Caswell

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dimethoate - Assessment of Mutagenicity

TB Proj. No.: 7-0037
Caswell No.: 358

FROM: Irving Mauer, Ph.D. 07-16-87
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Karen Hamernik, Ph.D., Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

The potential for dimethoate to induce mutagenic or genotoxic effects has been reexamined in a critical review and evaluation of the available data base.

Dimethoate had previously undergone Special Review ("RPAR" i.e., Rebuttable Presumption Against Registration/ Reregistration) because, in addition to mutagenicity, a number of other risk criteria had been exceeded under that regulatory process (oncogenicity, as well as reproductive/ fetotoxic effects). Subsequent review by the Agency (as well as by the FIFRA Science Advisory Panel) of registrants' rebuttals indicated the mutagenicity risk presumption had not been rebutted, but mutagenic risk appeared to be very low. However, at that time (1980) there were insufficient data upon which to base a mutagenicity risk assessment, and additional studies were required to be submitted in order to estimate the nature and magnitude of this potential. The Special Review of Dimethoate was concluded with Position

Document 4 (issued in September 1980), in which the Agency required the submission of the following additional mutagenicity data:

1. Gene mutation studies in mammalian systems

2. Dominant lethal assays

3. Studies designed to detect spindle effects (e.g., bone marrow cytogenetic studies for aneuploidy).

These requirements were also specified in the Dimethoate Registration Standard (issued August 31, 1982).

The data required by these regulatory requirements have since been submitted, reviewed, and evaluated (as indicated in the attached review).

Attachment

Dimethoate Mutagenicity: Review of Studies

A total of 21 studies assaying the mutagenicity of dimethoate are available. These studies have been reviewed and evaluated according to the categories of genetic effect ("study type") required under FIFRA Testing Guidelines (163.84-1 through -4) and summarized on the tabulation found on the following pages.

1. Assays for Gene Mutation

All but one of the studies for gene mutation involved bacterial systems.

Dimethoate has been shown to be mutagenic in reverse mutation spot assays using two excision-repair-deficient strains of Escherichia coli, namely WP 67 (Hanna and Dyer 1975) and WP 2 uvrA (Hanna and Dyer 1975; and American Cyanamid 1977). Mutagenicity was also demonstrated in quantitative reverse mutation plate assays using E. coli WP 2 uvrA as well as Salmonella typhimurium TA100 (American Cyanamid 1977). A low potency was confirmed in these latter studies by noting that the positive control, N-methyl-N'-nitro-nitrosoguanidine (MNNG) at 20 ug/plate produced greater than 1000 revertants/plate while 10,000 ug/plate of dimethoate produced only an average of 310 revertants/plate (American Cyanamid 1977). Thus, dimethoate is at least 1600 times less potent than MNNG under the conditions of this assay. The results with S. typhimurium TA100 displayed a similar relationship but were more difficult to quantify since a decrease in the dose response curve was seen at 1000 ug dimethoate/plate and the cells were killed at 10,000 ug/plate. In the TA100 S9-activated system, the highest number of revertant colonies observed was 594 at 1000 ug/plate dimethoate (although this figure appeared to be reduced by toxicity); MNNG, at 20 ug/plate, produced > 3000 revertants/plate. The unactivated TA100 assay suggested mutagenicity but cannot be considered reliable since the concurrent negative controls had an extremely high background count (522 revertants/plate).

Dimethoate has also been shown to induce forward mutations in E. coli K-12, as detected by resistance to 5-methyltryptophan (Mohn 1973). The potency, however, was also relatively low compared to the positive controls, MNNG and methyl methanesulfonate (MMS) (Mohn 1973). For example, 1×10^{-3}M dimethoate was required to produce a mutation frequency similar to that produced by only 1.7×10^{-7}M MNNG; thus dimethoate appears to be about 2000 times less potent than MNNG under the conditions of this assay.

Many other strains of bacteria have been tested with negative results. For example, Ashwood-Smith et al. (1972) reported that dimethoate was negative for mutagenic effects in a reverse mutation spot test assay using the repair-competent strain

DIMETHOATE: MUTAGENICITY STUDIES

Study Type	Test System	Reported Results	TB Evaluation	Reference
1. GENE MUTATION	Bacteria: Plate test in E. coli K-12 for forward mutation to 5 MT resistance.	Positive (weak) response at 10^{-3} M (= 5000 µg per plate).	Acceptable	Mohn 1973
	Bacteria: Spot tests for reverse mutations in E. coli (7 strains) and S. typhimurium (8 strains).	Positive in E. coli WP2 uvrA and WP67; negative in all other strains. Required long incubation (72 hr).	Inconclusive	Hanna and Dyer 1975
	Bacteria: Spot (disc) tests for reverse mutations in S. typhimurium (5 strains) and E. Coli WP2 uvrA. Plate tests in same strains.	Weakly positive in E. coli uvrA and in S. typh. TA100, but only at cytotoxic doses (5000 µg/disc).	Acceptable	American Cyanamid 1977
	Bacteria: Reverse mutation in spot tests with E. coli WP2.	Negative at the single dose tested (1000 µg/disc).	Unacceptable	Ashwood-Smith et al., 1972
	Bacteria: E. coli; and Saccharomyces.	(Secondary review of published data.)	Unacceptable, since Survey Article only	Wild 1975
	Bacteria: Spot tests in B. subtilis H17/M45, E. coli WP2/hcr, and S. typhimurium (4 TA strains).	Negative in all strains, but no MA used, and doses not stated.	Unacceptable	Shirasu et al., 1976

DIMETHOATE: MUTAGENICITY STUDIES (cont'd)

Study Type	Test System	Reported Results	TB Evaluation	Reference
1. GENE MUTATION (cont'd)	Bacteria: Oral host mediated (in mice), with S. typhimurium G46.	Positive (mutation frequency = 3.44X control) at a single dose to host (155 mg/kg, divided over 3 days). Incomplete reporting/ design deficiencies.	Inconclusive	Usha Rani et al., 1980(a)
	Mammalian cells: CHO/HGPRT forward mutation.	Negative. Although slight increases noted at 2700 and 3500 µg/mL, values were within normal background and historical control rates.	Acceptable	Allen and Johnson 1985

DIMETHOATE: MUTAGENICITY STUDIES (cont'd)

Study Type	Test System	Reported Results	TB Evaluation	Reference
2(a). CHROMOSOME ABERRATIONS (SOMATIC)	Cytogenetics in mouse bone marrow cells (ip injection).	Positive at 1 cc/100 g bw (i.e., no dose stated) for centromeric fission and "stretching," highest response reported at 24 hr postdosing. However, poorly reported and results of questionable validity.	Inconclusive	Bhunya and Behera 1975
	Cytogenetics in rat bone marrow cells (ip injection).	Negative for chromosome breaks up to clinically toxic doses.	Acceptable	San Sebastion et al. 1985
	Cytogenetics in male mouse bone marrow cells and spermatogonia (10 mg/kg, once; 0.6 ppm in drinking water for 7 weeks.	Negative for structural chromosome damage for both dose schedules, but no clinical effects observed; other design/reporting deficiencies.	Unacceptable	Degraeve and Moutschen (1981?)
	Cytogenetics in bone marrow cells of ♀ Syrian hamsters (ip, four doses, up to LD_{50}, 160 mg/kg, once).	"Weakly" positive. Non-dose-related minimal increases, but gaps were also included. Unstated number of animals (and sexes) tested. Only a commercial preparation (Bi 58EC, 37% ai) tested.	Unacceptable	Dzwonkowska and Huebner 1986

DIMETHOATE: MUTAGENICITY STUDIES (cont'd)

Study Type	Test System	Reported Results	TB Evaluation	Reference
2(a). CHROMOSOME ABERRATION (SOMATIC) (cont'd)	Micronuclei (MN) in PCE of mice (treated at oral doses of 51.8 and 77.8 mg/kg).	Positive dose-related increase in MN at normal PCE/NCE ratios of six animals (unstated sex). No positive controls.	Inconclusive	Usha Rani 1980(b) et al.
	Micronuclei in PCE of mice (treated at 55 mg/kg once, or twice, ip).	Negative at a dosage (2 x 55 mg/kg) causing cytotoxicity (decreased PCE/NCE ratios).	Acceptable	Pharmakon 1985

DIMETHOATE: MUTAGENICITY STUDIES (cont'd)

Study Type	Test System	Reported Results	TB Evaluation	Reference
2(b). CHROMOSOME ABERRATION (GERMINAL)	Dominant lethals in mice (treated at a single dose of 80 mg/kg, or 6.66 mg/kg/day for 30 days, both ip).	Positive; increased resorptions. However, no positive controls; other deficiencies.	Inconclusive	Gerstengarbe 1975
	Dominant lethals in mice (treated at single doses of 30 and 60 mg/kg; or 5 daily doses of 6 mg/kg/day; or 3 doses of 18 mg/kg/day, all ip).	Negative with all dosage schedules. Number of males unstated; no clinical toxicity at any dose; other deficiencies.	Unacceptable	Fischer and Scheufler 1981
	Dominant lethals in mice (treated at a single dose of 10 mg/kg, ip; or 0.6 ppm in drinking water for 7 weeks).	Negative with both dosage schedules. No information on (unstated) number of treated males. No clinical effects at either dosage. No positive control for chronic test. Insufficient number of pregnancies.	Unacceptable	Degraeve and Moutschen (undated); also, Degraeve et al. 1979
	Dominant lethals in mice (treated at 5, 10, and 20 mg/kg/day for 5 days by oral gavage).	Negative up to level of clinical toxicity (decreased body weight).	Acceptable	Becker and Schafroth 1985

DIMETHOATE: MUTAGENICITY STUDIES (cont'd)

	Study Type	Test System	Reported Results	TB Evaluation	Reference
3.	PRIMARY DNA DAMAGE	Mitotic gene conversion in yeast (Saccharomyces cerevisiae D4) (40 mM to 100 mM).	Positive dose-related response, but no positive controls, and other deficiencies (nature of test chemical, etc.).	Inconclusive	Fahrig 1973
		Unscheduled DNA synthesis (UDS) in SV-40-transformed human cells, VA-4 (treated at 10, 100, and 1000 mM).	Positive for UDS at 100 and 1000 mM, but only with activation. No positive controls. No data presented (results expressed as +/- only).	Unacceptable	Ahmed et al., 1977
		Sister chromatid exchange in Chinese hamster V79 cells in vitro (10, 20, 40, and 80 µg/mL).	Dose-related positive for SCE; slight cell cycle delay at the HDT.	Inconclusive, since no testing with S9.	Chen et al., 1981

DIMETHOATE: MUTAGENICITY STUDIES (cont'd)

Study Type	Test System	Reported Results	TB Evaluation	Reference
4. OTHER EFFECTS	Seed setting and germination in beans (Phaseolus vulgaris) treated at 0.1 and 0.5 percent, sprayed on mature plants.	Positive for reduced germination and chromosomal abnormalities ("fragments," "stickiness," and "anaphase bridges"). However, no controls and nature of test substance unstated.	Inconclusive	Agarwal et al., 1973
	Cytological effects in cotton (Gossypium barbadense) and bean (Vicia faba) seeds treated with either the technical (in the case of beans) or the formulated product (40% ai, both beans and cottonseeds) at concentrations of 0.0625% to 0.5%).	Positive dose-related effects on chromosomal abnormalities (abnormal mitoses, spindle effects). However, no positive controls and no data on negative controls.	Inconclusive	Amer and Farah 1974

10

of E. coli WP 2 try⁻. Although this result conflicts with other investigations using the same test system (e.g., Hanna and Dyer 1975; Shirasu et al. 1976), Ashwood-Smith and colleagues used only a single dose in spot (disc) tests.

Shirasu et al. (1976) reported that dimethoate was negative for mutagenic effects using Bacillus subtilis H 17 Rec⁺ and B. subtilis M45 Rec⁻. They also reported negative results in a reverse mutation assay using E. coli WP2 B/r try⁻. E. coli WP2 try⁻hcr is the same strain used by Hanna and Dyer (1975) and American Cyanamid (1977) (the latter investigators using, however, the notation WP 2 uvrA⁻) in studies which showed positive results after incubation of the plates for 3 days. Shirasu et al., however, incubated their plates for only 2 days; thus, the negative results may have been due to insufficient incubation time for this weak mutagen. The study by Shirasu and colleagues, however, is considered inadequate since no metabolic activation was used, and the doses used were not clearly stated.

Usha Rani et al. (1980a) tested dimethoate in a host-mediated assay employing Salmonella typhimurium G46 as indicator organism and an unspecified number of Swiss albino mice as host, treated by gavage with a single oral dose of 155 mg/kg divided over 3 days. They reported a significant increase in reversion frequency over control (3.44X), interpreting their results as indicating a metabolite (or metabolites) of dimethoate may be the mutagenic agents, rather than the parent compound. Although suggestive of mutagenicity, this study is considered inconclusive because of both incomplete reporting and experimental design deficiencies.

In an elaborate series of experiments to evaluate the mutagenic potential of dimethoate in a forward mutation assay in mammalian cells, investigators at American Cyanamid treated Chinese hamster ovary cell (CHO) cultures with dimethoate, both in the absence and presence of rat hepatic S9 activation (Allen and Johnson 1985). Although statistically significant increases in mutant colonies over concurrent controls were observed at the highest (cytotoxic) doses (2700 and 3500 ug/mL), the test values were within both referenced as well as historical laboratory background, and thus the assay is considered negative.

2. Assays for Chromosome Aberrations

Bhunya and Behera (1975) studied the effect of an unspecified formulation of dimethoate ("Rogor") on bone marrow cell chromosomes of male and female adult mice (unspecified strain). Although this short article (little more than an abstract) reported a "substantial number of chromosome breakage effects at the centromere" as caused by dimethoate, the experiment was inadequately reported and the results are of questionable

//

validity. The authors, for example, stated that controls were performed, but no control data were presented. Thus, this report is suggestive of chromosome damage, but is considered an inconclusive report because of the indicated deficiencies.

Only one of the three more recent cytogenetic studies in bone marrow cells of treated animals is considered adequate.

San Sebastion and colleagues (1985) administered technical dimethoate by ip injection to Sprague-Dawley rats up to a clinically toxic level (150 mg/kg), with no clastogenic (structural chromosome breakage) effects noted.

Degraeve and Montschen (1981) also reported negative results for clastogenicity in bone marrow cells (as well as spermatogonia) of male mice (Q-Edinburgh strain) given dimethoate as a single ip dose of 10 mg/kg, or in the drinking water at 0.6 ppm for 7 weeks. Since no clinical effects were observed at either dosage schedule, it appears that dosing may have been inadequate; as well, other design deficiencies (e.g., number of animals treated was unstated) render this study unacceptable.

Most recently, Dzwonkowska and Huebner (1986) reported "weakly" positive results for chromosome breakage in female Syrian hamsters injected ip at single doses up to the LD_{50} (160 mg/kg). However, this study is considered inadequate since chromosomal gaps (unstained separations not necessarily indicating true chromosome breakage) were also included in the data; as well, the increases noted were minimal and not dose-related, and an unstated number of animals of only one sex were used. Finally, only a commercial preparation (Bi 58 EC, 37% ai) was tested, rather than the technical.

The induction of micronuclei (MN) in polychromatic erythrocytes of mice treated with dimethoate was examined in two available studies. Usha Rani and colleagues (1980b) reported dose-related increases in animals treated at two oral doses of 51.8 or 77.8 mg/kg given 24 hours apart. Although suggestive of positive effects, this study is considered inadequate, because (1) the source, identity, and purity of the test article was not stated, (2) an insufficient number (6) of animals of unstated sex were employed, and (3) there were no positive controls.

On the other hand, in an adequate controlled assay, investigators at Pharmakon (1985) treated CD-1 mice (both males and females) at ip doses of 55 mg/kg (once, or twice) with no effects on MN induction, but clearly altered PCE/NCE ratios (evidence of cytotoxicity).

Germinal evaluations of dimethoate in the form of several dominant lethal assays (DLT) in mice were also available for this review.

Gerstengarbe (1975) reported that dimethoate induced a significant increase in resorption rates in pregnancies from the first through fifth weeks of mating ip-treated strain AB Jena/Halle strain males (given single doses of 80 mg/kg, or 6.66 mg/kg/day for 30 days) to groups of untreated females. However, since the source, nature and identity of the test material was not clearly specified, and no positive controls were reported, this study has been considered inconclusive evidence of a dominant lethal effect.

Three more recent mouse dominant lethal assays all reported negative results although only one of these can be considered adequate.

In one inadequate study submitted by American Cyanamid, AB Jena-Halle and DBA males were treated at three dosage schedules: single ip doses of 30 or 60 mg/kg dimethoate, or 5 daily doses of 6 mg/kg/day, or 3 doses of 18 mg/kg/day (Fischer and Scheuffler 1981). Although reportedly negative with all dosage schedules, this study is considered unacceptable because of major deficiencies, such as the number of males treated was unstated, and no clinical toxicity was apparently induced.

A second inadequate study also reported negative results for dominant lethals in mice given single ip doses of 10 mg/kg, or 0.6 ppm in drinking water for 7 weeks (Degraeve and Moutschen 1979, et seq.). This study, however, had no information on the number or condition of males treated, reported no clinical toxicities, had no positive controls, and generated an insufficient number of pregnancies for adequate interpretation.

The only well-controlled dominant lethal study available was that of Becker and Schafroth (1985) who reported negative results in NMRl mice at daily oral doses up to a level of dose-related clinical toxicity (5, 10, and 20 mg/kg/day for 5 days).

3. Assays for Primary DNA Damage

A limited number of assays for primary DNA damage in microbial and mammalian in vitro test systems was available for this review.

Fahrig (1973) treated cultures of Saccharomyces cerevisiae D4 with dimethoate at seven dosage levels (ranging from 40 mM to 100 mM), and reported dose-related induction of mitotic gene conversions. The potency of dimethoate, however, was low, 50 mM dimethoate inducing about the same number of conversions as 0.5 mM of the MMS control. Since no positive controls were apparently included in this assay, and the nature of the test substance was unspecified, this study is considered presumptively positive but inconclusive evidence of DNA damage.

Ahmed et al. (1977) reported an increase in unscheduled DNA synthesis in SV-40 transformed human cells (VA-4) after administration of 100 uM and 1000 uM dimethoate in the presence of metabolic activation. Results were negative at these concentrations without metabolic activation, and no significant increase was reported after administration of 10 uM with or without metabolic activation. This study is considered inadequate, however, because quantitative data were not presented, rather the results were reported only as positive or negative (+/-). In addition, no positive controls were used, and thus the activity of the pesticides studied cannot be related to known mutagens.

The study by Ahmed et al. does indicate, however, that dimethoate has a potency at least 100X less than other pesticides that were found to increase unscheduled DNA synthesis in this particular assay. Chlordane, aldrin, dieldrin, carbaryl, diquat, 2,4-D, and captan were all reported as positive at the lowest level tested (1 uM) while dimethoate was reported as negative at 10 uM and positive only at 100 uM and higher. However, the use of a virus-transformed cell line leaves unclear the potency of dimethoate in a normal (nontransformed) system.

Dose-related increases in sister chromatid exchanges were also reported in Chinese hamster V79 (lung) cells treated without metabolic activation at 10, 20, 40, and 80 ug/mL (Chen et al. 1981). Slight cell cycle delay was recorded at the HDT.

4. Assays for Other Potentially Genotoxic Effects

Studies not directly related to mutagenesis but suggestive of indirect effects were also available for review.

Amer and Farah (1974) studied the cytological effects of dimethoate on cotton (Gossypium barbadense) and broad beans (Vicia faba). Bean seeds were treated at concentrations of 0.5, 0.25, 0.125, and 0.0625% using both pure and formulated (a solution containing 40% active ingredient) dimethoate. Cottonseeds were treated with the formulated product at concentrations of 0.25, 0.125, and 0.0625% dimethoate. Both pure and formulated dimethoate inhibited cell division in beans. The mitotic index for bean seeds treated with pure dimethoate ranged from 18.4 (0.5% dimethoate) to 62.6 (0.0625% dimethoate) compared with a mitotic index of 94.1 for controls. The mitotic index for beans treated with formulated dimethoate ranged from 8.0 (0.25% dimethoate) to 42.0 (0.0625% dimethoate) compared with 56.4 for controls. A dose-response effect was seen in the percentage of abnormal mitoses, but the effects appeared to be due to mitotic spindle disturbances. Some fragmentation and bridge formation were seen but were not dose-related.

The same authors conducted further studies on the effect of dimethoate on the cytology of V. faba. In meiosis, spindle "disturbances" were the primary effect, but low percentage of fragmentation was also reported. It was not possible to determine the background levels for these effects, since their distribution in negative controls was not described. The authors also reported that the transmission of these effects to following generations was very low.

Agarwal and colleagues (1973) studied the effect of dimethoate on seed setting and germination on mature plants of the common bean, Phaseolus vulgaris, treated at 0.1% and 0.5% by foliar spray. Seeds from treated plants were then collected and soaked in water up to 48 hours at room temperature. Dimethoate treatment reduced germination 23 to 28 percent below controls, and chromosomal abnormalities (including fragments, stickness, and anaphase bridge formation) were seen in 12.8 to 27.5 percent of the treated series. No abnormalities were reported to have been observed in the controls, but no data were presented for either negative or positive controls. In several other respects, this cytological study was inadequately reported.

Dimethoate: Risk Characterization

From this reexamination and assessment of the mutagenicity data base available to the Agency, dimethoate does not appear to interact directly with mammalian genetic material (DNA or chromosomes), as demonstrated in acceptable in vitro and in vivo studies assaying for gene mutation and structural chromosome aberrations in both somatic and germinal tissue. On the other hand, there are a number of studies that strongly suggest an indirect mechanism of action affecting genetic mechanisms and/or cellular processes involved in DNA and/or cellular repair, and/or microtubule assembly associated with the mitotic spindle which can predispose to numerical chromosome aberrations (aneuploidy, etc.). Thus, the Agency concludes that, relative to human risk, the potential for dimethoate to induce direct mutagenic effects is very low, but cannot assess the magnitude of the risk for indirect effects in the absence of pertinent (and acceptable) studies assaying such activity.

Thus, available data indicate technical dimethoate is mutagenic in a minority of bacterial test systems [4 of 22 strains tested--Mohn 1973; Hanna and Dyer 1975; American Cyanamid 1977; Ashwood-Smith 1972; Wild 1975; Shirasu et al. 1976; Usha Rani et al. 1980(a)], but not in mammalian cells (Allen and Johnson 1985). Both cytogenetic analysis for chromosome aberrations in rodent bone marrow (San Sebastion et al. 1985; Degraeve and Moutschen 1981) as well as micronucleus assays (Pharmakon 1985) indicate dimethoate has little to no clastogenic activity, and several dominant lethal assays (Fischer and Scheuffler 1981; Degraeve et al. 1979; Becker and Schafroth 1985) indicate the compound has little to no potential for transmissible chromosomal damage.

Evidence of indirect interaction with genetic mechanisms, principally but not exclusively the mitotic spindle (i.e., disturbances in microtubular synthesis, assembly, or function), has been provided by positive studies for gene conversion (Fahrig 1973), sister-chromatid exchanges (Chen et al. 1981), unscheduled DNA synthesis in virally transformed cells (Ahmed et al. 1977), and plant studies (Amer and Farah 1974, Agarwal et al. 1973). Suggestive evidence for interference with the mitotic spindle in mammals was provided by the short, inadequate report of Bhuaya and Behera (1975); unfortunately, the more recent (negative) study for chromosome effects in rats (San Sebastion et al. 1985) did not provide any data on numerical aberrations (i.e., aneuploidy potential).

Bibliography

Agarwal, K.B.; Mehrotra, H.N.; Paul, B.K. (1973) Effect of organic insecticides on cytology of bean (Phaseolus vulgaris L.). Labdev J. Sci. Tech. 11-B(3-4):67-79.

Ahmed, F.E.; Hart, R.W.; Lewis, N.J. (1977) Pesticide-induced DNA damage and its repair in cultured human cells. Mutation Res. 42;161-174.

Allen, J.S.; Johnson, E. (American Cyanamid) (1985) Mutagenicity testing of dimethoate (AC 12,880) in the in vitro CHO/HGPRT Mutation Assay. Unpublished study by American Cyanamid, Princeton, NJ. EPA Accession No. 256594.

Amer, S.M.; Farah, O.R. (1974) Cytological effects of pesticides. VI. Effect of the insecticide "Rogor" on the mitosis of Vicia faba and Gossypium barbadense. Cytologia 39:507-514.

American Cyanamid Co. (1977) Mutagenicity testing of CL 12,880 (dimethoate). American Cyanamid Co., Princeton, NJ (unpublished).

Ashwood-Smith, M.J.; Trevino, J; Ring, R. (1972) Mutagenicity of dichlorvos. Nature 240:418-420.

Becker, H.; Schafroth, P; (RCC Research and Consulting). (1985) Dominant lethal study with dimethoate technical in the mouse. Unpublished study performed for the Dimethoate Task Force, Farmoplast, Milano, Italy. EPA Accession No. 259921.

Bhunya, S.P.; Behera, J. (1975) Centromeric sensitivity of mouse chromosomes to the systemic insecticide dimethoate (Rogor). Cur. Sci. 44(23):859-860.

Chen, H.H.; Hsueth, J.L.; Sirianni, S.R.; Huang, C.C. (1981) Induction of sister-chromatid exchanges and cell cycle delay in cultured mammalian cells treated with eight organophosphorus pesticides. Mutat. Res. 88:307-316.

Degraeve, N; Moutschen, J. (undated) "Genotoxicity of organophosphorus insecticide Dimethoate in mouse," (Universite de Liege, Laboratoire de Genetique, 15, rue Forgeur, B-4000 Liege, Belgium), unpublished study submitted by American Cyanamid Co. on behalf of the Dimethoate Task Force. EPA Accession No. 249601.

Degraeve, N.J.; Moutschen, J.; Moutschen-Dahmen, M; Gilot-Delhalle, J.; Colizzi, A.; Housbrechts, N.; Cholett, M.C. (1979) Genetic effects of organophasphate insecticides in mouse. Mutation Res. 64(2);131 (abstract only).

Degraeve, N; Moutschen, J. (undated) Genotoxicity of organophosphorus insecticide dimethoate in mouse. Unpublished study submitted by American Cyanamid in 1983. EPA Accession No. 249601.

Dzwonkowska, A.; Huebner, H. (1986) Induction of chromosome aberrations in the Syrian hamster by insecticides tested in vivo. Arch. Toxicol. 58:152-156.

Fahrig, R. (1973) Evidence of a genetic effect of organophosphorus insecticides. (Transl. from German.) Naturwissenchaften 60:50-51.

Fischer, G.W.; Scheufler, H. (1981) Effect of Dimethoate and O-Demethyldimethoate in the Dominant Lethal Test on Mice (translated from the German: "Zur Wirkung von Dimethoat und O-Desmethyldimethoat im Dominanten Letaltest an der Maus"), Wiss. Z. - Martin-Luther Univ. Halle-Wittenberg, Math.-Naturwiss. 30:21-29.

Gerstengarbe, S. (1975) Mutagenesis caused by dimethoate -- identified by means of the dominant lethal test in the mouse (Mus musculus L.). (Transl. from German.) Arch. Sci. J., Univ. Halle, M Series.

Hanna, P.J.; Dyer, K.F. (1975) Mutagenicity of organophosphorus compounds in bacteria and Drosophila. Mutation Res. 28:405-420.

Mohn, G. (1973) Mutagenic activity of monofunctional alkylating agents including organophosphorus insecticides. Mutation Res. 20:7-15.

Pharmakon Research International, Inc. (1985) Micronucleus Test with Dimethoate, CL 12,880 (Lot #611A). Unpublished study performed for the Dimethoate Task Force. EPA Accession No. 257603.

San Sebastion, J.R.; Naismith, R.W.; Matthews, R.J. (Pharmakon Research International, Waverly PA) (1985) In vivo bone marrow cytogenetics rat metaphase analysis: Dimethoate CL 12,880. Unpublished study performed for the Dimethoate Task Force, Farmoplast, Milano, Italy. EPA Accession No. 259921.

Shirasu, Y.; Moriya, M.; Kato, K.; Furuhashi, A.; Kada, T. (1976) Mutagenicity screening of pesticides in the microbial system. Mut. Res. 40:19-30.

Usha Rani, M.V.; Reddy, O.S.; Reddy, P.P. (1980a) Mutagenicity studies involving aldrin, endosulfan, dimethoate, phosphamidon, carbaryl, and ceresan. Bull. Environ. Contam. Toxicol. 25:277-282.

Usha Rani, M.V.; Reddy, P.; Pentiah, P.R. (1980b) Cytogenetic effects of organophosphate insecticides on bone marrow erythrocytes of mice. Cell and Chrom. Newsl. 3:11-13.

Wild, D. (1975) Mutagenicity studies of organophosphorus insecticides. Mutation Res. 32:133-150.

APPENDIX A

ANEUPLOIDOGENIC AGENTS AND OTHER SUBSTANCES SUSPECTED OF INTERFERING WITH MICROTUBULAR PROCESSES INVOLVED WITH THE MITOTIC SPINDLE

Actinomycin-D
Amitrole
Amphotericin-B
Azathioprine

Benomyl (the metabolite, MBC/other benzimidazole carbamates)

Chloral hydrate
Chloramphenicol
Chloroform
Colchicine/Colcemid
Cycloheximide
Cyclophosphamide
Cytochalasin-D

Diazepam
Diethylstilbestrol (and other, estradiol, estrogens)
Dimethoate
p,p'-DDT

Ethidium bromide
Ethylenethiourea

p-Fluorophenylalanine
5-Fluorodeoxyuridine

Griseofulvin

Halothane
Heparin

Lindane (and other organochlorines, such as dieldrin, heptachlor, etc.)

Maytansin
Mercaptoethanol
Methyl mercury chloride

Nocodazole

Oryzalin

Podophylotoxin (Podophyllin)

Rotenone
Retinoic acid

Sodium cyclamate

Taxol
12-o'-Tetradecanoyl phorbol 13-acetate (TPA)
Thiabendazole
Thiophanate-methyl
Trifluralin
Trimethoprim
Tubulozole

Vinblastine
Vincristine