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ATE:

Petitions Control Branch (SC-13)

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Petitions Review Branch (SC-970)

Chlordane: Toxicological evaluation of new rat reproduction and chronic dog feeding studies in connection with assessing safety of present tolerances on chlordane.

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(AF 15-229)

Introduction:

Three years ago, the "Advisory Committee* on Chlordane," made extensive review of available information on identity, toxicity, and usage of chlordane. The committee recommended that the tolerances on chlordane be continued at the existing level of 0.3 ppm unless evidence of new studies then underway or others done in the future should provide evidence of potential hazard.

Following this, the Food and Drug Administration legally restricted content of the hexachlorocyclopentadiene intermediate in chlordane to a maximum of 1%. The intermediate was allegedly responsible for greater toxicity of chlordane manufactured before 1959 than that made since. Except for specifying this impurity limit in chlordane, FDA made no change in regulations affecting chlordane tolerances then (1965).

These studies--rat reproduction and dog chronic feeding--are now complete. They are briefly outlined and "no-effect levels" for each estimated below. An evaluation of safety of existing chlordane tolerances is then made, based on results of these studies and on other available information.

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*Cf. Report of the Food and Drug Administration's Advisory Committee Appointed to Review the Proposal to Repeal the Tolerances for Residues of Chlordane on Certain Fruits and Vegetables and to Establish a Tolerance of Zero on Each Crop Listed in Pesticide Regulation Section 120.22, February 21, 1965.

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Chlordane Reproduction Study - Rat*

Duration: 3 generations, 6 litters (plus a small (ca. half-size) 7th litter).

Levels Fed: 0, 0.3, 3, 15, 30, and 60 ppm of "technical" chlordane (levels checked and confirmed by analysis of diets).

Numbers of Rats: 10 males and 20 females/group.

Indexes:

	F.I. x G.I.**						V.I.***						
Level Fed (ppm) =		0	0.3	3	15	30	60	=	0	0.3	3	15	30	60
Litter														
F_{1a}	=	75	75	70	75	75	70	=	73	85	78	93	72	74
F_{1b}	=	70	70	65	80	70	65	=	82	76	87	81	87	71
F_{2a}	=	80	80	75	70	75	75	=	69	79	71	70	79	74
F_{2b}	=	74	75	75	75	75	80	=	75	83	83	80	79	74
F_{3a}	=	80	75	70	80	75	75	=	69	68	74	76	65	84
F_{3b}	=	80	85	75	75	80	79	=	73	83	74	81	80	85
$F_{3c}1$****	80	-	-	-	-	-	-	83	-	-	-	-	-	-
$F_{3c}2$	78	-	-	-	-	-	-	91	-	-	-	-	-	-
$F_{3c}0$	70	-	-	-	-	-	-	95	-	-	-	-	-	-

*Performed by Dr. L. Ingle of University of Illinois at Urbana.

**F.I. x G.I. = per cent litters of pairs mated.

***V.I. = per cent pups alive at 5 days of number born.

****$F_{3c}1$ is from parental F_{2b} females on 60 ppm chlordane; $F_{3c}2$ is from parental F_{2b} females removed from chlordane after weaning of F_{3b} litters; and $F_{3c}0$ is from parental F_{2b} female controls. Presumably, females were bred to males on similar dietary intake, but this is not explicitly stated.

Chlordane Rat Reproduction Study

Indexes: (Cont'd)

Level Fed (ppm)	-	L.I.* 0	0.3	3	15	30	60
Litter							
F_{1a}	-	97	94	96	94	97	99
F_{1b}	-	95	99	99	98	99	97
F_{2a}	-	98	98	97	98	93	89
F_{2b}	-	97	98	100	99	99	94
F_{3a}	-	98	99	99	98	98	83**
F_{3b}	-	97	99	97	95	100	87***
$F_{3c}1$	****77	-	-	-	-	-	-
$F_{3c}2$	100	-	-	-	-	-	-
$F_{3c}0$	97	-	-	-	-	-	-

Weights:

Level Fed (ppm)	-	Mean body wt of weanlings (g) 0	0.3	3	15	30	60
Litter							
F_{1a}-F_{3b}, inc.	-	55.9	55.4	55.3	55.1	54.6	56.5
$F_{3c}1$	50.2	-	-	-	-	-	-
$F_{3c}2$	53.9	-	-	-	-	-	-
$F_{3c}0$	56.1	-	-	-	-	-	-

	-	Mean liver wt of weanlings, % of body wt 4.3	4.3	4.6	4.6	4.6	4.6
F_{3b}							
$F_{3c}1$	5.4+	-	-	-	-	-	-
$F_{3c}2$	4.6	-	-	-	-	-	-
$F_{3c}0$	4.4	-	-	-	-	-	-

+ Differs significantly

*L.I. = percent survivors at 21 days of pups alive at 6 days (less number killed thereafter to reduce litter size to 10).
**Two (of 17) pups deaths said to be due to chlordane.
***Fourteen (of 14) pups deaths ascribed to chlordane.
****Nine (of 10) pups dead of chlordane.

Chlordane Rat Reproduction Study

Average Number Pups/Litter:

Level Fed (ppm)	-	0.3	3	15	30	60
Litter						
F_{1a}-F_{3b}, inc.	-	9.3	9.7	9.3	9.4	9.2
$F_{3c}1$	6.6	-	-	-	-	-
$F_{3c}2$	9.7	-	-	-	-	-
$F_{3c}0$	10.6	-	-	-	-	-

Neoplasms: None attributable to chlordane.

Histopathology: Negative except for those 60-ppm pups which died from effects of chlordane. For them, liver cells of the central zone showed slight-to-moderate peripheral concentration of cytoplasmic granules and perinuclear vacuolization. Some centrolobular and midzonal cell hypertrophy occurred; lung tissue showed vascular congestion and small areas of hemorrhage.

Mortality: None in parental rats ascribed to chlordane. Of pups, dead of chlordane (and, also, following intermittent exposure to high frequency sound which caused hyper-excitability and convulsions), two were from F_{3a} pups, 14 of F_{3b} pups, and 9 from $F_{3c}2$ pups.

No-effect level: 30 ppm.

Effect level: 60 ppm (effect consisting of decreased L.I. and greatly increased mortality of pups from chlordane intoxication in F_{3b} litters; although $F_{3c}1$ litter is not part of "standard" reproduction test, effects here were increased mortality of pups, decreased litter size, and slight liver hypertrophy in pups.)

Chlordane Chronic Feeding Study - Dog

Study Performed by:	International Research and Development Corporation, Mattawan, Michigan
Number of Animals:	Total of 35 female and 25 male young, purebred beagles used.
Feeding Levels:	0 (10 animals), 0.3 (8 animals), 3, 15 (10 animals per level), and 30 ppm (14 animals) "reference grade (presumably technical) chlordane" fed.
Duration:	2 years overall. At 12 months, one male and one female from each dietary group were sacrificed and autopsied. At 72 weeks, four dogs at 30 ppm were returned to the control diet for remainder of study.
Behavior, Appearance:	No effects due to chlordane in this study.
Mortality:	Negative. One male dog at 30 ppm was sacrificed at 12 months because of severe dermatitis, progressive from time of first appearance at 15 weeks.
Body Weight:	No effects due to chlordane occurred.
Food Consumption:	No significant effects due to chlordane.
Organ Weight:	Increased liver weight relative to body weight at 2 years in all dogs at 30 ppm, in 4 of 8 at 15 ppm, and in all 4 dogs, formerly at 30 ppm, which had been withdrawn from chlordane dosage at 72 weeks on test. Likewise, compound-related increases in relative liver weight seen in both a male and a female dog at 30 ppm, killed at 1 year.

Clinical Laboratory Tests:*

All hematological values--hemoglobin, hematocrit, total and differential leukocyte count, reticulocyte count, and erythrocyte sedimentation rate--unaffected by dietary chlordane in this study. No effect of chlordane on plasma levels of glucose (fasting) or urea nitrogen. Borderline decrease in both total serum protein and in albumin-globulin ratio only in females at 30 ppm at 24 months on test. (Cf. Table I-2.) Liver function tests (serum glutamic-oxalic transaminase, serum glutamic-pyruvic transaminase, thymol turbidity, cephalin flocculation, bromsulfalein retention, prothrombin time, and serum lactic dehydrogenase) showed no effect due to chlordane, except in isolated instances, chiefly in dogs

*See attached tables for summary of results of selected tests at selected time intervals.

Clinical Laboratory Tests (Cont'd)

at 15 or at 30 ppm. (Cf. Tables 3-8.) Alkaline phosphatase, on the other hand, showed apparent dose-related increase at all levels of chlordane intake above 0.3 ppm in male and female dogs at 15 and at 24 months; differences from controls were significant ($p < 0.05$) for dogs at 15 and at 30 ppm. (Cf. Table 9.) Results of urinalysis, including glucose, albumin, and bilirubin assays, test for occult blood, and microscopic examination, were negative.

Storage: No data given*

Metabolism: No results included.

Liver Biopsy: Negative for 30-ppm dogs (1 male plus 1 female) at 1 month and at 3 months. At 6 months, 1 male (of 1) and 2 females (of 3) at 30 ppm had eosinophilic homogeneous hyaline bodies in the cytoplasm of liver parenchymal cells. Two dogs at 15 ppm showed no liver lesions at 6 months.

Histopathology: Microscopic examination of tissues in dog killed at 2 years, showed compound-related changes only in the liver. They occurred in 5 of 8 dogs at 15 ppm, in 8 of 8 dogs at 30 ppm, and in all 4 dogs which had been removed from chlordane 8 months before end of 2-year feeding period. Liver changes consisted of enlargement of centrolobular hepatocytes with vacuolation and margination of coarse cytoplasmic granules. Oil-red-O-stained frozen sections of liver did not show lipid in the ballooned hepatocytes. Nor was there necrosis. Lesions appeared to be equally severe in dogs in the 15-ppm, 30-ppm, and withdrawn-from-30-ppm groups. One (of 2) dogs at 30 ppm, killed at one year, had lesions as described plus some liver cells with cytoplasmic hyaline bodies.

No-effect level: 3 ppm.

Effect level: 15 ppm. Effect consisted of compound-induced, microscopically detectable lesions in livers of a majority of dogs killed after 2 years' feeding of chlordane at 15 ppm. Liver hypertrophy also occurred.

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*However, Velsicol Chemical Corporation, sponsor of study has samples (e.g., of perineal fat) from this dog study (and, also from rat reproduction study) to be analyzed. They will inform us shortly of any available results (Dr. M. Eisler, Toxicologist at Velsicol, to M. Quaife, DFT, per telephone on April 19, 1968).

Clinical Laboratory Tests (Cont'd)

Comment: These effects (liver) appear not to be merely signs of a physiological adaptation process in that no evidence of their reversibility is presented. Strong suggestive evidence they are not reversible is provided by the failure of dogs on 30 ppm chlordane, liver lesions in which were no more severe than they were in dogs at 15 ppm, to show evidence of reversal of lesions after 8 months off a chlordane-containing diet.

EVALUATION:

"No-effect levels" for technical chlordane as presently (at least from 1965-on) manufactured appear to be 30 ppm (1 mg/kg body weight) for rat reproduction and 3 ppm (0.075 mg/kg body weight) for chronic dog feeding study. Although the Committee* did not evaluate Dr. Ingle's chronic rat feeding study done in 1955** as providing a definite "no-effect level," it did conclude, "...there (is) justification for accepting at least 2 ppm and very likely a substantially higher figure as a no-effect level in the rat."

Thus, 3 ppm appears to be the "no-effect level" of the more sensitive animal species, the dog, and 3 ppm or higher "no-effect" for the rat. The ADI would be 0.01 ppm, whole diet basis, if a 100-to-1 safety factor is used.

For a 60-kg man, 0.03 ppm x 1.5 kg diet = "total ADI" = 0.045 mg/day or $\left(\frac{0.045 \text{ mg}}{60 \text{ kg body wt}} =\right)$ 0.00075 mg/kg body wt/day. By way of comparison, a figure of 0.001 mg/kg/day is given as the ADI for chlordane by a recent WHO/FAO report.***

The portion of the human U.S. diet presently covered by tolerances for chlordane at 0.3 ppm is about 31.4% or, roughly 1/3, on the average. Thus, on the average, the theoretically maximum possible intake of chlordane by a human being in the U.S. is (0.3 ppm x 1/3 =) 0.1 ppm (whole-diet basis). This is 3 x the ADI.

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*Cf. "Report of the Food and Drug Administrations Advisory Committee Appointed to Review the Proposal to Repeal the Tolerances for Residues of Chlordane on Certain Fruits and Vegetables and to Establish a Tolerance of Zero for Each Crop Listed In Pesticide Regulation Section 120.22," dated February 21, 1965.

**and made available to the Committee as Exhibit 4C in "A Digest of Information Relating to Chlordane," furnished by the Velsicol Chemical Corporation.

***Cf. "Draft Report of the 1967 Joint Meeting of the FAO Working Party of Expert Committee on Pesticide Residues, Rome, 4-11 December 1967," dated January 10, 1968, p. 2, Appendix III.

EVALUATION: (Cont'd)

Actual intake of chlordane by humans in the U.S. was regarded by the 1965 Advisory Committee as constituting only a fraction of the theoretically maximum possible intake. The conclusion was based both on very low figures in various total diet ("market-basket") studies for chlordane content and on "lack of detection of chlordane" in a series of 300 human fat samples (from U.S. residents) analyzed then.

Assays of current total diets in U.S. indicate actual chlordane intake to still be relatively very low.

For example, in analyses of total U.S. diets covering June, 1966, through April, 1967, chlordane was noted as being present in only 2 out of 360 food composites examined.* One level was 0.02 ppm and the other, 0.0005 ppm.

Since samples were collected from 30 different U.S. cities, this suggests virtual absence of chlordane in the average U.S. diet. (It does not, of course, mean chlordane may not or will not occur in various individual diets.)

We, therefore, do not regard results of these toxicological studies on chlordane as showing evidence of potential hazard to humans in the U.S. such that present (April, 1968) tolerances for chlordane in raw agricultural commodities should be reduced or revoked.

We do suggest as additional desirable toxicological information to help assess safety of chlordane intake, a determination of the "no-effect" level, on chronic feeding, of chlordane to a mammal other than the rodent with respect to induction of "liver-processing" enzymes.

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*Martin, R. J., and Duggan, R. E., "Pesticide Residues in Total Diet Samples (III)," Pesticides Monitoring Journal, March, 1968, Vol. 1, pp. 11-20.

Acknowledgment: Mr. Thomas E. Berry and Mr. J. Winbush kindly provided assistance in checking and interpreting values for various laboratory analysis from the chronic dog study; Petitioner sent these as a complete computer print-out comprising about 400 pages.

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ADDENDUM

Although no analyses of human tissues directly for chlordane are known to be extant, a recent paper* suggests the intriguing possibility that presence of chlordane in fat may have been demonstrated for a large number of human autopsy samples collected in the U.S.A.

We infer this for the following reasons:

1. A peak in the gas chromatograms (with "relative retention time" of 1.20) identical to that for heptachlor expoxide occurred in assay of over 300 samples of the authors' series of human fat samples.

2. Such peak was shown in certain samples of fat examined by thin-layer chromatography (or analyzed by colorimetric method for heptachlor epoxide) to consist only partially of heptachlor epoxide.

3. Finally, Velsicol personnel,** in analysis of tissues from chlordane-fed dogs or rats, found that a peak in gas chromatograms which was first thought to correspond to heptachlor epoxide was, in fact, chlordane. PSI AS

Since these fat samples analyzed by Hoffman et.al. were collected in the period, 1962 through 1966, if our inference is correct, there is evidence for possible presence of chlordane in fat of humans in the U.S.A.

By extension, chlordane must occur in the diet in finite, but unknown amounts.

Velsicol people (conference of May 14, 1968) mentioned finding a 1:1 ratio of dietary chlordane to chlordane in fat of rats fed it. If true, and if the ratio for humans is of similar magnitude, an estimate of chlordane content of the human diet could be made following assay of human fat samples for chlordane; such estimate would, of course, be speculative at best.

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*Hoffman, W., Adler, H., Fishbein, W. I., and Bauer, F.A., "Relation of Pesticide Concentrations in Fat to Pathological Changes in Tissues," Archives of Environmental Health 15, 758-65 (1967).

**Cf. memo of Conference of Bureau of Science with persons from Velsicol Chemical Corporation on May 14, 1968, by H. R. Gittes, filed in CPT folder on chlordane.

9

Table I

Dogs - total serum protein (g/100 ml)

Males at: ppm chlordane:		0 mos.	12 mos.	24 mos.
	0	5.1	5.8	6.4
	0.3	4.8	5.7	6.1
	3	5.0	5.8	6.4
	15	5.0	6.2	6.4
	30	4.9	5.8	6.1

Females at: ppm chlordane:		0 mos.	12 mos.	24 mos.
	0	4.9	5.7	6.3
	0.3	5.1	5.9	6.3
	3	4.8	5.6	6.1
	15	4.9	6.0	6.4
	30	5.1	5.4*	5.8*

*Value differs from control value at $p < 0.05$.

Table 2

Dogs - serum albumin-globulin ratio

Males at: ppm chlordane:		0 mos.	12 mos.	24 mos.
	0	1.1	0.93	1.4
	0.3	1.0	1.0	1.5
	3	1.0	0.81	1.2
	15	1.0	0.87	1.1
	30	1.0	1.0	1.1

Females at: ppm chlordane:		0 mos.	12 mos.	24 mos.
	0	1.1	0.96	1.8
	0.3	1.0	0.76	1.3
	3	1.0	0.87	1.3
	15	1.3	1.0	1.3
	30	1.1	1.0	1.2

*Value differs significantly from control value at $p < 0.05$.

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Table 3

Dogs - serum glutamic-oxaloacetic transaminase*.

Males at:		0 mos.	12 mos.	24 mos.
ppm chlordane:	0	19.2	16.4	13.8
	0.3	20.8	15.5	12.3
	3	18.8	14.2	12.8
	15	21.2	14.6	11.0**
	30	18.9	14.2	11.0**
Females at:		**0 mos.**	**12 mos.**	**24 mos.**
ppm chlordane:	0	19.8	16.4	16.3
	0.3	21.6	16.0	12.3
	3	17.8	13.5	11.0**
	15	20.0	12.4**	12.8
	30	20.0	15.7	10.0**

*Expressed as Sigma-Frankel units/ml.
**Value differs from control value at $p < 0.05$.

Table 4

Dogs - serum glutamic-pyruvic transaminase*.

Males at:		0 mos.	12 mos.	24 mos.
ppm chlordane:	0	17.4	18.2	17.0
	0.3	19.6	20.0	10.5
	3	17.8	1,014	17.0
	15	20.8**	17.0	17.3
	30	18.3	20.4	20.5**
Females at:		**0 mos.**	**12 mos.**	**24 mos.**
ppm chlordane:	0	20.0	16.4	18.7
	0.3	20.5	15.2	12.6
	3	19.0	15.6	13.5
	15	18.2	13.4**	15.8
	30	18.6	34	18.3

*Expressed as Sigma-Frankel units/ml.
**Value differs significantly from control value at $p < 0.05$.

Table 5

Dose - thymol turbidity (units)

Males at:		12 mos.	24 mos.
ppm chlordane:	0	0.8	0.7
	0.3	1.1*	1.0
	3	1.1	0.6
	15	0.7	0.7
	30	1.5*	0.8*
Females at:		**12 mos.**	**24 mos.**
ppm chlordane:	0	0.7	0.70
	0.3	1.0*	0.63
	3	1.2*	1.2
	15	1.0*	0.80*
	30	1.3*	0.80*

*Value differs from control value at $p < 0.05$.

Table 6

Dose - bromsulfalein retention at 30 minutes (%)

Males at:		0 mos.	12 mos.	24 mos.
ppm chlordane:	0	0.7	2.1	2.3
	0.3	0.9	1.2*	1.5
	3	1.1	2.0	1.8*
	15	1.1	2.9	1.7
	30	1.0	1.9	2.1
Females at:		**0 mos.**	**12 mos.**	**24 mos.**
ppm chlordane:	0	1.0	2.0	1.9
	0.3	0.5*	1.6	1.4
	3	0.9	2.3	2.0
	15	0.7	3.2	2.3
	30	0.9	3.4*	2.4*

*Value differs from control value at $p < 0.05$.

Table 7

Dogs - prothrombin time (seconds)

Males at:		12 mos.	24 mos.
ppm chlordane:	0	6.8	7.0
	0.3	7.0	7.3
	3	6.8	7.3
	15	7.0	6.8
	30	8.3*	7.3

Females at:		12 mos.	24 mos.
ppm chlordane:	0	6.5	7.0
	0.3	6.0	6.7
	3	6.3	6.5
	15	7.5	7.0
	30	8.0**	9.0**

*Value is significantly different from control value at $p < 0.05$.
**Ditto, but at $p < 0.06$.

Table 8

Dogs - serum lactic dehydrogenase

Males at:		14 mos.	Females at:	14 mos.
ppm chlordane:	0	143		118
	0.3	-		-
	3	-		-
	15			
	30	115		118

*Value different from control value at $p < 0.05$.

Table 9

Dose - serum alkaline phosphatase*

Males at:		18 mos.	24 mos.
ppm chlordane:	0	3.0	2.0
	0.3	4.7**	2.6
	3	4.3**	3.5
	15	16.5**	9.3**
	30	23.3**	14.0**

Females at:		18 mos.	24 mos.
ppm chlordane:	0	5.0	3.2
	0.3	3.3	1.7
	3	7.8**	5.3
	15	15.6	7.8**
	30	21.7**	11.3**

*Expressed as King-Armstrong units/ml.
**Value is significantly different from control value at $p < 0.05$.

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SC-950 (Dr. Jacobson)
VM-100

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