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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

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THRU: Pauline Wagner Pauline Wagner 3/17/94
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SUBJECT: Carcinogenicity Peer Review Document on PHOSMET

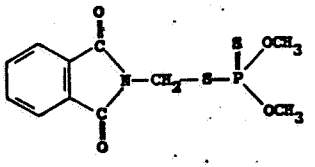
The PHOSMET draft document has two significant problems: (i) the chemical structure is not totally accurate, and (ii) some important information on structure activity relationship (SAR) analysis has not been included (see attachment). They should be corrected before finalizing the document.

Attachments

cc: K. Dearfield
P. Wagner

The 3/7/94 draft document on PHOSMET has two significant shortcomings that should be corrected.

(1) The chemical structure of PHOSMET shown in the draft document is not totally accurate. It appears to have been taken out of the Farm Chemicals Handbook which is known to contain some poorly, and sometimes incorrectly, drawn chemical structures. To most chemists, the chemical structure, as drawn in the draft document, indicates a six-membered ring fused to a seven-membered ring (visually, it looks like two six-membered rings) whereas the phthalimide ring should really be a six-membered ring fused to a five-membered ring. This is particularly awkward on p.13 which is supposed to show that PHOSMET and FOLPET have a common phthalimide ring. You should probably use the structure in Merck Index (see below) if you do not want to redraw the structure.



(2) The structure-activity relationships section made structural analogy between PHOSMET and FOLPET because of the common phthalimide ring and then correctly pointed out that the analogy is more or less irrelevant because the carcinogenic activity of FOLPET can be contributed to its $-SCl_3$ sidechain. What is missing is to point out the fact that PHOSMET is **a methyl ester of thiophosphoric acid and is expected to be a methylating agent.** This can readily explain the potent **in vitro mutagenic activity** of the compound. Structural analogy should be made to other organophosphate pesticides (e.g., DIMETHOATE) which have been shown to be carcinogenic/mutagenic.

It is very important to point out that there is a very poor correlation between animal carcinogenicity and in vitro mutagenicity of organophosphate pesticides. This can be attributed to the fact that organophosphate pesticides are readily detoxified (see enclosed excerpt of my 1989 review). Once an organophosphate pesticide loses one of its three ester groups, it is no longer an alkylating agent (e.g., dimethyl phosphate, in contrast to trimethyl phosphate, is not a methylating agent). In the case of PHOSMET, it contains a side chain, $-N-CH_2-S-$, which is known to be readily hydrolyzed in acidic condition to yield formaldehyde and dimethyl thiophosphate. This can probably explain why PHOSMET has very weak carcinogenic activity despite of the fact that it is a potent mutagen under in vitro conditions. Has the submitter provided any information about the metabolism of PHOSMET? This information is crucial in assessing the carcinogenic potential of PHOSMET in whole animal.

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Chapter 11

Role of Structure–Activity Relationship Analysis in Evaluation of Pesticides for Potential Carcinogenicity

Yin-tak Woo and Joseph C. Arcos

Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC 20460

Structure-activity relationship (SAR) analysis is essential for the development of pesticides and for the evaluation of cancer hazard and risk assessment. The critical factors that should be considered in SAR analysis and the profile of typical potent carcinogens are discussed. A scheme combining structural and functional criteria for suspecting chemical compounds of carcinogenic activity is presented. Selected classes of pesticides with carcinogenic potential are reviewed to exemplify structural and/or functional features responsible for their carcinogenic activity.

Structure-activity relationship (SAR) analysis is a critical tool in the research and development of new industrial and agricultural chemicals and is the first line of approach in the cancer hazard evaluation of chemicals. Careful SAR analyses can spot or reveal potential health hazard of new chemicals early in the research and development stage. SAR considerations are also essential for designing and selecting appropriate batteries of tests to study the potential toxicity of chemicals and to elucidate their molecular mechanisms of action.

There are various ways to approach SAR analysis. This chapter focuses on principles and concepts of mechanism-based SAR analysis along with an overview of the structural features and critical factors that should be considered in the evaluation of pesticides for potential carcinogenicity. Most of principles and

(3) The "Guilt-by association" criterion points to the possible carcinogenic potentiality of compounds which, although found inactive under some "standard" conditions of animal bioassay (and/or mutagenicity testing), belong in chemical classes in which several other compounds were found to be potent and multi-target carcinogens, for example the 5-nitrofuran type urinary antibacterials. These compounds should be reevaluated to determine if retesting under more strigent conditions is warranted.

Brief Overview of Structural Features of Selected Classes of Carcinogenic Pesticides

1. Organophosphorus pesticides: For various reasons, organophosphorus pesticides represent a very difficult group for the evaluation of potential carcinogenicity based on SAR analysis. Table II summarizes the results of U.S. National Cancer Institute/National Toxicology Program (NCI/NTP) bioassays of 13 organophosphorus pesticides. Of these, two (tetrachlorvinphos and, by gavage, dichlorvos) gave positive results while four or five yielded equivocal results. Industry data submitted to EPA indicate that at least a number of other organophosphorus pesticides are/may be carcinogenic (22). Overall, virtually all these positive or equivocal organophosphorus pesticides are methyl or ethyl esters of phosphoric or thiophosphoric acids. Most of these pesticides are electrophilic and/or mutagenic but the correlation between carcinogenicity and electrophilicity/mutagenicity seems to be poor.

At least five critical factors affect the potential carcinogenicity of organophosphorus pesticides (see Fig. 4). The alkyl groups (R) are generally believed to be a major contributor to the genotoxicity of organophosphorus compounds although the role of the electrophilic phosphoryl moiety cannot be excluded. In general, the alkylating activity of organophosphorus compounds decreases with an increase of the size of the R group. Alkyl esters of thiophosphoric acids are not as effective alkylators as the phosphates. They can, however, be metabolically converted to phosphates by oxidative desulfuration or by thiono-thiolo rearrangement. The alkylating activity of organophosphorus compounds can be increased by increasing the electron-withdrawing capability of the leaving group. However, the same parameter can also make the phosphoryl moiety more susceptible to alkaline hydrolysis. Once the leaving group departs, the resulting dialkyl phosphate is no longer an alkylating agent.

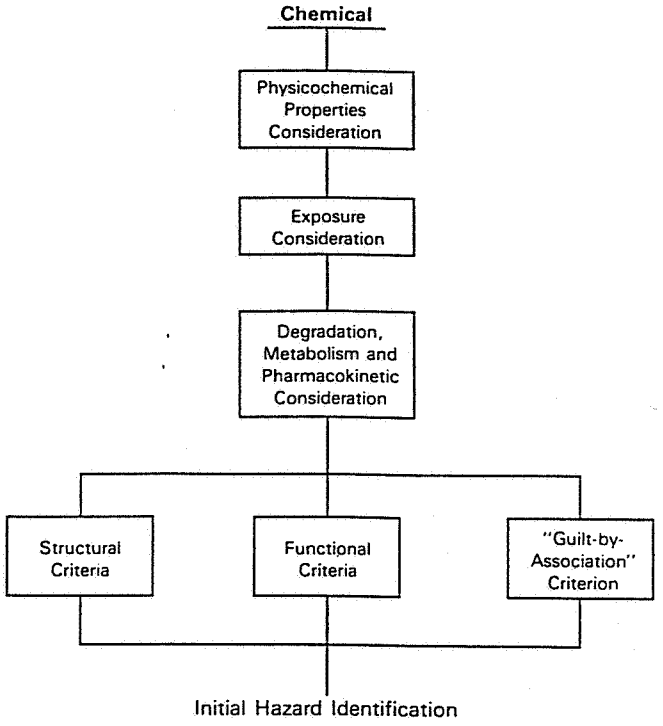
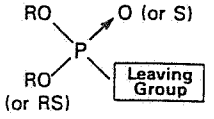


Fig. 3. Scheme for Hazard Identification Based on Structural, Functional and "Guilt-by-association" Criteria.

Organophosphorous Pesticides



Critical Factors:

1. Nature of R group: size, branching, halogenation.
2. Phosphate versus thiophosphate.
3. Electron - withdrawing capability of the leaving group.
4. Detoxification by hydrolysis or -SH compounds.
5. Breakdown products.

Fig. 4. Critical Factors Affecting Carcinogenic Activity of Organophosphorus Pesticides.

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TABLE II

Organophosphorus Pesticides That Have Been Tested by U.S. National Cancer Institute/National Toxicology Program

pesticide	tr	salm	route	mr	fr	mm	fm
AZINPHOSMETHYL	069	+w	FEED	E	N	N	N
COUMAPHOS	096	-	FEED	N	N	N	N
DIAZINON	137	-	FEED	N	N	N	N
DICHLORVOS	010	+	FEED	N	N	N	N
DICHLORVOS	342	+	GAV	SE	EE	SE	CE
DIMETHOATE	004	++	FEED	N	N	N	N
DIOXATHION	125	+	FEED	N	N	N	N
FENTHION	103	-+w	FEED	N	N	E	N
MALAOXON	135	-	FEED	N	N	N	N
MALATHION	024	-	FEED	N	N	N	N
MALATHION	192	-	FEED	N	N		
METHYL PARATHION	157	+	FEED	N	N	N	N
PARATHION	070	+w-	FEED	E	E	N	N
PHOSPHAMIDON	016	+	FEED	E	E	N	N
TETRACHLORVINPHOS	033	-	FEED	N	P	P	P

Code: tr = NCI or NTP technical report numbers; salm = Ames test (+, positive; +w, weakly positive; -, negative; ONT, on test); mr = male rats; fr = female rats; mm = male mice; fm = female mice; evidence for carcinogenicity (CE, clear evidence; P, positive; SE, some evidence; E or EE, equivocal evidence; IS, inadequate study; N or NE, negative).

Detoxification of organophosphorus pesticides before they can reach their target sites is probably the main reason for poor correlation between carcinogenicity and electrophilicity/mutagenicity. The problem is further complicated by the fact that several different enzymes are involved in the metabolic detoxification of organophosphorus pesticides. For example, paraoxon, tetrachlorvinphos and dimethoate are preferentially detoxified by A-esterase (paraoxonase), GSH-dependent S-alkyltransferase and carboxyesterase (aliesterase), respectively whereas chlorfenvinphos is mainly detoxified by NADPH-dependent oxidative dealkylation (23). Furthermore, significant species differences have been observed in the rate of detoxification; for example, the relative rates of dealkylation of chlorfenvinphos by rat, mouse, rabbit and dog livers are 1, 8, 24 and 88, respectively (23). A systematic study of detoxification pattern of organophosphorus pesticides is critically needed before we can obtain more accurate prediction from SAR analysis.

Terminal or vicinal halogenation of the R group can be expected to increase the electrophilic reactivity of the breakdown product(s). Besides the alkyl group, other breakdown products of organophosphorus pesticides may also contribute to carcinogenicity. For example, it has been suggested (24) that the leaving group of tetrachlorvinphos (Fig. 5) can contribute to carcinogenicity by generating the electrophilic intermediate, chloromethyl 2,4,5-trichlorophenyl ketone.

2. Carbamate/thiocarbamate pesticides: Considerably less information on carbamate pesticides is available in the open literature for establishing a clear SAR pattern. However, from studies on urethan and its analogs (3,25), some structural features that favor carcinogenicity/ mutagenicity can be discerned (see Fig. 6). They are: (i) a small alkyl group at the carboxy end. Vinyl carbamate is by far the most potent carbamate carcinogen. Urethan (ethyl carbamate) has been postulated to be metabolically activated by dehydrogenation to vinyl carbamate. However, the demonstration of carcinogenicity of methyl carbamate by NTP (Technical Report No. 328) suggests that dehydrogenation is not an obligatory route for carbamate activation. Two carbamate pesticides (asulam, benomyl) reported to be carcinogenic (22) are N-substituted derivatives of methyl carbamate. (ii) Some fourteen 1,1-diaryl-2-acetylenic carbamates of the structure given in Fig. 6 have been shown to be carcinogenic. The aryl and acetylenic moieties are probably involved in stabilizing the carbonium ion which would arise after departure of the carbamoyloxy moiety. (iii) Replacement of the alkoxy group by chlorine yields a potent carcinogen,

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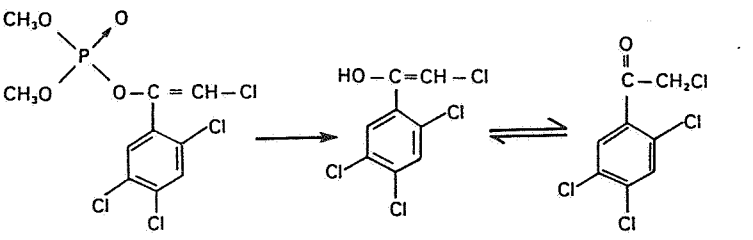
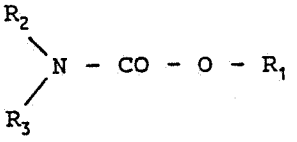


Fig. 5. Leaving Group as Potential Electrophilic Carcinogenic Intermediate of the Organophosphorus Pesticide, Tetrachlorvinphos.

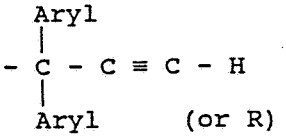
CARBAMATES



Salient structural features known to contribute to or affect carcinogenicity/mutagenicity:

1. Carcinogenic if R_2, R_3 = H; R_1 = vinyl, ethyl or methyl.

2. Carcinogenic if R_2, R_3 = H or alkyl; R_1 =



3. Carcinogenic if R_2, R_3 = CH_3; - OR_1 replaced by - Cl.

4. N,N-Disubstitution with bulky group decreases carcinogenicity/mutagenicity.

5. Mutagenic if either R_2 or R_3 is acyloxy.

Fig. 6. Salient Structural Features Known to Contribute to or Affect the Carcinogenicity and/or Mutagenicity of Carbamates.

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Carcinogenicity and Pesticides

Principles, Issues, and Relationships

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