period, the mice were sacrificed and assessed for liver toxicity responses.

The mice dosed with the alpha-isomer when dosed at either 250 or 500 ppm all developed "nodular hyperplasia" and "hepatocellular carcinoma" with most of the mice in these groups being affected. None of the mice dosed with either beta, gamma or episilon alone (at 100, 250, or 500 ppm) developed these lesions. Mice dosed with combinations of 250 ppm each of the alpha-isomer and either beta, gamma or episilon also developed the neoplastic lesions. Thus, the alpha-isomer is concluded to be the active ingredient in technical BHC which may result in liver neoplasms in mice.

The study is SUPPLEMENTARY. No individual animal data presented. The alpha-isomer, but not the gamma-isomer was shown to be associated with increased liver tumors in mice.

R-29

Study Type: Oncogenicity - Mouse [Translated from German "Contributions to Ecological Chemistry II. Hepatoma development in mice after administration of HCH isomers in high dosages.]

Accession No.:

MRID No.:

Sponsor: None

Contracting Lab.: None - Study conducted at Gakushin University, Tokyo, Japan

Date: As published in Chemosphere 1(6):279-282 (1972). (authors M. Goto, M. Hattori, T. Miyagawa, and M. Enomoto).

Summary of Study and Results

This study was a followup of a previous study by the same principle author which demonstrated that the alpha-isomer of BHC induced liver tumors (hepatoma) in mice after feeding 600 ppm for 3 months.

In this study (the current study) 8 groups of 20 males of ICR-JCL strain mice (5 weeks of age at start) were dosed with either 600 ppm of either technical grade HCH(I), alpha-isomer of HCH(II), beta-isomer of HCH(III), gamma-isomer of HCH(IV), a mixture of delta and epsilon HCH(V), 1,2,4-trichlorobenzol(VI), 2,3,5-trichlorophenol(VII), 2,4,5-trichlorophenol(VIII) or 300 ppm of gamma -HCH(IX). After 26 weeks 10 mice were sacrificed from each group and their internal organs examined. In summary, all of the mice in group I and II developed hepatoma, 8 of 10 mice in group V and 5 of 10 mice in group IX (lindane) developed "liver tumors." Coincident with the presence of the liver tumors, the liver weight was also elevated.

This study is SUPPLEMENTARY. There are no supporting individual animal data and other technical details are not available in this report. The study provides data of interest in that it demonstrates that lindane was associated with increased incidences of neoplasms in mouse liver.

R-30

Study Type: Oncogenicity - Mouse [Note: Induction of hepatoma in mice with benzene hexachloride.]

Accession No.:

MRID No.:

Sponsor: None

Contracting Lab.: None: Study conducted at the Department of Pathology, Osaka University School of Medicine

Date: As published in GANN 64:511-13, October 1973 (authors M. Hanada, C. Yutani, and T. Miya).

Summary of Study and Results:

In this study, groups of dd mice were dosed with diets containing 100, 300, or 600 ppm of crude BHC, or pure alpha, beta or gamma isomers of BHC for 36 to 38 weeks.

The mice in the groups receiving crude BHC, alpha- and gamma-isomer were reported to develop "hepatoma" or liver tumors. The response in the groups receiving crude BHC and the pure alpha-isomer were most pronounced with all of the male and female mice in the high-dose group developing the neoplastic condition. At least one male mouse receiving 100 ppm alpha-isomer had a hepatoma. Three of four males and one of three females receiving 600 ppm of pure gamma-isomer (lindane) were reported as developing hepatoma. None of the controls or mice receiving 100 or 300 ppm of gamma-isomer developed hepatomas.

This study is SUPPLEMENTARY. No individual animal data were provided and too few test animals per dose were used. The study provides an indication that lindane may produce neoplasms in mouse liver.

Study Type: Oncogenicity - Mice [Carcinogenicity of Benzene Hexachloride (BHC).]

Accession No.:

MRID No.:

Sponsor: None

Contracting Lab.: None: Study conducted at Nara Medical University, Nara, Japan

Date: As published Proceedings of the Second International Symposium of the Princess Tokamatsu Cancer Research Fund in Topics in Chemical Carcinogenesis, 1972. (authors H. Nagasaki, S. Tonrii, T. Mega, M. Marugami, and N. Ito)

Summary of Study and Results

Four groups of male dd mice were dosed as control, 6.6 ppm, 66 ppm, and 660 ppm of benzene hexachloride composed of alpha-(67%), beta-(11%), gamma-(15%), episilon-(6%) and other isomers (< 1.0%) for 24 weeks. After sacrifice, the liver, brain, heart, kidneys, spleen, and testes were weighted and assessed microscopically.

The principle finding of this investigation was that all of the mice dosed with 660 ppm of benzene hexachloride (technical) developed "hepatoma." None of the mice in the groups receiving the basal diet of 6.6 or 66 ppm of benzene hexachloride developed hepatomas. All dosed mice had increased liver weight (+ 14% for the low-dose group and plus 16% for the mid- and + 245% for the high-dose group). Other pathological changes found in the liver included "oval cell infiltration," "bile duct proliferation," "cellular hyperplasia," and "nodular hyperplasia." None of the other organs examined had either weight or pathological differences from the control group.

One other interesting aspect of this paper is that data were presented to show that the alpha- and beta-isomer accumulate in the liver. The beta-isomer, although it was present in the original test material in lower proportions than the alpha-isomer, was present in a higher concentration than the alpha-isomer. The gamma-isomer was not detected at concentrations greater than 0.03 ppm.

This study is SUPPLEMENTARY. No individual animal data were submitted to support the findings. The study confirms other reports that technical benzene hexachloride (containing the alpha-isomer) results in liver neoplasms when administered to mice in the diet.