12-16-85

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlordane/Heptachlor Mutagenicity

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Jay Mauer 12-16-85

TO: Henry Spencer, Ph.D., Pharmacologist
Section 7, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Jane E. Harris, Ph.D.
Section Head, Toxicology Branch
Hazard Evaluation Division (TS-769C)

This is in response to your request for an expeditious appraisal of the positive mutagenicity studies for chlordane and heptachlor/heptachlor epoxide, as reported in the CAG document ("Carcinogenicity Risk Assessment for Chlordane and Heptachlor/Heptachlor Epoxide"). The negative reports listed in that document were also scanned.

Based upon recent Velsicol submissions (Accession Nos. 254320 and 254324) and other information available to me (NTP, EMIC), two general conclusions are warranted from this preliminary assessment (see below for a summary of available data):

1. Although the adequacy of the data base as reported could not be undertaken (e.g., DER's) because of the timely response requested, it appears that all the genetic end-points we require to be assayed (gene mutation, chromosomal aberrations, DNA damage/repair) have been addressed.

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2. Consistent with the results of mutagenicity testing previously reported for other agents of this chemical class (organochlorines such as Lindane, chloroform, inter alia), the mutagenic potential of these chemicals are low to unsubstantial. Hence, from the available data it would appear their oncogenic potential derives from mechanisms other than "genetic initiation" (direct interaction with DNA). [There is suggestive evidence in mammalian systems (the few promotion assays available) of an "epigenetic" mode of action for these chemicals.]

Survey of Available Literature

1. Chlordane-technical (but not reagent-grade alpha-chlordane, gamma-chlordane, and gamma-chlordene) was positive in adequate bacterial (Ames) assays (three positive, one negative), but consistently so only in the most sensitive strain (TA-100), and only at very high concentrations (5000 ug/plate and above). Testing for mutagenicity in mammalian cells in culture (four reports) revealed inconsistent results (two positive, two negative), due in part to the different test systems employed. The two negatives were reported for HGPRT in ARL (adult rat liver) and for both thioguanine and diphtheria-toxin resistance in V79 (Chinese hamster lung) cells. One of the positives was for ouabain resistance in V79 cells, but reported for only a single concentration of an unstated formulation, at less than 50 percent cell survival; the second was found in non-activated mouse lymphoma cells (L5178Y-TK), but the study is incomplete since metabolic activation was not employed.

The plant systems may activate chlordane to mutagenic derivatives is suggested by a single article reporting both gene conversion in Saccharomyces cerevisiae D4 cultures exposed to a reagent grade (presumably alpha-chlordane according to the CAS Number stated), and reversion to wild type in pollen grains from the homozygous "waxy" strain W22 of Zea mays (corn) exposed to a "commercial-grade formulation." However, too few procedural details were reported to adequately interpret these results.

Although reportedly negative for gross chromosomal damage in vivo (two mouse DLT's) and in vitro (CHO cells), technical chlordane or one of its principal components (alpha-chlordane) apparently has DNA-damaging activity, as revealed in a single study reporting increased dose-dependent sister-chromatid exchanges (SCE) in intestinal cells sampled from

exposed central mudminnows (Umbra limi), as well as in CHO cells in culture and in a human lymphoid cell line (one report each). Whereas the positive SCE in fish was recorded at nonlethal concentrations (added to aquarium water), both in vitro studies were only marginally positive (less than twofold above controls) at toxic concentrations. Only one of the four studies assaying for unscheduled DNA synthesis (UDS) in vitro was positive (in VA-4 cells, an SV-transformed human cell line), but only in the absence of metabolic activation (negative with MA). Technical chlordane was negative for UDS in primary hepatocyte cultures from rats, mice, and hamsters (two reports) as well as in a human fibroblast cell line (D-550). Finally, gene conversion was reported in yeast cells (S. cerevisiae D4) exposed to an activation system (negative without), but too few data are included in the article to properly interpret the results.

2. Heptachlor/Heptachlor Epoxide. In contrast to the positive results for (gene) mutagenicity reported for chlordane (both technical and reagent grades), the available data for heptachlor and its epoxide is consistently negative (three Ames and/or E. coli, one B. subtilis rec assay, 1 ARL-HGPRT, two Drosophila SLRL, 1 HPC/UDS). Reversions to histidine prototrophy in Salmonella TA-1535 and 100 under activation conditions to an unstated dose range, as well as to the wild-type (nonwaxy) phenotype in corn pollen grains were reported by the same investigator who found "commercial" chlordane positive.

Adequate reports on dominant-lethal assays in mice were negative, but an abstract from a meeting reported positive results for both germinal (DLT) and somatic (bone marrow) chromosome damage in rats fed 1 and 5 ppm of an unstated formulation of heptachlor for three generations. A Russian study also reported positive chromosome damage in bone marrow cells from "white male mice" treated i.p. with "heptachlor" (also of unstated source and purity) at a single dose level stated to be "4% of the LD_{50}." Too few procedural details are included in these "positive" studies to interpret the results reported.

As with chlordane, negative UDS results have been reported for heptachlor technical in primary rodent hepatocytes (two reports), but a positive recorded in VA-4 cells (virus-transformed human fibroblasts) for both the technical and epoxide, but only with metabolic

activation (in contrast to chlordane, positive only in the absence of activation). [The mutagenicity data base for heptachlor epoxide is less than adequate to satisfy FIFRA guidelines.]

Discussion

Recent studies have suggested that organochlorines (Lindane, chlordane, heptachlor, inter alia) do not interact directly with DNA (i.e., are not "genotoxic"), but rather act "epigenetically" by mechanisms affecting cell membrane permeability and/or following an irreversible "initiating event" (i.e., are "promoters" ensuring survival of preexisting transformed cells). The following citations employing chlordane heptachlor available for this "quick-and-dirty" review (and listed in the CAG document) are consistent with this suggestion:

1. Inhibition of metabolic cooperation in mixed cultures consisting of thioguanine-resistant and TG-sensitive cells (several reports from both Williams' and Trosko's labs).

2. The putative positive UDS results in cells already "initiated" (e.g., the SV-40 transformed cell line, VA-4).

3. Inhibition of DNA synthesis and/or cell cycle mechanisms by severely toxic concentrations leading to perturbation of repair (increased UDS, SCE).

cc: Dr. Amy Rispin
Science Intergration Staff
Hazard Evaluation Division (TS-769C)

PRELIMINARY EVALUATION OF REPORTED POSITIVE STUDIES

Compound	Test Material	Assay	Test System	Dose/Conc. Range	Reference	TB Evaluation	Comments
CHLORDANE	Technical	Gene Mutation	Ames (all)	20-100	Simmon et al. (77)	ACCEPT.	POS. only in TA 100
	Technical	Gene Mutation	Ames (all)	a) 10-5000 b) 1000-50,000	Simmon and Tanaka (77)	ACCEPT.	a) EQUIV in TA 98 b) POS. in TA 100
	Technical	Gene Mutation	Ames (TA 98, 100)	5-10,000	Maruyama (80)	ACCEPT.	POS. in TA 100; NEG. in TA 98
	Reagent (alpha)	Gene Mutation	Sacch. - D4	(Unstated)	Gentile et al. (82)	INCONCL.	POS. only + S9; too few data
	"Commercial"	Gene Mutation	Z. mays Pollen	(Unstated)	Ibid	INCONCL.	Tech not tested; too few data
	(Unstated)	Ouabain resist.	V79 cells	(Only 1 dose reported)	Ahmed et al. (77b)	INCONCL.	"Weak" at < 50% survival; few data
	(Unstated)	Gene Mutation	ARL/HGPRT	10^{-3}-10^{-6} M	Telang et al. (82)	ACCEPT.	NEG. for HGPRT, but POS. for promotion
	Technical	In vivo SCE	Mudminnow	$5.4/10^{-12}$-10^{-9}	Vigfusson et al. (83)	ACCEPT.	Dose-dependent POS.
	Reagent (alpha)	In vitro SCE	LAZ-007 cells	10^{-6}-10^{-3} M	Sobti et al. (83)	INCONCL.	< 2-fold increase; no dose response
	(Unstated)	In vitro UDS	WA-4 cells	1,10,100,1000 uM	Ahmed et al. (77a)	ACCEPT.	POS. only without S9
	Reagent (gamma)	Cell cycle inhib.	L5178Y cells	4 ug/ml	Brubaker et al. (70)	INCONCL.	NEG. for DNA, POS. for G2 arrest; only 1 dose
	Reagent (alpha)	In vitro CA/ SCE	CHO cells	(Unstated)	NTP (85)	INCONCL.	NEG. for CA; POS. for SCE
	Reagent	Gene Mutation	L5178Y/TK	(Multiple)	NTP (85)	INCONCL.	Not tested with S9

PRELIMINARY EVALUATION OF REPORTED POSITIVE STUDIES (cont'd)

Compound	Test Material	Assay	Test System	Dose/Conc. Range	Reference	TB Evaluation	Comments
HEPTACHLOR	Technical	Gene Mutation	Ames (all)	(Unstated)	Gentile et al. (82)	INCONCL.	POS. in TA 1535/100, but only with S9
	(Unstated)	Gene Mutation	ARL/HGPRT	10^{-7} to 10^{-4} M	Telang et al. (82)	ACCEPT.	NEG. for HGPRT; POS. for promotion
	(Unstated)	in vitro UDS	WA-4 cells	100,1000 uM	Ahmed et al. (77a)	INCONCL.	POS. only with S9, but no values for UDS
	Epoxide	in vitro UDS	WA-4 cells	10,100,1000 uM	ibid.	INCONCL.	POS. only with S9, but no values for UDS
	(Unstated)	in vivo CA	Mouse BM	5 mg/kg	Markarjan (66)	INCONCL.	Too few procedural details; 1 dose
	(Unstated)	DLT/BM-CA	Rat repro. study	1, 5 ppm	Carey (74)	INCONCL.	[Abstract]
	Technical	in vitro CA/SCE	CHO cells	(Multiple)	NTP (85)	INCONCL.	POS. for both CA and SCE, but too few details available