December 19, 2001

MEMORANDUM

SUBJECT: The Determination of Whether Dithiocarbamate Pesticides Share a Common Mechanism of Toxicity

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This memorandum summarizes the position of the Office of Pesticide Programs (OPP) with respect to the grouping of the dithiocarbamate pesticides based on a common mechanism of toxicity (final position document: *The Determination of Whether Dithiocarbamate Pesticides Share a Common Mechanism of Toxicity,* Attachment A). The results of this hazard assessment are intended to provide direction to the Special Review and Reregistration Division (SRRD) as it proceeds in conducting the reassessment of tolerences for these pesticides and to the Registration Division (RD) as it considers any tolerance actions involving these pesticides. This memorandum describes the information considered by scientists, the process followed in developing a position on support for grouping the dithiocarbamate pesticides, and the conclusions regarding this scientific issue.

Background

The Food Quality Protection Act (FQPA) amended the laws under which EPA evaluates the safety of pesticide residues in food. Among other types of information EPA is to weigh when making safety decisions, the new amendments direct EPA to consider "available information concerning the cumulative effects of such residues and other substances that have a common mechanism of toxicity." Sec. 408(b)(2)(D)(v) of the Federal Food Drug and Cosmetic Act. FQPA also directs EPA to apply the new safety standard to tolerances established prior to the passage of FQPA. Further, in carrying out the tolerance reassessment provisions of FQPA, EPA "shall give priority to review of the tolerances or exemptions that appear to pose the greatest risk to public health." Sec. 408(q)(2).

The carbamate pesticides represent a class of food use pesticides that have been given high priority by OPP for the reassessment of tolerances in accordance with the mandates FQPA. Within the class, there are three distinct subgroups: N-methyl carbamates, thiocarbamates, and dithiocarbamates. As part of the reassessment, OPP scientists considered whether it would be appropriate to group the dithiocarbamate pesticides because the dithiocarbamates operate by a common mechanism of toxicity. In reviewing this issue, OPP scientists were guided by several relevant science policies, including:

- *Guidance for Identifying Pesticide Chemicals and Other Substances that Have a Common Mechanism of Toxicity* (issued for public comment in August 1998; issued in revised form in February 1999, Attachment B). Internet: http//www.epa.gov/fedrgstr/EPA-PEST/1999/February/Day-05/6055.pdf **or** Document No. 6055, Fax-on-Demand, (202) 401-0527.

- *Proposed Guidance on Cumulative Risk Assessment of Pesticide Chemicals that have a Common Mechanism of Toxicity* (USEPA, January 22, 2000, Attachment C). Internet: http://www.epa.gov/fedrgstr/EPA-PEST/2000/June/Day-30/6049.pdf

- *A Science Policy on a Common Mechanism of Toxicity: The Carbamate Pesticides and the Grouping of Carbamate with Organophosphorus Pesticides*, Attachment D. Internet: http://www.epa.gov/scipoly/sap/1999/September/carbam.pdf

Review of the dithiocarbamate pesticides

EPA's Office of Pesticide Programs (OPP) prepared the preliminary document *The Grouping of a Series of Dithiocarbamate Pesticides Based on a Common Mechanism of Toxicity* in response to a September 1999 recommendation from the FIFRA Scientific Advisory Panel (SAP) that the Agency specifically address effects other than acetyl cholinesterase inhibition reported in studies conducted on the

dithiocarbamates before initiating a cumulative risk assessment on this group of pesticides (SAP Report No. 99-05, November, 1999). Thus, the approach to the assessment of the dithiocarbamate pesticides was to consider whether these substances cause a common effect, other than acetyl cholinesterase inhibition, that might be attributable to a common mechanism. A review of data provided in studies submitted by registrants and in studies reported in the literature suggested that treatment of rats with the dithiocarbamate pesticides may induce a common effect (neuropathology) by a common mechanism (metabolism to carbon disulfide or other reactive metabolite commonly formed by the dithiocarbamates).

On September 7, 2001, the Agency presented to the SAP a draft cumulative hazard assessment of the dithiocarbamates. The SAP commented that it disagreed with the Agency that there was sufficient evidence that the dithiocarbamates induce neuropathology via a common mechanism of toxicity and questioned whether formation of carbon disulfide could be linked to the neuropathology induced by most members of this group of pesticides. (SAP Report No. 2001-11 dated November 1, 2001, Attachment E). The SAP pointed out the conflicting evidence that metabolism to carbon disulfide was a critical step in the induction of neuropathology. For example, ferbam is reported to generate carbon disulfide *in vivo* at equivalent or higher rates than other dithiocarbamates but the chemical has not been shown to induce neuropathology. Further, the SAP noted that a consistent pattern of neuropathology has not been demonstrated among the dithiocarbamates. The panel commented that some members of the group produce axonal lesions, others primary demyelination, and still others neuronal lesions. The panel also pointed out that the neuropathology changes reported in some studies with the dithiocarbamates are not consistent with those seen with carbon disulfide exposure. However, the SAP commented that the quality of the histopathology reports and an apparent lack of consistent pathological examinations hindered the evaluation of common neuropathology effects. It was suggested that the Agency use a Pathology Working Group (PWG) to provide input regarding similarities in the pathology effects caused by different dithiocarbamtes.

Comments received from the public also raised issues regarding the grouping of the dithiocarbamates based on a common mechanism of toxicity (McDermott, *et al.,* 2001[1]; R.T. Vanderbilt Company, Inc., 2001[2]). These commenters also pointed out that none of the dithiocarbamates reviewed induce the neuropathy characteristic of carbon disulfide and that the metabolism to carbon disulfide of ferbam and dimethyl-dithiocarbamate, which are not neuropathic, refutes the hypothesis that the

[1]Memo from Edward M. Ruckert, McDermott, Will and Emery and Counsel to the EBDC/ETU Task Force, to Anne Overstreet, OPP/EPA, dated November 1, 2001.

[2]Memo from David Bower and Sharen Breyer, R.T. Vanderbilt Company, Inc., to Paul Lewis OSCP/EPA, dated September 27, 2001.

dithiocarbamates can be grouped on their potential to produce a common neuropathic metabolite. Both commenters also questioned whether some of the dithiocarbamates produce neuropathic effects.

Based on the recommendations of the SAP and comments received from the public, OPP reevaluated the existing data suggesting that the dithiocarbamates can be grouped based on a common mechanism of toxicity. The dithiocarbamates included in this final review are Mancozeb, Maneb, Metiram, Na-Dimethyldithiocarbamate, Ziram, Thiram, Ferbam, and Metam sodium. OPP concludes that the available evidence shows that the neuropathology induced by treatment of rats with the dithiocarbamates can not be linked with the formation of carbon disulfide because: a) the neuropathology induced by the dithiocarbamates is not consistent with the neuropathology induced by exposure to carbon disulfide, b) there is a lack of concordance between doses of the dithiocarbamates that induce neuropathology and the amounts of carbon disulfide formed during metabolism and c) there is evidence that more than one mechanism of toxicity could be operative that accounts for dithiocarbamate induced neuropathology because there is no consistent pattern of neuropathology reported in studies with this subgroup of carbamates. Accordingly, the available evidence does not support grouping the dithiocarbamates based on a common mechanism for neuropathology.

As discussed in the attached document, the Agency has also considered support for grouping the dithiocarbamate pesticides based on a common mechanism of toxicity other than neuropathology. There are several dithiocarbamate pesticides that produce a common effect (thyroid cancer) via formation of a common metabolite (ETU). OPP addressed this issue previously in a regulatory action that led to a reduction in the use of those dithiocarbamates (mancozeb, maneb, and metiram). Although treatment of rats with ferbam and ziram induce neoplastic responses in thyroid tissue, these pesticides are not metabolized to ETU and can not be considered to induce a neoplastic response by a common mechanism. The inhibition of cholinesterase was determined to be a common effect for only two of the dithiocarbamate pesticides, ziram and metam sodium. Although central nervous system (CNS) defects were reported in some studies with the dithiocarbamates, the type of defects observed were not consistent among the chemicals and the mechanism(s) for the production of the defects are unknown. Therefore, there is insufficient data to show a common mechanism as to the CNS defects.

Regarding the recommendation that the Agency consider using a PWG to provide input on the specific neuropathology formed by the dithiocarbamates, the Agency will consider seeking such assistance when and if more evidence becomes available that a single, common mechanism is shown to be involved in one or more toxic effects that are induced by the dithiocarbamates.

Conclusion

OPP has considered the recommendations of the SAP and comments received from the public and the document, *The Grouping of a Series of Dithiocarbamate Pesticides Based on a Common Mechanism of Toxicity*, has been revised accordingly. OPP believes that persuasive arguments were presented by the SAP and public commenters concerning the lack of support for grouping the dithiocarbamate pesticides based on a common mechanism of toxicity for the production of neuropathology. It is OPP's position that the dithiocarbamate pesticides should not be included in a common mechanism group based on a potential to induce neuropathology. There is also no evidence, or data are lacking, to show that exposure to the candidate common mechanism group of dithiocarbamate pesticides can lead to other common effects by a common mechanism. However, there is sufficient evidence that three dithiocarbamates, maneb, mancozeb, and metiram can induce a common effect (thyroid cancer) by the formation of the common metabolite, ETU. These three chemicals should be grouped, as in the past, when conducting a risk assessment. Ziram and metam sodium also share a common mechanism of toxicity, the inhibition of cholinesterase.

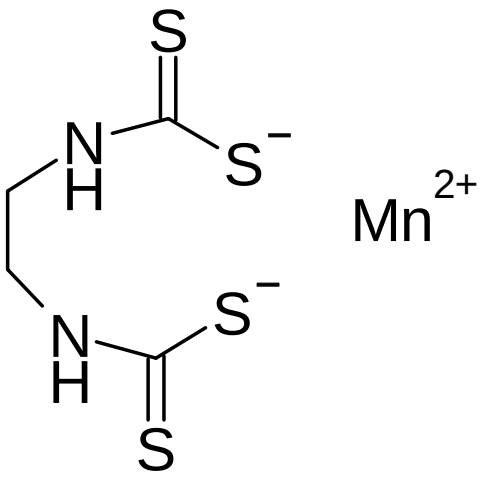
OPP will place this memorandum and its attachments in a public docket and will post the memorandum on OPP's website. In addition, OPP will notify its stakeholders of this determination using the Pesticide Program Update messaging system and will announce the availability of these documents to the media. Further, OPP will invite the public to submit comments on this determination, as well as any relevant new data or analyses over the next 60 days. Finally, as OPP moves ahead, the Office will consider fully all comments and information submitted by the public.

Attachments:

a) *The Determination of Whether Dithiocarbamate Pesticides Share a Common Mechanism of Toxicity*. December 1, 2001
b) *Guidance for Identifying Pesticide Chemicals and Other Substances that Have a Common Mechanism of Toxicity.* January 29, 1999
c) Proposed Guidance on Cumulative Risk Assessment of Pesticide Chemicals that have a Common Mechanism of Toxicity. June 22, 2000
d) *A Science Policy on a Common Mechanism of Toxicity: The Carbamate Pesticides and the Grouping of Carbamate with Organophosphorus Pesticides*, August 30, 1999
e) SAP Report No. 2001-11, FIFRA Scientific Advisory Panel September 7, 2001; Report Issued November 1, 2001

Attachment A

The Determination of Whether Dithiocarbamate Pesticides Share a Common Mechanism of Toxicity



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December 1, 2001

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Executive Summary

This document discusses the available scientific evidence for determining whether a common mechanism of toxicity exists among certain dithiocarbamate pesticides. The weight-of-the-evidence (WOE) analysis follow generally the approach outlined in the January 29, 1999 ***Guidance for Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity*** [http//www.epa.gov/fedrgstr/EPA-PEST/1999/February/Day-05/6055.pdf]. The dithiocarbamate pesticides covered in the current document are: **mancozeb, maneb, metiram, Na-dimethyldithiocarbamate, thiram, ziram, ferbam, and metam sodium.**

Treatment of laboratory animals with these dithiocarbamates may result in effects such as neuropathology, thyroid toxicity, and central nervous system developmental toxicity. Based on a preliminary review, only the pesticides **mancozeb, maneb, metiram, ziram, thiram** and **metam sodium** were identified as a candidate group that may induce a common effect by a common mechanism of toxicity. Evidence from studies submitted to the Agency and from studies reported in the literature suggested that each of these dithiocarbamates induces distal peripheral neuropathy and, for most, that this effect may be associated with the formation of a common metabolite, carbon disulfide (CS_2). CS_2 is known to induce distal peripheral neuropathy in laboratory animals. Although Na-dimethyldithiocarbamate may also be metabolized to carbon disulfide, 90-days treatment of rats up to 98.75 mg/kg/day with the chemical did not induce neuropathology. Ferbam has also not been shown to induce neuropathy in laboratory animals when tested for two years up to a dose-level of 331 mg/kg/day in the diet of rats. OPP also examined other possible mechanisms of toxicity and concluded that available evidence does not support the existence of other common mechanisms of toxicity for the dithiocarbamates. However, OPP pointed out that Mancozeb, Maneb, and Metiram should be grouped based on their metabolism to the common metabolite, ethylene thiourea, and the production of a common effect, thyroid toxicity.

On September 7, 2001, the Agency presented to the SAP the preliminary cumulative hazard assessment of the dithiocarbamates. The SAP commented that it disagreed with the Agency that there was sufficient evidence that the dithiocarbamates induce neuropathology via a common mechanism of toxicity and questioned whether formation of carbon disulfide could be linked to the neuropathology induced by most members of this group of pesticides. (SAP Report No. 2001-11 dated November 1, 2001). The SAP pointed out the conflicting evidence about whether metabolism to carbon disulfide was a critical step in the induction of neuropathology. For example, ferbam is reported to generate carbon disulfide in vivo at equivalent or higher rates than other dithiocarbamates but the chemical has not been shown to induce neuropathology. Further, the SAP noted that a consistent pattern of neuropathology has not been demonstrated among the dithiocarbamates. The panel commented that some members of the group produce axonal lesions, others primary demylination, and still others neuronal lesions. The panel also pointed out that the neuropathology changes

reported in studies with the dithiocarbamates are not consistent with those seen with carbon disulfide exposure. However, the SAP commented that the quality of the histopathology reports and an apparent lack of consistent pathological examinations hindered the evaluation of common neuropathology effects.

Although the dithiocarbamate pesticides are also metabolized to other reactive moieties including carbonyl sulfide and isothiocyanate, data are not sufficient to evaluate the relative role these other moieties may have in inducing neuropathy.

Based on the recommendations of the SAP and a reevaluation of the existing data, OPP concludes that the available evidence shows that the neuropathology induced by treatment of rats with the dithiocarbamates can not be linked with the formation of a common metabolite because a) the neuropathology induced by the dithiocarbamates is not consistent with the neuropathology induced by exposure to carbon disulfide, b) there is a lack of concordance between doses of the dithiocarbamates that induce neuropathology and the amounts of carbon disulfide formed during metabolism, c) there is evidence that more than one mechanism of toxicity is operative that accounts for thiocarbamate induced neuropathology because there is no consistent pattern of neuropathology reported in studies with this subgroup of carbamates, and d) evidence that ferbam and dimethyldithiocarbamate, which are not neuropathic pesticides, can be metabolized to carbon disulfide contradicts the hypothesis that formation of carbon disulfide by the dithiocarbamates is a common neuropathic agent.

As discussed in this document, the Agency has also considered support for grouping the dithiocarbamate pesticides based on a common mechanism of toxicity other than neuropathology. There are several dithiocarbamate pesticides that produce a common effect (thyroid cancer) via formation of a common metabolite (ETU). OPP addressed this issue previously in a regulatory action that led to a reduction in the use of those dithiocarbamates (mancozeb, maneb, and metiram). Although treatment of rats with ferbam and ziram induce neoplastic responses in thyroid tissue, they are not metabolized to ETU and can not be considered to induce a neoplastic response by a common mechanism. Although CNS defects were reported in some studies with the dithiocarbamates, the type of defects observed were not consistent among the chemicals and the mechanism(s) for the production of the defects are unknown. Two dithiocarbamates, ziram and metam sodium, inhibit cholinesterase, a common mechanism of toxicity.

In summary, it is OPP's position that the dithiocarbamate pesticides should not be included in a common mechanism group based on a potential to induce neuropathology. However, there is sufficient evidence that three dithiocarbamates, maneb, mancozeb, and metiram can induce a common effect (thyroid cancer) by the formation of the common metabolite, ETU. These three chemicals should be considered to share a common mechanism of toxicity and OPP intends to consider the cumulative effects of exposure to these compounds as part of its reassessment process. Finally, ziram and metam sodium should be considered to have a common mechanism of toxicity, the inhibition of cholinesterase.

The Grouping of a Series of Dithiocarbamate Pesticides
Based on a Common Mechanism of Toxicity

I. Introduction

A. Background

The Food Quality Protection Act of 1996 (FQPA) requires EPA to consider "available information concerning the cumulative effects of [pesticide] residues and other substances that have a common mechanism of toxicity." Sec. 408(b)(2)(D)(v) of the Federal Food Drug and Cosmetic Act. Central to performing this task is the process of identification of those pesticide chemicals that can be grouped based on a common mechanism of toxicity.

At a meeting of the FIFRA Scientific Advisory Panel (SAP) convened to solicit advice on a guidance document regarding the evaluation of a common mechanism of toxicity of the carbamate pesticides, a recommendation was made that the Agency specifically address effects other than cholinesterase inhibition reported in studies conducted with the thiocarbamates and the dithiocarbamates (US EPA, 1999b). The SAP stated that "groupings of carbamates based on non-cholinergic endpoints such as reproductive, thyroid, developmental, and broad-spectrum neurotoxicity could possibly be appropriate for certain carbamates, especially the low-potency, thio- and dithiocarbamate fungicides and herbicides, whose ability to inhibit acetylcholinesterase is weak or absent." On September 7, 2001, the Agency presented to the SAP a draft cumulative hazard assessment of the dithiocarbamates. The SAP commented that it disagreed with the Agency that there was sufficient evidence that the dithiocarbamates induce neuropathology via a common mechanism of toxicity and questioned whether formation of carbon disulfide could be linked to the neuropathology induced by most members of this group of pesticides. (SAP Report No. 2001-11 dated November 1, 2001). The current document describes the results of EPA's reevaluation of common effects induced by the dithiocarbamates by a common mechanism of toxicity and conclusions regarding the evidence supporting the grouping of the dithiocarbamates based on a common mechanism of toxicity for neuropathology, reproductive/developmental, or CNS effects.

B. Purpose

The purpose of this document is to present the position of the Agency on whether the dithiocarbamate pesticides share a common mechanism of toxicity .
OPP has used a weight-of-evidence (WOE) approach that considers all pertinent information to determine whether chemicals act via a common mechanism of toxicity. A stepwise process is outlined in the 1999 Guidance document that starts with an initial grouping of chemicals based on having shared structural, toxicological and/or pesticidal properties *{Guidance for Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity* , January 29, 1999 [http//www.epa.gov/fedrgstr/EPA-PEST/1999/February/Day-05/6055.pdf **or**

Document No. 6055, Fax-on-Demand, (202)401-0527]}. In a second phase, the steps that define the mechanism of toxicity for one or more chemicals in the group is identified. Finally, structural, toxicological and pharmacokinetic/pharmacodynamic data for the remaining chemicals in the group are examined to determine by WOE which of these possess the same mode of toxic action as the other compound(s) in the group. All those chemicals found to share the same mode of action for a common toxic effect are considered to have been grouped by a common mechanism of toxicity.

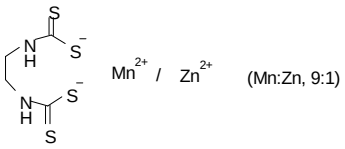
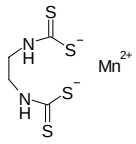
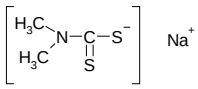
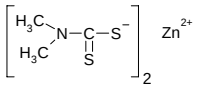
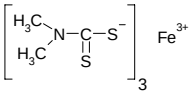
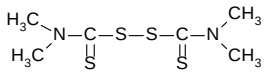
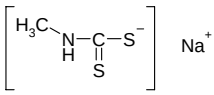
It should be noted that “mechanism of toxicity” is defined in the Guidance document (USEPA,1999) as “the major steps leading to an adverse health effect following an interaction of a pesticide with biological targets. All steps leading to an effect do not need to be specifically understood. Rather, it is the identification of the crucial events following chemical interaction that are required in order to describe a mechanism of toxicity.”

II. The Candidate Group of Pesticides

The dithiocarbamates reviewed in this document are all registered with the Agency for use as fungicides. Metam sodium and Na-dimethyl-dithiocarbamate (DMDTC) also have insecticidal uses. Thiram has insecticidal and herbicidal uses. The mechanism of action associated with the pesticidal activity of the dithiocarbamates is the inhibition of metal-dependant and sulfhydryl enzyme systems in fungi, bacteria, plants, and insects, as well as mammals (Miller, 1982).

The compounds shown inTable 1 are the **registered Dithiocarbamate** pesticides considered for grouping via a common mechanism of toxicity. This group, hereafter referred to as the candidate group, initially was selected based upon them all possessing the **dithiocarbamate moiety**.

Table 1. Structures of the Dithiocarbamates in the Candidate Group

Chemical	Structure	CAS No.	PC Code
EBDC's[1]			
Mancozeb		8018-01-7	14504
Maneb		12427-38-2	014505
Metiram	-[2]	9006-42-2	014601
DMDTC's[1]			
Na-Dimethyl-dithiocarbamate		128-04-1	034804
Ziram		137-30-4	034805
Ferbam		14484-64-1	034801
Thiram		137-26-8	079801
MMDTC's[1]			
Metam sodium		137-42-8	039003

[1] EBDC's = Ethylene-(bis)-dthiocarbamates. DMDTC's = Dimethyldithiocarbamates. MMDTC= monomethyldithiocarbamates

[2] Mixture of ammoniates of zinc-ethylene-(bis)-dithiocarbamates with ethylene-(bis)-dithiocarbamic acid bimolecular and trimolecular cyclic anhydrides and disulfides.

III. Lines of Evidence

In this section, the various available lines of evidence used in evaluation of a common mechanism of toxicity for the compounds under consideration are presented.

A. Structure Activity Considerations

In general, based on structure-activity relationships (SAR), the pesticides in a given class may be grouped according to their likelihood to generate a common type of toxic molecule or reactive intermediate or their ability to mimic a common biologically active molecule that interferes with the normal homeostasis of the cell (e.g., via receptor binding, enzyme induction, etc.).

For the candidate group of dithiocarbamates at least three modes of eliciting toxic action, other than cholinesterase inhibition, may be conjectured. These include: (a) the capacity to generate carbon disulfide (CS_2), (b) biotransformation to ethylenethiourea (ETU), (c) the ability to chelate physiologically important ions (e.g., Copper)

1. Capacity to Generate CS_2

There are data that shows *in vivo* release of CS_2 by 3 (ziram, ferbam, and thiram) of the 4 DMTCs in Table 1 . Although no data have been found showing *in vivo* release of CS_2 by the 3 EBDCs, there is data showing *in vivo* release of CS_2 by the related compound zineb (zinc EBDC). Because 2 of the EBDCs (metiram and mancozeb, Table 1) consist in part of zinc EBDC, some *in vivo* release of CS_2 may be expected from these compounds. For the remaining compounds in Table 1, arguments are presented in Section **III.C.2.a**. and in Table 2 to show that some *in vivo* release of CS_2 may also be expected from them, allowing us to conclude that *in vivo* release of CS_2, has been seen or is expected for the dithiocarbamates included in this review. *In vivo* release of CS_2 is important in the context of this document because CS_2 has been shown to cause neuropathies (Schaumburg and Berger, 1992) and may be the agent through which some of the dithiocarbamates are neuropathic.

2. Biotransformation to ETU

The three ethylenebis dithiocarbamates (EBDCs) can be biotransformed to ethylenethiourea (ETU). ETU is of toxicological concern due to its carcinogenicity, teratogenicity and antithyroid properties. The Office of Pesticide Programs (OPP) has previously cumulated risks that may result from dietary and residential exposures to the EBDCs, Mancozeb, Maneb, and Metiram.

3. Chelation

At least 7 of the dithiocarbamates can participate in chelation of physiologically important polyvalent cations. Dithiocarbamate chelates can be formed with copper, zinc, cadmium and lead, resulting in lipophilic species that may be responsible for redistribution of heavy metals into the brain. This shift in the distribution of heavy metals has been found also in peripheral neuropathies associated with CS_2 exposure.

Table 2. Dithiocarbamate compounds demonstrated to produce CS_2 *in vivo* .

Compound	Species and System	Proportion recovered as CS_2 (%)	Reference
EBDCs			
Zineb	Rat. Identified CS_2 chemically	Not quantified	Truhaut et al.(1973)
DMDTCs			
Thiram	Rat. ^{14}C -labeled C=S carbon. Recovery at the CS_2 trap	7.4-11.4	MRID 42235701
Ferbam	Rat. ^{35}S-labeled C=S.	18.1	Hodgson et al. (1975)
Ziram	Rat. ^{14}C -labeled C=S carbon. Recovery at the CS_2 trap (act. charcoal)	15.6-15.7	MRID 42391001
Disulfiram	Man	46-53	Merleveda and Casier, 1961 (cited in Hayes, 1982)
Disulfiram	Rat	2	Stromme, 1965 (cited in Hayes, 1982)
MMDTC			
Metam sodium	Rat	20	HED Doc No. 014062

B. Toxicological Considerations

Table 3 summarizes the toxic effects observed in subchronic or chronic studies with the candidate dithiocarbamates. Observed effects that were evaluated included neuropathology, thyroid effects, CNS developmental effects, and cholinesterase inhibition.

1. Neuropathology

Degeneration and/or demyelination of sciatic or spinal nerve tissue is a toxic effect observed in rat studies performed with the dithiocarbamate mancozeb, maneb, metiram, ziram, thiram, and metam sodium (Table 3). The data available on the neuropathological effects from studies submitted to OPP, as well as published data, are limited for ferbam but in a literature review, it was suggested that ziram and ferbam may induce neuropathology because both chemicals are oxidized to thiram, a neuropathic chemical (Miller, 1982). However, results of studies with ferbam reported in the literature do not indicate that ferbam induces neuropathology following treatment of rats or dogs. Hodge *et al.*, 1956, reported that two or more months of dietary (food) treatment of rats with either ziram or ferbam induced neurological changes (abnormal hindleg grasping action) at the same dose (0.25% of the diet) but histopathology revealed no lesions in nervous tissue. Furthermore, neuropathology was not observed in rat or dog subchronic or chronic studies at doses of up to 331 mg/kg/day in the feed (Hodge *et al., 1956;* Lee et al., 1978).

Table 3. Dithiocarbamates: toxicity endpoints and NOAELs/LOAELs (mg/kg/day)

Group/Chemical	Neuropathology	Thyroid Effects	CNS Effects	ChEI	RfD*
Ethylenebis-dithiocarbamates					
mancozeb	8.2/49.7 degeneration/demyelination of sciatic, tibial nerves, etc. - 90-day rat neurotoxicity. MRID 42034101	4.4/30.9 thyroid effects - 2 yr rat. MRID 41903601	128/512 atrophy of brain tissue, cranial edema, dilated ventricles -rat. MRID 00246663	not measured	4.4/30.9 thyroid effects -2 yr rat. MRID 41903601
maneb	23/100 digestion chambers in tibial, sciatic, peroneal nerves (minimal response) - 90-day rat neurotoxicity. MRID 43947602	<8.6/8.6 thyroid effects-18 mo. mouse. MRID 42642401	400/770 exencephaly, hydrocephaly rat -single dose to dam on GD-11. Larsson et al., 1976	no ChEI up to 100 - 90-day rat neurotoxicity MRID 43947602	<8.6/8.6 thyroid effects-18 mo. mouse MRID 42642401
metiram	27.3/88.8 decreased myelination of sciatic and tibial nerves - 90-day rat. MRID 40290601, 42539101	no consistent effects on thyroid**	none reported	not measured	0.4/6.7 reduced grip strength - 90 day rat. MRID40290601, 42539101
Dimethyl-Dithiocarbamate					
Na-dimethlydithio-carbamate	no neuropathology up to 98.75 -90-day rat neurotoxicity. MRID 435550501	no effects	none reported	no ChEI up to 98.75 MRID 43550501	2/20 Decreased maternal body weight, decreased ossification (fetal)
ziram	10.2/34.6 histopathology of spinal cord, axonal degeneration of the sciatic nerve (minimal)- - 2 yr rat. MRID 43404201	11/22 C-cell carcinomas - 2 yr rat. NTP Rpt. No. 238, 1983	none reported	6/16 - 15 % brain; at 40, 23% brain -90-day rat. MRID 43463701	1.6/6.6 decreased body weight gain - 1 yr dog. MRID 42823901
thiram	25.5/66.9 degeneration/demyelination of the sciatic nerve and the axis cylinders; degeneration of the ventral horn of the lower lumbar region of the spinal cord of rats - 80 week rat (Lee, C.C. and P.J. Peters, 1976)	equivocal evidence (no dose response for hyperplasia) of thyroid effects - 2 yr rat. MRID 42157601	<12.5/12.5 anophthalmia, microphthalmia in rat develop-mental study . MRID 00259810	not measured	0.84/2.61 elevated cholesterol and increase in liver/body weight ratio - 1 yr dog. MRID 41967901
ferbam	no neuropathological lesions up to 331 mg/kg/day - 90-day rat. MRID 00143817	8/32 squamous metaplasia 80-week rat. MRID 00143817	11/114 hydrocephalus in rat developmental study. MRID 00143816	no study	not established
N-methyl-dithiocarbamate					
metam sodium	0.056/0.19 increase in sciatic nerve degenration - 2 year rat MRID 43275802	no effects	no effects	324/647 acute neurotoxicity MRID 42977801	not established

* noaels and loaels used as basis for RfD; **because metiram is an EBDC and can be metabolized to ethylene thiourea (ETU), a thyroid carcinogen, the Cancer Assessment Review Committee recommended that each EBDC should be classified as a Group B2 carcinogen and the Q_1* for ETU should be used for linear extrapolation after applying the metabolic conversion factor for EBDC;

2. Thyroid Effects

The ethylene bisdithiocarbamates (EBDCs), mancozeb, maneb, and metiram, but not the dimethyl dithiocarbamates (DMDTCs), ziram, thiram,and ferbam or the monomethyl-dithiocarbamate, metam sodium, are metabolized to ethylene thiourea (ETU), a carcinogen that acts directly on the thyroid (Miller, 1982). Treatment of rats or mice with the dithiocarbamates mancozeb, maneb, and ziram has been associated with thyroid toxicity (see Table 3). RfDs were established based on thyroid effects for mancozeb and maneb. Although the data submitted to OPP do not show that metiram induces thyroid effects, the chemical can be metabolized to the common metabolite ETU. OPP has previously cumulated risks that may result from dietary and residential exposures to mancozeb, maneb, and metiram. Results of a 2-year rat carcinogenicity feeding study with ziram, conducted by the National Toxicology Progam, provides evidence that ziram induces an increased incidence of C-cell carcinomas (NTP Technical Report No. 238, 1983). The results of an 80-week feeding study conducted by Lee *et al.,* 1978, showed that ferbam also induces an increased incidence of thyroid squamous metaplasia. Metam sodium has not been shown to produce thyroid effects.

3. CNS Developmental Effects

The dithiocarbamates maneb and mancozeb (registered as pesticides) and propineb and zineb (not registered as pesticides) have been reported in the literature to induce hydrocephalus (Miller, 1982; Larsson *et al., 1976)*. Results of developmental toxicity studies submitted to OPP show that mancozeb, thiram, and ferbam induce central nervous system (CNS) defects in rats. However, the CNS effects are variable among these chemicals (atrophy, exencephaly/hydrocephaly, anopthalmia, hydrocephaly) No CNS developmental effects were reported in acceptable studies conducted with metiram, Na-dimethyldithiocarbamate, ziram, or metam sodium. Thus, there is not support for grouping the dithiocarbamates based on their potential to produce common CNS developmental effects.

4. Cholinesterase Inhibition

The absence of ChEI data in studies submitted to the Health Effects Division (HED) on many of the dithiocarbamates reviewed precludes an evaluation of the relative contribution of ChEI to the toxicity of all of the dithiocarbamates (Table 3). However, Miller (1982) postulated that the dithiocarbamates have little or no cholinesterase-inhibiting capabilities. There are, however, data showing that ziram and metam sodium inhibit cholinesterase but that maneb and Na-dimethyldithiocarbamate do not inhibit cholinesterase.

5. Relative Sensitivities of Common Effects

Neuropathy and thyroid effects are the two effects identified as possible common effects. NOAELs for neuropathology range from 0.056-27.3 mg/kg/day. NOAELs for thyroid effects are somewhat less than or about equal to the NOAELs for neuropathology for mancozeb, maneb, and ziram and treatment of rats with ferbam has been reported to induce thyroid effects but no neuropathological lesions. NOAELs used to define RfDs for these chemicals range from 0.4 to <8.6 mg/kg/day (8.6 mg/kg/day was a LOAEL in the case of maneb; a NOAEL or an RfD has not been established for ferbam). NOAELs used to define RfDs for five of the dithiocarbamates are not orders of magnitude lower than the NOAELs for neuropathological or thyroid effects. ChEI produced by ziram is produced at a somewhat lower does than is neuropathy but ChEI produced by metam sodium occurs at a much higher dose than does neuropathy.

C. Metabolism and Pharmacokinetics Considerations

Metabolism and pharmacokinetics considerations are important in determining common mechanisms of toxicity in a candidate set of chemicals. Information on the disposition of a chemical helps to elucidate issues of target site dose delivery. The study of the biotransformation of the chemicals will determine if a putative common toxic metabolite or its precursor are produced.

As will be discussed below, the candidate dithiocarbamates have many metabolic similarities, as well as some differences.

1. Absorption

Absorption of these chemicals after oral dosing is moderate. Measurement of excretion of radioactivity in urine (an approximate measure of absorption) for ^{14}C-(C=S)-labeled thiram, amounted to 18-24% of the dose; for ^{14}C-(methyl)-labeled thiram urinary excretion amounted to 43% of the dose.

Several observation suggest that the dithiocarbamates, may undergo extensive breakdown in the gut prior to absorption.

i. Brocker and Schlatter (1979) reported that when ^{54}Mn-labeled maneb is administered orally to rats, no manganese complex was absorbed from the GI tract. On the other hand, when ^{14}C-labeled maneb was administered orally, about 50% of the radioactivity appeared in the urine and 1% in the expired air.

ii. Izmirnova and Marinov, (1972, cited in Hayes, 1982) reported that, following administration of 35S-Ziram, 5 chloroform-soluble metabolites were found in the gastric contents, suggesting that a part of the breakdown of ziram takes place in the gut.

The plausibility of these observations is supported by the high rate of decomposition of dialkyldithiocarbamates in acidic solution. Aspila et al. (1969) measured the first order rate constants for the dissociation of dialkyl dithiocarbamate into CS_2 and dialkylamine in aqueous acidic solutions. Their findings translate into half-lives of 15.5 and 7.3 seconds for dimethyl- and diethyl-dithiocarbamate, respectively, at pH 2.2 and to half-lives of 36 and 13.9 seconds for dimethyl- and diethyl-dithiocarbamate, respectively, at pH 3.4. These values are consistent with some degree of breakdown at the pH of the stomach, followed by absorption of some or all of the breakdown products.

2. Biotransformation

Dithiocarbamates undergo extensive biotransformation in rats. Little or no untransformed parent is reported. As summarized below numerous metabolites have been detected.

As expected from the SAR considerations, the dithiocarbamates undergo cleavage with generation of CS_2 and further metabolism of the carbon moieties.

a. Generation of CS_2

One feature in common to all dithiocarbamates, used in their analytical chemistry, is the decomposition of these compounds by hot mineral acid to the respective amine and carbon disulfide (Thorn and Ludwig, 1962). There is experimental data (Table 2) indicating that some of the dithiocarbamates decompose *in vivo* into CS_2 after oral dosing. For others this decomposition will be inferred. Various authors, discussed below, have studied the effect of milder acidic conditions on the decomposition of the dithiocarbamates, and their results provide evidence that the dithiocarbamates, may undergo extensive breakdown with release of CS_2 at the acidic conditions existent in the stomach of the rat [pH 3.8-5] following oral ingestion.

i. Case of the DMDTCs and MMDTCs

In the case of the 4 DMDTCs in Table 1, there is actual data indicating formation of CS_2 in rats after oral administration of 3 of them (ziram, ferbam and thiram). Examination of Table 2 indicates that $^{14}C(C=S)$-labeled thiram and ziram and $^{35}S(C=S)$-labeled ferbam produced radioactivity in expired air recoverable at the CS_2 trap. Additionally, this conclusion for thiram is supported by the finding (Table 2) that disulfiram (Figure 5), an ethyl analog of thiram, produces CS_2 *in vivo* in rats.

Concerning the fourth DMDTC in Table 1, sodium DMDTC , Lopatecki and Newton (1952) studied the acid-dependant decomposition of sodium diethyldithiocarbamate (sodium DEDTC, an analog of the subject chemical sodium DMDTC) at pH 5.0. The release of CS_2 was confirmed by the solubility properties of the gaseous product and Reith's test. Based on the limited difference betwen the methyl and ethyl groups, one may conclude that the subject chemical, sodium DMDTC, will also release CS_2 under acid conditions. This conclusion is supported by the observation that other members of the group (ziram and ferbam), in spite of being chelated species (i.e. they release the ligand slowly), are able to release CS_2 *in vivo*. The non-chelated sodium species would be expected to release its CS_2 even faster.

Concerning the relative rates and extent of release of CS_2 by the DMDTCs, Lopatecki and Newton (1952) studied the acid-dependant decomposition of the subject chemicals ziram and ferbam and of sodium diethyldithiocarbamate (sodium DEDTC, an analog of the subject chemical sodium DMDTC) at pH 5.0. In the case of sodium DEDTC, incubation in phosphate buffer resulted in release of CS_2 with suggestion of a plateau by 35 minutes. In the case of the chelates ziram and ferbam, although CS_2 was released by both compounds, the rate of release decreased in the order sodium DEDTC > ziram > ferbam. Thus although the DMDTCs , will release its CS_2 the rate of this release, based on pH considerations only, will be compound dependent. There is actual data showing the formation of CS_2 metam sodium. Twenty percent of an oral dose of metam sodium was metabolized to CS_2 when administered to rats.

ii. Case of the EBDCs

In the case of the 3 subject EBDCs in Table 1 (mancozeb, maneb and metiram), no data on the *in vivo* release of CS_2 were found. The following data are used to infer that CS_2 is likely to be formed in vivo from these chemicals.

Lopatecki and Newton (1952) studied the acid-dependant decompositon of sodium EBDC, (Nabam) the parent compound of the 3 EBDCs depicted in Table 1. These authors observed that nabam decomposed at pH 5.0 into approximately equimolar amounts of CS_2 and H_2S. The presence of CS_2 was confirmed by the solubility properties of the gaseous product and Reith's test. The rate of this decomposition is expected to increase with decreasing gastric pH, based on the results of Miller and Latimer (1962) that the rate of decomposition of nabam increases with decreasing pH in aqueous media.

In the absence of experimental data, one may extend qualitatively the results obtained with nabam to the three subject EBDCs (mancozeb, maneb and metiram) to conclude that it is plausible that CS_2 may be produced after dietary administration of any of the three subject EBDCs to rats. This conclusion is based on:

- The three subject EBDCs have the same EBDC ligand as sodium EBDC (nabam), they differ only in the kind of ion attached to the ligand (Table 1)

- The three subject EBDCs have limited but finite solubilities in water, with the EBDC moiety in equilibrium with the respective cation (Thorn and Ludwig, 1962). Thus, the EBDC moiety is free to interact with the acidic medium and generate CS_2.

Two sets of observations support the above extrapolation:

- The findings of Truhaut et al. (1973) using the related EBDC compound, zineb (zinc EBDC). This author administered zineb to rats by gavage and identified CS_2 in the expired air. This observation confirms that indeed at least one EBDC can generate CS_2 *in vivo*. Having observed that zineb releases CS_2 in vivo, one may postulate that mancozeb and metiram will do so to some extent, because both compounds consist, in part, of zinc complexed with the EBDC moiety (Table 1).

- Furthermore, work by Brocker and Schlatter (1979) with maneb, supports the idea that this compound is not absorbed as a complex. These authors reported that when ^{54}Mn-labeled maneb is administered orally to rats, no manganese-labeled material was absorbed from the GI tract (as measured by the absence of radioactivity in urine, blood, or tissues . On the other hand, when ^{14}C-labeled maneb was administered orally, about 50% of the radioactivity appeared in the urine and 1% in the expired air. One may conclude that if maneb does not persist as a complex in the stomach, some of

the released EBDC anion, will undergo cleavage into CS_2 under the acidic conditions of the stomach, as observed *in vitro* for the sodium salt, nabam,

- Johnson et al. (1996), measured the excretion of 2-thiothiazolidine-4-carboxylic acid (TTCA, a CS_2 metabolite and biomarker of exposure to CS_2) to study the bioavailablity of the CS_2 released from the dithiocarbamates. These authors dosed rats orally with CS_2, disulfiram, N,N-diethyldithiocarbamate, and N-methyldithiocarbamate and measured the urinary levels TTCA excreted after dosing. These authors concluded that the CS_2 generated *in vivo* by these dithiocarbamates had, on the average, comparable bioavailability as an equimolar dose of the pure CS_2 administered orally to the rats.

b. Metabolic Pathways

Figure 1 depicts the biotransformation of the dimethyldithiocarbamates, as exemplified by Thiram,

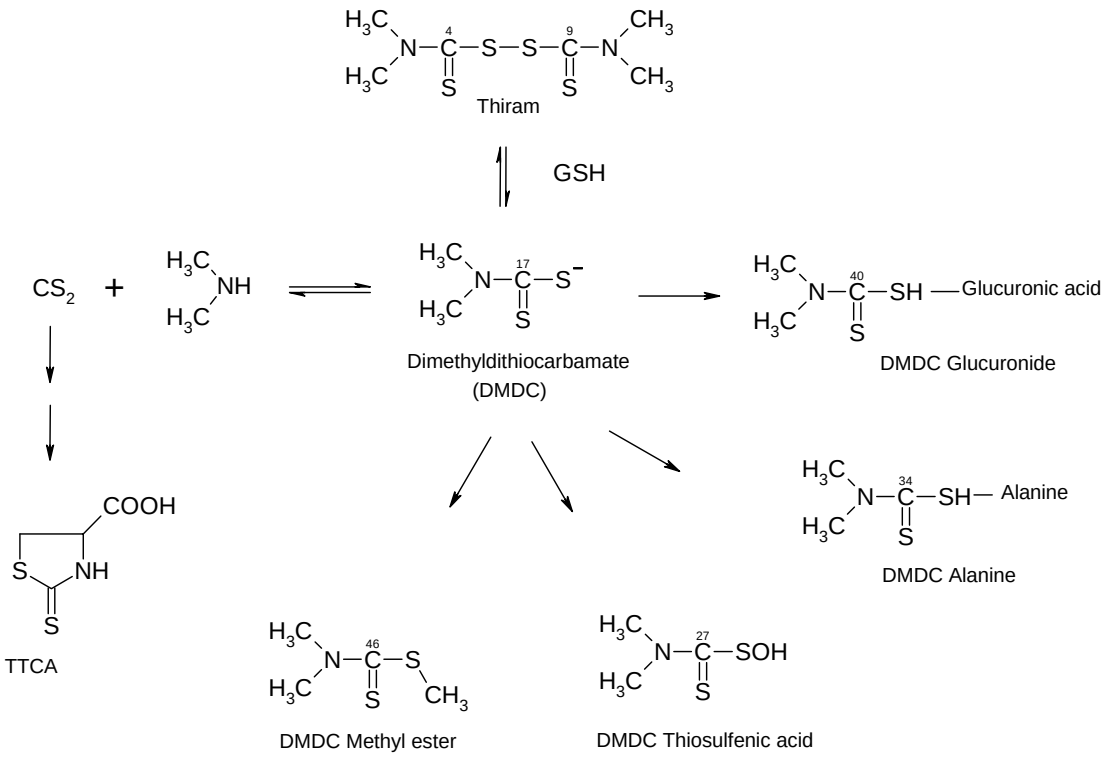


Figure 1. Biotransformation of Thiram [Adapted from Johnson et al. (1996) and MRID 42235701]

As exemplified by Mancozeb, Figure 2, depicts the biotransformation of the ethylenebis dithiocarbamate (EBDCs).

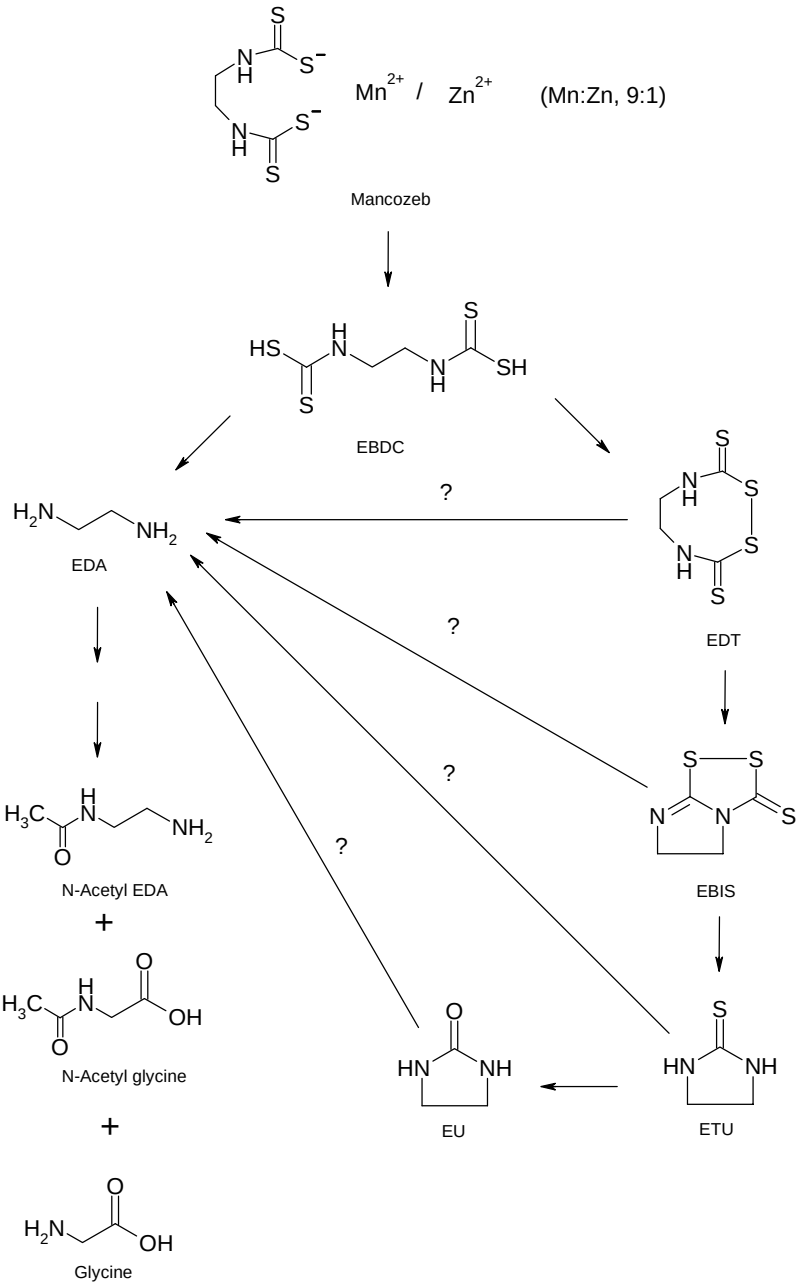


Figure 2.

Biotransformation of Mancozeb. Adapted from Accession Nos. 262834 and 262835. EBDC: Ethylenebisdithiocarbamic acid; EDA: Ethylenediamine; ETU: Ethylenethiourea; EU: Ethyleneurea. Conversion of Mancozeb to EBDC and then to EDA or EDT are known chemical, nonenzymatic conversions. For reactions with a " ? ", it is not known to what extent do these reactions take place.

c. Metabolite Excretion

Metabolism data are available for six of the dithiocarbamate pesticides reviewed in this document. The metabolic profiles for these six follow.

i. **Thiram** (see Figure 1)

14C-labeled (at the C=S bond) thiram was administered to SD rats of both sexes at 1.9 mg/kg as a single oral gavage dose. Recovery in urine amounted to 18.8-24.2% and in tissues to 3.6-4.2% at 7 days. In male rats dosed with 2.1-2.5 mg/kg, recovery in expired air amounted to an average 61.3% of the administered dose. In a separate bioavailability study, male Crl:CDBR rats were administered 30 ppm 14Cthiram in the diet for 1 hour. After 1 hour of feeding and at 72 hours post dosing, radioactivity in urine, feces, expired air [CO2 and CS2], and carcass contained 41, 38, 20 and 6% of the administered dose, respectively.

Twenty four-hour urine samples were collected and analyzed for metabolites. Metabolites in urine amounted to (as % of radioactivity in urine): **[MRID 42235701**].

	Male	Female
2-thio-oxothiazolidine-4-carboxylic acid (TTCA) 33.6	[8.1][1] 30.2 [5.7]	
DMDC-glucuronide	3.2 [0.8]	4.2 [0.8]
DMDC-thiosulfenic acid	0.92 [0.2]	9.08 [1.7]
DMDC-methyl ester	ND	ND[2]
DMDC-alanine	34.81 [8.4]	42.07 [7.9]

[1] Values in square brackets are % of dose; other numbers are % of radioactivity in urine.
[2] Detected at 125 mg/kg at 2.4-3.4% of the dose.

ii. Ferbam

Hodgson et al. (1975) studied the metabolism of Ferbam using 35S-labeled and 14C-labeled (at the methyl carbon) compound. Charles River female rats were given a single 500 mg/kg oral gavage dose of 35S or 14C ferric ferbam. Biliary excretion, blood distribution, placental transfer and milk secretion were studied. In rats dosed with 35S, at 24 hours post dosing, radioactivity in feces, urine, expired air, whole blood and bile amounted to 16.9, 22.7, 18.1, 0.6 & 1 % of the applied dose, respectively. In rats dosed with 14C, at 24 hours post dosing , radioactivity in the same matrices amounted to 20.0, 42.9, 0.6, 0.9 & 1.4% of the applied dose, respectively. The total of whole blood, liver, kidneys, muscle and brain amounted to about 3.8% of the dose.

Analysis of the expired air indicated that CS_2 represented more than 99.9% of the sulfur containing metabolites in the expired air. A trace of COS was also detected. Analysis of urine indicated three ferbam metabolites: inorganic sulfate, dimethylammonium ion (dimethyl amine) and a glucuronide of DMDC. The authors concluded that after absorption, some of the CS_2 is eliminated in the expired air, while the remainder is oxidized to inorganic sulfate. They also speculated that some of the N,N-dimethyldithiocarbamate is absorbed intact and then conjugated with glucuronic acid. The authors speculated that the conversion of ferbam to N,N-dimethyldithiocarbamate followed by decomposition to diethylamide and CS_2 probably takes place in the gut, since ferbam is known to decompose to CS_2 and diethylamide under acidic conditions.

iii. Ziram

14C-labeled (at the C=S bond) ziram was administered to SD rats of both sexes at 15 mg/kg as a single oral gavage dose. Recovery in urine amounted to 26.3-25.5% and in tissues to 0.8-0.9% at 7 days. Expired air was trapped as CO_2 (ethanolamine:ethoxyethanol) and in activated carbon. Radioactivity as CO_2 amounted to 21.7 - 20.7% of the dose, and volatiles (presumably CS_2 amounted to 15.6-15.7% of the dose over a 5-day collection period. In a separate study by Izmirnova and Marinov, (1972, cited in Hayes, 1982), following administration 35S-Ziram, 5 chloroform-soluble metabolites were found in the gastric contents, suggesting that a part of the breakdown of ziram takes place in the gut **[MRID 42391001]**.

iv. Metiram

Groups of 5 male and 5 female rats were given single oral doses of 14C-metiram (ethylene label) 5 or 50 mg/kg for the main study. Additionally three male rats were administered single oral doses of 14C-ETU at 0.5 mg/kg for ETU metabolism. Additionally 3 rats/sex were dosed with 14C-metiram at 5 or 50 mg/kg for bile duct excretion studies. For the main study, at the low dose, males excreted 32.6 and 63.5% of the dose in urine and feces, respectively, over 48 hours. Females excreted 42.8 and 52.7% of the dose in urine and feces, respectively, over 48 hours. About 14.3 and 7.1% of the dose for males and females, respectively, was excreted in bile, in bile cannulated rats over 48 hours. About 20% of the urinary radioactivity [about 6.5% of the dose in males) was identified as N-Acetyl-ETD plus ETD and 20-30% of the urinary radioactivity [about 6.5-9.8% of the dose in males] had similar behavior as glycine, a known metabolite of ETD. Thus, about 13-16% of the dose accounts for ETD and its metabolites. ETU and EU constituted about 18% and 5-10%, respectively, of the urinary radioactivity (about 5.8 and 1.6-3.3%, respectively, of the dose) . Assuming that metiram does produce CS_2, based on about a 20% conversion of ETU to ETD and metabolites (i.e about 2% of the dose), one may estimate that 11-14% of the dose of ETD and its metabolites results from degradation of EBDC by loss of CS_2 **[Accession No. 259892]**

v. Maneb

Groups of rats were administered a single oral dose of 250 mg/kg14C-maneb by gavage. The major metabolite in urine was ETU. CS_2 formation was not studied. **[Accession No.263913]**

vi. Mancozeb (see Figure 2)

17 male and 17 female SD rats were administered single oral doses of [14C-ethylene]mancozeb by gavage at 1.5 mg/kg. Recoveries of radioactivity in urine and feces amounted to 48.9 and 55.3 % of the dose, respectively, in males and 49.5 and 46.6% of the dose, respectively, in females. About 6.3-8.8% of the dose was excreted in bile. Thus, most of the material in feces is unabsorbed material. Based on 48.9% of the dose being excreted in urine in males , metabolites in urine comprised the following percentages of the dose: EBIS (0.7%), ETU (18.0%), EU (4.0%), EDA (3.8%), N-AcEDA (3.8%), N-ForEDA plus N-AcGly (tentatively identified, 4.8%), Glycine (tentatively identified, 2.2%). Thus, about 14.6% of the dose is accounted for EDA and its postulated metabolites.

Assuming that indeed mancozeb generates CS_2 and that the generation of EDA from ETU and EU is very limited , at least 10% of the dose of mancozeb is being converted to CS_2 **[Accession Nos. 262834 and 262835]**.

IV. Mechanisms of the Common Toxic Effects

This section discusses some potential mechanistic aspects for the common toxic endpoint by which the dithiocarbamates might be grouped at this time. In particular, this section addresses several issues:

- The role of CS_2 in producing distal, peripheral axonopathies
- The *in vivo* generation of CS_2 by dithiocarbamate
- Molecular events associated with CS_2- and dithiocarbamate- induced distal peripheral axonopathies.
- Are these molecular events, sufficiently similar between administered CS_2 and dithiocarbamate to suggest that CS_2 mediates the distal peripheral neuropathies produced by the dithiocarbamate?

A. The role of CS_2 in producing distal, peripheral axonopathies

As noted by Bus (1985), although CS_2 produces toxicity in a variety of organs in animals and humans, toxicity to the central and peripheral nervous systems is the major toxic endpoint associated with CS_2.

The production of peripheral neuropathy in laboratory animals exposed repeatedly to CS_2 is very well documented. As described by Anthony et al. (1996), experimental animals exposed repeatedly to CS_2 become progressively weak, beginning at the hindlimbs; and may experience weakness in more proximal muscle groups with continued exposure. In exposed humans, there is an initial stocking-and-glove distribution of sensory loss, that progresses to involve more proximal sensory and motor axons.

Numerous reports (summarized in GDCh, 1991) describe clinical signs and histopathology of the spinal cord and peripheral long nerves in various species following inhalation of CS_2. Gottfried et al (1985) reported swelling and degeneration of axons of spinal cord and peripheral nerves, increase of neurofilaments, reduction of microtubuli and thinning of the myelin, penetration of Schwann cells into the axoplasma in rats dosed with CS_2 by inhalation at doses of 900-2400 mg/m^3, for 90 days. Wronska-Nofer et al. (1973) describe muscle atrophy and paresis of the hind legs in rats dosed with CS_2 by inhalation at 1500 mg/m^3 for 5-14 months. Jirmanova and Lukas (1984) observed swelling of giant axons, degeneration of nerves, intramuscular nerve endings and muscles; thinning of myelin sheats (vacuolization, demyelination) in rats exposed to CS_2 bay inhalation for 6 months.

More recently, Anthony et al. (1996) have noted that the distal axonopathy produced by CS_2 is identical pathologically to that caused by hexane. The cellular changes involve the development of neurofilament aggregates in the distal subterminal axon, which produce massive swellings of the axon, often just proximal to the node of Ranvier. The neurofilament-filled axonal swellings results in marked distortion of the nodes, including retraction of paranodal myelin. These processes of neurofilament accumulation and degeneration of the axon are followed by the development of the clinical peripheral neuropathy.

B. The *in vivo* generation of CS_2 by dithiocarbamates

There is data (Table 2) that shows *in vivo* release of CS_2 by 3 (ziram, ferbam, and thiram) of the 4 DMTCs and the one MMDTC (metam sodium) in Table 1 . Although no data have been found showing *in vivo* release of CS_2 by the 3 EBDCs, there is data showing *in vivo* release of CS_2 by the related compound zineb (zinc EBDC). Because 2 of the EBDCs (metiram and mancozeb, Table 1) consist in part of zinc EBDC, some *in vivo* release of CS_2 may be expected from these compounds. For the remaining compounds in Table 1, arguments are presented in Section **III.C.2.a**. to show that some *in vivo* release of CS_2 may also be expected from them, allowing us to postulate that *in vivo* release of CS_2, has been seen or is expected for all dithiocarbamates subject of this document. In fact, Hayes (1982) has noted in a review, that it appears that CS_2 and its metabolites are the only compounds in common to the metabolism of all dithiocarbamate fungicides.

Furthermore, several authors have observed that this dithiocarbamate-generated CS_2 is very similar in its behavior to that of CS_2 administered as the pure compound:

- Johnson et al. (1996) dosed rats orally with CS_2, disulfiram, N,N-diethyldithiocarbamate, and N-methyldithiocarbamate and measured the urinary levels TTCA (a biomarker of exposure to CS_2) excreted after dosing. These authors concluded that the CS_2 generated *in vivo* by these dithiocarbamates had, on the average, comparative bioavailability as an equimolar dose of the pure CS_2 administered orally to the rats.

- Johnson et al. (1998) reported that electrophoretic comparison of crosslinked neurofilament protein preparations prepared from diethyldithiocarbamate (DEDC) or CS_2 - treated rats showed them to be identical (i.e. identical electrophoretic patterns were obtained).

C. Molecular events associated with CS_2- induced distal peripheral axonopathies

Several possible mechanisms have been presented in the past to explain the pathogenesis of CS_2-induced neurofilamentous axonopathies. These have included metal ion chelation and induction of Vitamin B6 deficiencies (Bus, 1985).

More recently, Graham et al. (1995) have advanced the idea that CS_2 exerts its effect in the long axons by crosslinking axonal proteins (Figure 3). These authors postulate that CS_2 reacts with amino groups in protein chains, to yield adducts that then undergo transformation to an electrophile (e.g. an isothiocyanate, Figure 4) that in turn reacts with protein nucleophiles (in a different protein) to produce crosslinked proteins . These authors further postulate that progressive cross-linking of the stable neurofilament during its anterograde transport in the long axons ultimately results in the accumulation of neurofilaments, that result in axonal swellings. Reaction with additional targets appears to be responsible for the degeneration of the axon distal to the swellings.

Valentine et al. (1997) studied the dose response and time course for crosslinking of neurofilaments and axonal degeneration in spinal nerves of rats dosed with CS_2 . Fischer 344 rats were dosed with CS_2 by inhalation at levels of 0, 50, 500, or 800 ppm for 2, 4, 8 and 13 weeks. Analysis of neurofilament proteins indicated crosslinking increased in dose-related fashion, increased with time and preceded the axonal damage. At 800 ppm crosslinking was significantly higher than in controls at 2 weeks after the initiation of treatment, and reached an approximate plateau at 8 weeks. At 500 ppm a plateau was also reached at 8 weeks. At 50 ppm, the degree of crosslinking was not significantly different from controls. No axonal swellings were observed in spinal cords to 50 ppm. Swellings were not detected in animals exposed at 500 or 800 ppm at 2 or 4 weeks. Thereafter, axonal swellings at the two higher doses increased with dose and time of exposure. It is not known how cross-linking might contribute to the observed structural changes.

Whether the cross-linking of axonal neurofilament proteins is a key event in the pathogenesis of CS_2-induced neurofilamentous axonopathies, is subject of debate at present (LoPachin et al., 2000; LoPachin , 2000). Thus, at present, there is no specific mechanism to explain the pathogenesis of CS_2-induced neurofilamentous axonopathies.

D. Molecular events associated with dithiocarbamate-induced distal peripheral axonopathies

Johnson et al. (1998) studied the dose response and time course for crosslinking of neurofilaments and axonal degeneration in spinal nerves of rats dosed orally with diethyldithiocarbamate (DEDC). As depicted in Figure 5, DEDC is the ethyl -analog of the DMDTC's. Furthermore, DEDTC is a metabolite of Disulfiram (DS) which is known to generate CS_2 in vivo and to produce peripheral neuropathies (Eneanya, 1981).

In the Johnson et al. (1998) studies DEDC was administered by gavage at 3 mmol/kg to SD rats once every other day for 8 or 16 weeks. Analysis of axonal proteins indicated that the level of crosslinked neurofilaments in spinal cord extracts was significantly higher in treated rats than in controls. Furthermore, the degree of crosslinked light neurofilaments increased with statistical significance between 8 and 16 weeks. Levels of crosslinked medium neurofilaments, although increased between 8 and 16 weeks of treatment were not statistically significantly differently between each other. Morphological examination of nerve tissue revealed no significant changes at 8 weeks of dosing. At 16 weeks, however, a significant increase in the number of swollen axons was observed in the posterior tibial nerve. As expected, if CS_2 is the crosslinking agent, electrophoretic comparison of crosslinked neurofilament protein preparations prepared from DEDC- or CS_2 -treated rats showed them to be identical (i.e. identical electrophoretic patterns were obtained). Although these experiments do not prove that neurofilament crosslinking is the key step in the pathogenesis of the disulfiram-induced distal axonopathies, they clearly indicate, that a dithiocarbamate and CS_2 leave similar footprints in their interaction with the axon. This evidence supports the idea that dithocarbamates act via CS_2 in the pathogenesis of distant peripheral neuropathies. However, a more recent report provides evidence that disulfiram neuropathy is not mediated through the formation of CS_2 (Tonkin, et al., 2000). These authors showed that disulfiram neuropathy lacks both the morphological changes and intermolecular cross-linking characteristics of CS_2 and appears to be a Schwann cell toxicant. Thus, these results show that the neuropathy produced by the dithiocarbamates can not be assumed to be associated with the formation of the formation of the common metabolite, CS_2. In addition, the findings of Tonkin et al. show that different mechanisms of toxicity are operative among the dithiocarbamates because at least one dithiocarbamate, diethyldithiocarbamate, has been shown to produce neuropathy via the formation of CS_2 (Johnson et al., 1998).

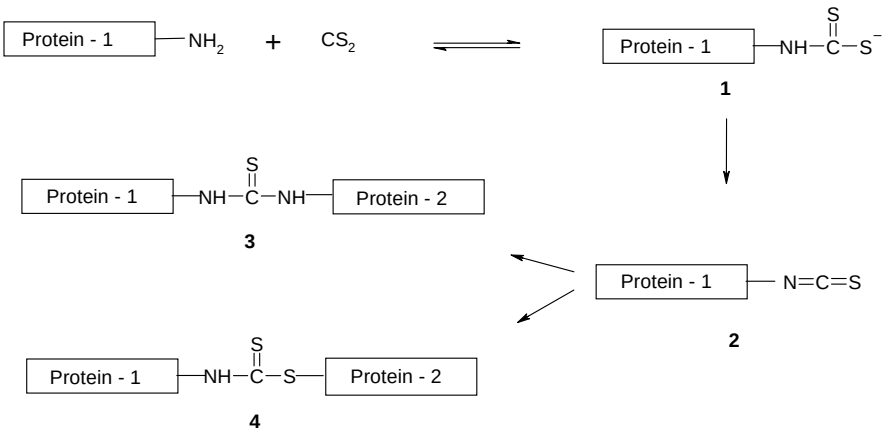


Figure 3. Proposed reaction sequence in CS_2 mediated cross-linking of proteins. Lysyl amino groups of proteins (e.g. protein 1) react with CS_2 to yield the dithiocarbamate 1, part of which is converted yo isothiocyanate 2. Conceptually, nucleophilic attack by amino or thiol groups in another protein (e.g. protein 2) may take place yielding the thiourea 3 or the N,S-dialkyl dithiocarbamate ester 4. Adapted from Amarnath et al. (1990).

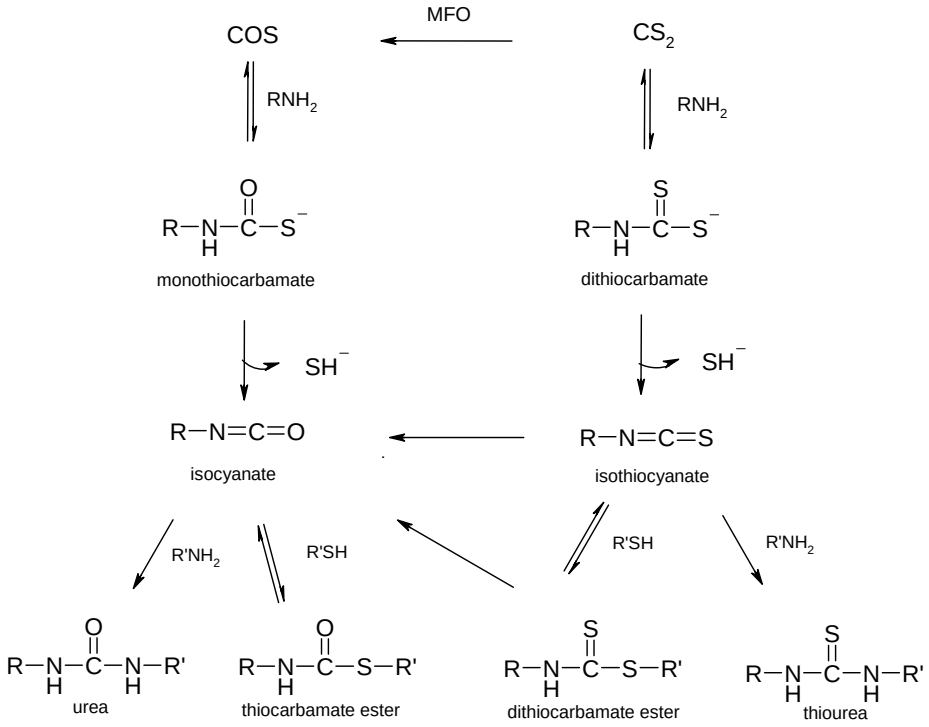


Figure 4. Cross linking reactions resulting from CS_2 exposure. RNH_2 and R'NH_2 are different protein backbones being crosslinked. Likewise, RNH_2 and R'SH are different protein backbones being crosslinked. In this diagram, crosslinking may occur via an isothiocyanate originated from CS_2 or via an isocyanate originated from COS. (Adapted from Graham et al. 1995).

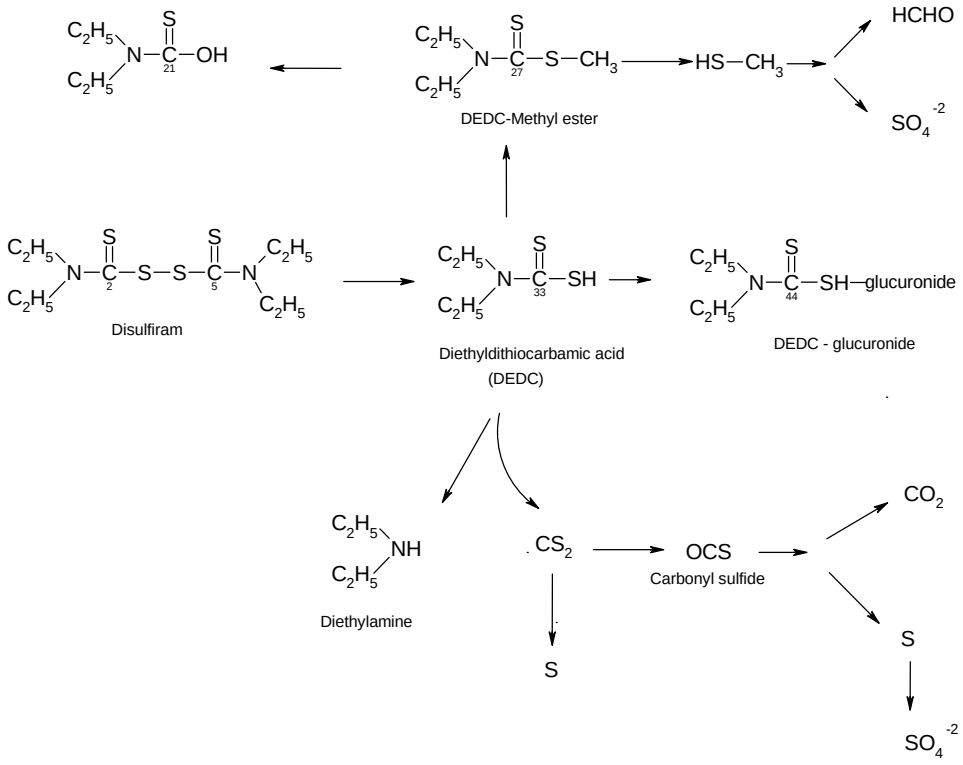


Figure 5. Metabolic fate of disulfiram. Adapted from Eneanya et al. (1981).

V. Grouping of the Dithiocarbamate Pesticides Based on the Potential to Induce a Common Effect

Table 4 summarizes the neuropathogical, thyroid, developmental (CNS), effects and effects on cholinesterase activity shown to be induced following treatment of rats and mice with the dithiocarbamate that have food tolerances.

Table 4. Summary of effects of treatment of rats or mice with dithiocarbamate

Chemical	Neuropathology	Thyroid	CNS - developmental	Cholinesterase inhibition
mancozeb	+	+	+	not measured
maneb	+	+	+	-
metiram	+	-	no study	not measured
Na-dimethyldithio-carbamate	-	-	-	-
ziram	+	+	-	+
thiram	+	±	+	not measured
ferbam	-	+	+	no study
metam sodium	+	-	-	+

Evaluation of the toxicities induced by the dithiocarbamate pesticides, as a group, does not support the use of a neuropathology, CNS developmental effects, or ChEI as common effects induced by a common mechanism of toxicity. The data do indicate, however, that a common mechanism of toxicity, thyroid toxicity and cholinesterase inhibition, exists for some chemicals. The reasons for these conclusions are discussed below.

- Direct evidence of neuropathology (lesions of brain, spinal cord, or peripheral neurons) exists for mancozeb, maneb, metiram, ziram, thiram, and metam sodium. However, a consistent pattern of neuropathology has not been shown among these pesticides. Some induce axonal lesions, others primary demyelination or neuronal lesions. Furthermore, results of a recent study conducted using the related compound disulfiram show that the axonal lesions induced by this dithiocarbamate are different than the lesions produced by diethyldithiocarbamate, which provides evidence that there is not an underlying common effect that is induced by the dithiocarbamates. Ferbam or Na-dimethyldithiocarbamate do not induce neuropathies even though these pesticides have been shown to produce carbon disulfide *in vivo*.

- Mancozeb, maneb, ziram and ferbam induce thyroid effects in rats. Although studies conducted with metiram have not shown effects on the thyroid, this dithiocarbamate can also be metabolized to ethylene thiourea (ETU), the metabolite associated with effects of the dithiocarbamates on thyroid tissue. However, because ziram and ferbam are not metabolized to ETU, these dithiocarbamate should not be grouped with the EBDCs based on a common mechanism of toxicity.

- Results of studies submitted to OPP or reported in the literature show that treatment of rats with mancozeb, maneb, thiram and ferbam does not result in the production of a common CNS effect. Effects on the brain reported are variable and include atrophy, dilated ventricles, microphthalmia or hydrocephalus. A common mechanism has also not been identified for CNS effects. Two dithiocarbamates, maneb and Na-dimethyldithiocarbamate do not appear to inhibit cholinesterase but ziram and metam sodium share the common mechanism of cholinesterase inhibition. Thus, the data available do not support cholinesterase inhibition as being a common effect of the entire group of dithiocarbamates.

VI. Grouping of Dithiocarbamate Pesticides Based on a Common Mechanism of Toxicity for Neuropathology

Results of the current hazard assessment show that ziram and thiram are converted to carbon disulfide and that mancozeb, maneb, and metiram potentially may be converted to carbon disulfide. Ferbam is metabolized to carbon disulfide also, but available data do not show that treatment of rats with ferbam induces neuropathies. Thus, only five dithiocarbamates can be considered to be metabolized to a common metabolite and have the potential to induce distal peripheral neuropathy.

Evidence has been presented suggesting that the dithiocarbamates may produce the axonopathy through their common metabolite, carbon disulfide but other evidence is available that does not support the metabolism to CS_2 as being the common, causative moiety that mediates the production of neuropathy. The reasons that formation of CS_2 can not be identified as a common mechanism of toxicity follow.

- Treatment of rats with N,N-diethyldithiocarbamate (DEDC), and analogue of dimethyldithiocarbamate, was shown to produce axonal effects consistent with the changes produced by CS_2 (Johnson et al., 1998). However, a recent investigation showed that CS_2 does not mediate the neuropathy induced by the dithiocarbamate, disulfiram (Tonkin, et al., 2000). Thus, the group of pesticidal dithiocarbamates can not be assumed to share a common mechanism of toxicity based on metabolism to the metabolite, CS_2.

- Although sodium dimethyldithiocarbamate and ferbam are metabolized to CS_2, these two pesticides have not been reported to induce neuropathy.

- There are no in vivo data that show that the EBDC's are metabolized to CS_2.

- The neuropathic effects induced by the dithiocarbamate group of pesticides (e.g. axonal degeneration, demyelination) are not consistent with the neuropathic effects induced by CS_2 (axonal swelling, disorganized masses of neurofilaments). Concordance in site specificity among the dithiocarbamate would strengthen support for assuming the neuropathic effects are attributable to a common mechanism

VII. Grouping Scenario of Dithiocarbamate Pesticides Based on Common Mechanism of Action

The weight-of-evidence supports grouping of **Mancozeb, Maneb, and Metiram,** by a common mechanism of toxicity for thyroid effects. Thyroid toxicity induced by the ethylenebisdithiocarbamates has been attibuted to the metabolism of these chemicals to ETU. Although the dimethyldithiocarbamates ziram and ferbam, and perhaps thiram, also produce thyroid effects in the rat, these chemicals are not metabolized to ETU and the mechanism by which the they induce thyroid effects has not been established. Thus, OPP concludes that there is sufficient evidence to support grouping only EBDCs mancozeb, maneb, and metiram based on a common mechanism for the induction of thyroid effects. The weight of evidence does not support grouping the dithiocarbamates by a common mechanism of toxicity for neuropathy because: a) the pattern of neuropathology that is produced by different dithiocarbamates is not consistent; b) although the neuropathy produced by diethyldithiocarbamate has been linked with the formation of CS_2, the neuropathy produced by disulfiram is not mediated by the formation of CS_2; c) there are no data showing that the EBDC's are metabolized to CS_2 in vivo; and d) Na-DMDC and ferbam are metabolized to CS_2 but these dithiocarbamates do not produce neuropathy. Ziram and metam sodium inhibit cholinesterase, an effect that the agency has previously established as a common mechanism of toxicity (USEPA, 2000).

VIII. Summary

As stated in the introduction to this review, the intent of this document is to provide a scientific basis for determining if the carbamates may be subgrouped based on the characteristic of some to produce effects other than cholinesterase inhibition. The data reviewed show that neuropathology and thyroid toxicity are common effects of the dithiocarbamate pesticides. Some, if not all, dithiocarbamates may be metabolized to carbon disulfide but the lack of concordance between the morphological profiles of the neuronal lesions produced by these pesticides and the neuropathy produced by carbon disulfide indicates carbon disulfide is not the sole toxicant that mediates the neuropathology. Additionally, Na-DMDC and ferbam are metabolized to carbon disulfide but do not produce neuropathy. This evidence refutes the hypothesis that the neuropathy produced by the group of pesticidal dithiocarbamates can be linked with the formation of CS_2. The dithiocarbamates may also be metabolized to sulfoxides, isothiocyanate, and COS but data are not sufficient to evaluate their relative roles in inducing neuropathy. Thus, at this time the dithiocarbamates cannot be grouped based on a common mechanism of toxicity for neuropathy. Thyroid effects attributable to a common metabolite (ETU) have been established for the EBDCs but not for the DMDTCs or metam sodium. The EBDC's should be continued to be grouped for cumulative risk assessments, as in the past, based on the formation of a common thyroid toxicant, ETU. Finally, there are data showing that ziram and metam sodium share a common mechanism of toxicity, the inhibition of cholinesterase.

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