# Artifact Formation in the GC/MS Analysis of Zolone EC (Phosalone) Insecticide Formulation

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In an investigation of potential causes for three grape-harvester pesticide poisoning incidents, acetone extracts of Zolone EC [active ingredient, phosalone [*O,O*-diethyl *S*-(6-chloro-2-oxobenzoxazolin-3-yl)-methyl phosphorodithioate]] were analyzed by GC/MS for the presence of possible impurities. Initial findings suggested the presence of isophosalone [*O,S*-diethyl *S*-(6-chloro-2-oxobenzoxazolin-3-yl)methyl phosphorodithioate], a phosalone isomer containing *S*-ethyl and oxon functionalities, as a formulation impurity. High-resolution and tandem mass spectrometry experiments confirmed the structure of isophosalone. An additional solid probe tandem mass spectrometry study and investigations of the effects of injector temperature, column length, and on-column injection verified that isophosalone was produced as an artifact of gas chromatography and was not initially present in the Zolone EC formulation. The results demonstrate the utility of high-resolution and tandem mass spectrometry experiments for structural confirmation and illustrate that the potential exists for formation of isomer artifacts of phosphorodithioate insecticides during the gas chromatographic process.

In California's Central Valley during August and September, 1987, three episodes of grape-harvester poisoning occurred that involved 78 workers. An investigation by the California Department of Food and Agriculture (CDFA) and staffs of the Agricultural Commissioners in Madera and Fresno counties indicated that all affected workers showed moderate to severe cholinesterase depression while the majority reported symptoms of a flu-like illness compatible with cholinesterase poisoning. Pesticide application histories of the three vineyards indicated that the cholinesterase-inhibiting insecticide phosalone [*O,O*-diethyl *S*-(6-chloro-2-oxobenzoxazolin-3-yl)methyl phosphorodithioate] was applied to all three vineyards and could have been responsible for the poisoning cases, although no violations of the 7-day worker re-entry interval were noted and vineyard residue levels of phosalone and its oxygen analogue phosoxalone [*O,O*-diethyl *S*-(6-chloro-2-oxobenzoxazolin-3-yl)methyl phosphorothioate] appeared to be within normal levels (O'Malley, 1988). The phosalone formulation applied in the vineyards was Zolone EC, an emulsifiable concentrate formulation containing 34.4% phosalone manufactured by Rhone-Poulenc Ag Co.

In addition to many other possible causative factors, it was hypothesized that the presence of impurities in the Zolone EC insecticide formulation used could have contributed to the poisoning cases by directly inhibiting cholinesterase enzymes or by potentiation of phosalone toxicity through inhibition of detoxifying esterase enzymes. The presence of impurities in several technical-grade organophosphate insecticides, including malathion and phenthoate, has been shown to increase organophosphate toxicity in mammals (Pellegrini and Santi, 1972; Umetsu et al., 1977; Talcott et al., 1979; Toia et al., 1980).

Initial GC/MS analysis by CDFA of acetone extracts of Zolone EC formulations used in the vineyards where the poisoning episodes occurred showed the presence of an unknown late-eluting peak in addition to phosalone and phosoxalone. The expertise of the University of California was solicited by CDFA for help in characterization and identification of the suspected unknown formulation impurity.

## MATERIALS AND METHODS

**Chemicals.** An acetone extract of Zolone EC batch 80203, in a volume of about 1 mL, was obtained from CDFA. The concentration of phosalone in the sample was approximately 3 $\mu g/\mu L$. Phosalone is a cholinesterase inhibitor and should be handled with caution in well-ventilated areas. The dermal $LD_{50}$ of phosalone in the rat has been reported to be 1500 mg/kg (O'Malley, 1988).

**Analysis.** Samples were analyzed by using several techniques, including gas chromatography/mass spectrometry (GC/MS), gas chromatography/tandem mass spectrometry (GC/MS/MS), and solid probe tandem mass spectrometry (MS/MS). Gas chromatography was performed by using an HP-5790A gas chromatograph (Hewlett-Packard, Avondale, PA). For most experiments, splitless injections of 1 $\mu L$ were made, and a 30-m DB-5 capillary column with 0.25 mm i.d. and 0.25 $\mu m$ film (J&W Scientific, Folsom, CA) was used. For high-resolution mass spectrometry experiments, injections were made with a 10:1 split. Injector temperature was 270 °C, and column temperature was programmed linearly from 80 to 250 °C at 20 °C/min. Other gas chromatography studies used a 15-m DB-5 column with 0.25 $\mu m$ film (J&W Scientific) and/or on-column injection.

All mass spectrometry experiments were performed by using a VG ZAB-HS-2F mass spectrometer (VG Analytical, Wythenshawe, U.K.). GC/MS experiments involved full scanning in the mass range of 40–450 Da and electron ionization at 70 eV. GC/MS/MS and solid probe MS/MS experiments provided daughter ion spectra of selected ions by linked scans of constant $B/E$. GC/high-resolution mass spectra were obtained at a resolution $(M/\Delta M)_{10\%\ \text{valley}}$ of 5000, scanning from $m/z$ 450 to 40 at 1.5 s/decade.

## RESULTS AND DISCUSSION

**Initial Findings.** The gas chromatogram of the acetone extract of the Zolone EC formulation showed the presence

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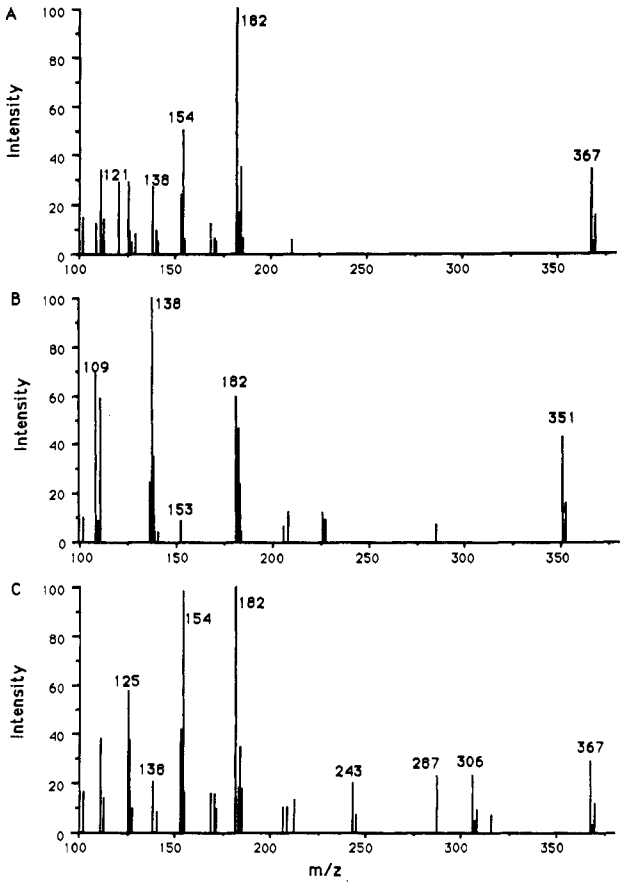


**Figure 1.** Electron ionization mass spectra of (A) phosalone, (B) phosoxalone, and (C) suspected formulation impurity.

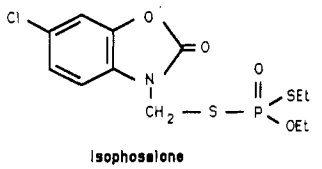


**Figure 2.** Structural assignment for proposed Zolone EC formulation impurity.

of phosoxalone ($t_r$ = 14.50 min), phosalone ($t_r$ = 15.85 min), and a later eluting peak suspected as a formulation impurity ($t_r$ = 17.52 min).

The electron ionization mass spectra of phosalone, phosoxalone, and the suspected formulation impurity are compared in Figure 1. The spectra of phosalone and the suspected impurity were similar yet distinct. Both compounds yielded a molecular ion at $m/z$ 367 accompanied by a $^{37}Cl$ isotope peak at $m/z$ 369.

Following analysis of the mass spectra for the three compounds, the suspected formulation impurity was assigned the structure of isophosalone [*O,S*-diethyl *S*-(6-chloro-2-oxobenzoxazolin-3-yl)methyl phosphorodithioate] (Figure 2), an isomer of phosalone possessing an oxon (P=O) coordinate covalent bond rather than a thion (P=S) and an *S*-ethyl rather than an *O*-ethyl ester linkage. This type of isomerization has been reported to occur with a number of organophosphate pesticides, including malathion (Umetsu et al., 1977) and parathion (McPherson and Johnson, 1956).

Two major lines of fragmentation evidence support this assignment. The first involves the presence of the $m/z$ 306 ion in the mass spectrum of isophosolone, which is absent in the mass spectrum of phosalone. This ion presumably arises from cleavage of the P–S-ethyl bond of isophosalone which is assumed to be much more prone to

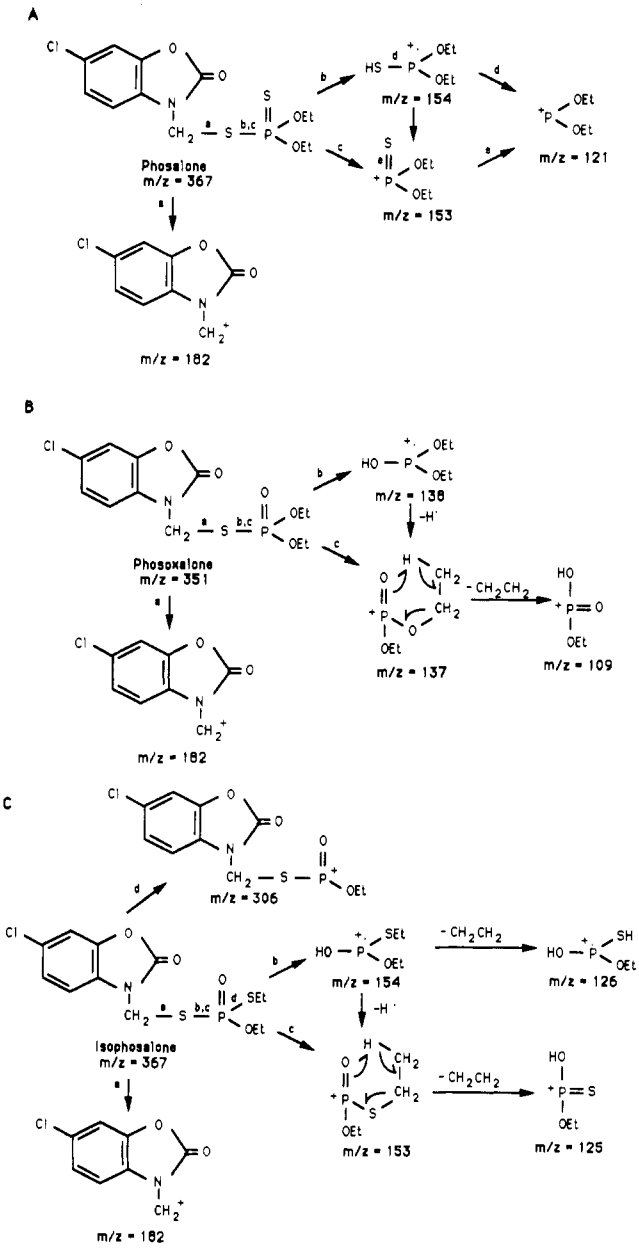


**Figure 3.** Proposed electron ionization fragmentation pathways for (A) phosalone, (B) phosoxalone, and (C) isophosalone.

fragmentation than the corresponding P–O-ethyl bond; similar fragmentation would not be possible for phosalone due to its lack of a P–S-ethyl bond. The preferred cleavage of the P–S rather than the P–O bond in phosphorothiolates has been reviewed in detail by Desmarchelier et al. (1976), and examples illustrating this preferential fragmentation were provided for a number of chemicals, including *O,S*-dimethyl phosphoroamidothioate (Monitor), *O,S*-dimethyl *N*-acetylphosphoroamidothioate (Orthene), and *O,O,S*-triethyl phosphorothioate.

Fragmentation data also indicate that the suspected formulation impurity probably exists as an oxon rather than as a thion. It is proposed that a major fragmentation pathway of the thion phosalone involves scission of the P–$SCH_2N$ bond which forms ions of $m/z$ 153 and 154 (assuming hydrogen rearrangement). These ions may rapidly eliminate S or HS to form an ion of $m/z$ 121 (Figure 3A). In the case of phosoxalone, a comparable ion at $m/z$ 121 is not seen. This is presumably due to the greater bond strength of the P=O bond relative to that of P=S. As a result, the ion of $m/z$ 137 can rearrange with loss of ethylene to yield an ion of $m/z$ 109 (Figure 3B). Frag-

**Table I. High-Resolution Mass Spectral Data for Molecular Ions and Selected Ions**

| ion | proposed formula | pred mass | obsd mass |
|---|---|---|---|
| phosalone | | | |
| 367 | $C_{12}H_{15}ClNO_4PS_2$ | 366.9869 | 366.9792 |
| 182 | $C_8H_5ClNO_2$ | 182.0009 | 181.9986 |
| 154 | $C_4H_{11}O_2PS$ | 154.0217 | 154.0156 |
| 121 | $C_4H_{10}O_2P$ | 121.0418 | 121.0407 |
| phosoxalone | | | |
| 351 | $C_{12}H_{15}ClNO_5PS$ | 351.0097 | 351.0096 |
| 182 | $C_8H_5ClNO_2$ | 182.0009 | 182.0011 |
| 138 | $C_4H_{11}O_3S$ | 138.0446 | 138.0391 |
| 109 | $C_2H_6O_3P$ | 109.0055 | 109.0077 |
| isophosalone | | | |
| 367 | $C_{12}H_{15}ClNO_4PS_2$ | 366.9869 | 366.9792 |
| 306 | $C_{10}H_{10}ClNO_4$ | 305.9757 | 305.9781 |
| 182 | $C_8H_5ClNO_2$ | 182.0009 | 182.0026 |
| 154 | $C_4H_{11}O_2PS$ | 154.0217 | 154.0251 |

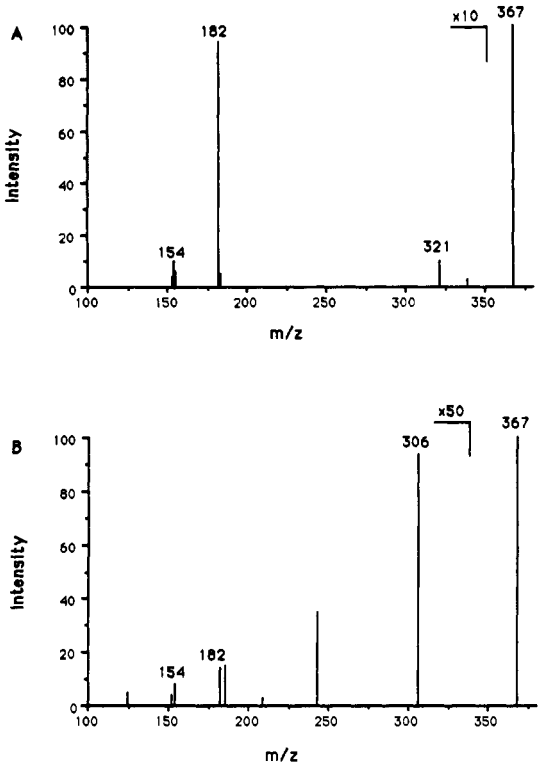


**Figure 4.** Daughter ion spectra of $m/z$ 367 ion of (A) phosalone and (B) isophosalone.

mentation of the $m/z$ 153 ion of the suspected formulation impurity appears to proceed along the same mechanism, consistent with the proposed isophosalone structure, yielding a rearranged product via loss of ethylene with $m/z$ 125 (Figure 3C). A scheme for identification of classes of organophosphate insecticides through observation of these and other characteristic fragmentation reactions has been proposed by Stan et al. (1977).

**Confirmation of Structure of Suspected Formulation Impurity as Isophosalone.** Several mass spectometry experiments were conducted to verify the proposed structure of the suspected formulation impurity as isophosalone. Results of high-resolution studies of the molecular ions and important fragment ions are shown in Table I. Results confirm the proposed empirical formulas of the molecular and fragment ions.

The daughter ion spectra of the molecular ions of phosalone and isophosalone ($m/z$ 367) are compared in Figure 4. Results indicate that the fragmentation of the two molecular ions produce distinct but different spectra. The ion of $m/z$ 306 from the fragmentation of the molecular

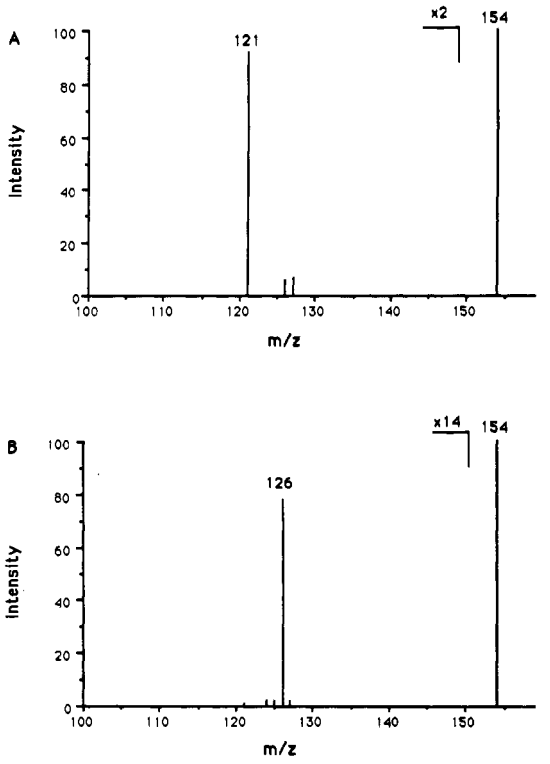


**Figure 5.** Daughter ion spectra of $m/z$ 154 ion of (A) phosalone and (B) isophosalone.

ion of isophosalone is not observed in the fragmentation of the molecular ion of phosalone, confirming, in combination with the high-resolution data, the loss of an *S*-ethyl fragment from the molecular ion.

The daughter ion spectra of the $m/z$ 154 ions of phosalone and isophosalone are compared in Figure 5. Although high-resolution data indicated that each ion has the same empirical formula, the fragmentation pathways of the two ions are markedly different. Fragmentation of the phosalone $m/z$ 154 ion yields predominantly the $m/z$ 121 ion, which is not produced in the fragmentation of the $m/z$ 154 ion of isophosalone. A major daughter ion of the $m/z$ 154 ion of isophosalone is observed at $m/z$ 126, which presumably arises from a rearrangement involving the loss of ethylene and is consistent with fragmentation proposed for an oxon (Stan et al., 1977).

The daughter ion spectra of the $m/z$ 137 ion of phosoxalone and its analogue, the $m/z$ 153 ion of isophosalone, are compared in Figure 6. Fragmentation of each ion shows a predominant loss of 28 Da, consistent with the proposed rearrangements supporting the assignment of the suspected formulation impurity as an oxon.

**Verification of Isophosalone as an Artifact of Gas Chromatography.** A chromatogram of the acetone extract of the Zolone EC formulation showing the separation of phosalone and isophosalone is presented in Figure 7. The chromatogram shows broad, tailing peaks, which could have arisen from column overload and/or column decomposition of phosalone. Additional studies were performed to determine if isophosalone was initially present in the insecticide formulation or if it was produced during the gas chromatographic process. Although it appeared that greater amounts of isophosalone resulted from injections made by using an injector temperature of 275 °C than from 220 °C, on-column injections of the acetone extract of the formulation also produced isophosalone, confirming that isophosalone did not result solely from formation in the injector. Differences in injection volume also appeared to influence the appearance of

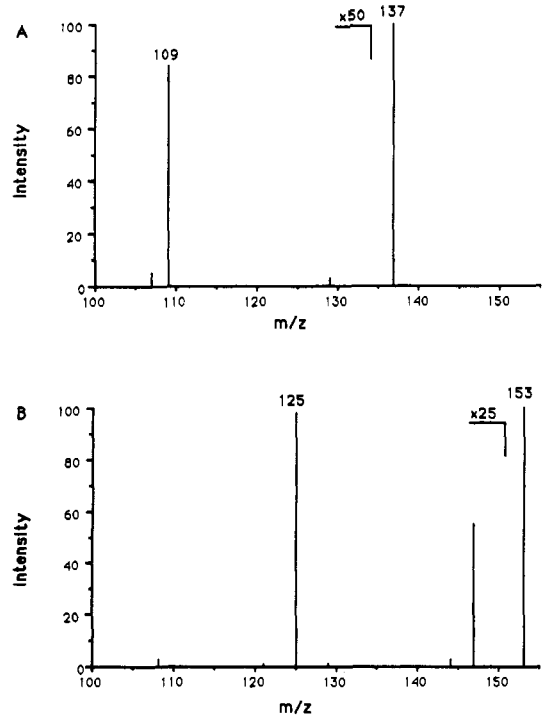


**Figure 6.** Daughter ion spectra of (A) $m/z$ 137 of phosoxalone and (B) $m/z$ 153 ion of isophosalone.

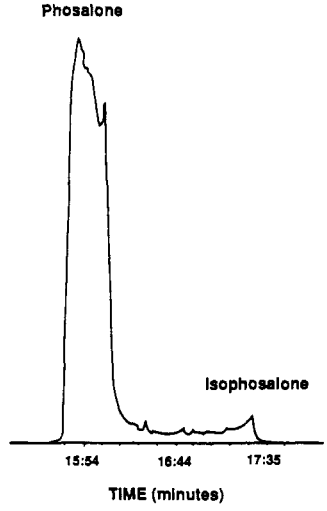


**Figure 7.** Selected ion chromatogram of $m/z$ 367 illustrating tailing in the GC/MS analysis of phosalone.

isophosalone; less isophosalone was detected relative to phosalone when lower injection volumes were used. The effects of column length were also compared. Isophosalone was readily detected by using a 30-m DB-5 column but was not observed when a 15-m DB-5 column was used. These results suggest that isophosalone may be produced as a gas chromatography artifact through reactions in the column. Furthermore, the chromatographic data (Figure 7) reveal a tail on the back side of the phosalone peak that spreads to the later eluting isophosalone peak. Such tailing is often observed when analytes decompose within the column. The ratio of the peak areas of phosalone to isophosalone decreased with increasing amounts of material injected on the 30-m column, suggesting that column overloading and perhaps bimolecular reactions are involved in the decomposition. These findings imply that gas chromatography alone may not yield conclusive evidence of the presence of isomers for some organophosphates.

As a final experiment to verify that isophosalone was produced as an artifact of gas chromatography, an MS/MS experiment was performed involving solid probe sample introduction of the acetone extract of the Zolone EC formulation and generation of daughter ion spectra of the $m/z$ 367 ($M^{\bullet+}$) ions. The spectrum generated from this experiment was essentially the same as that of the fragmentation of $m/z$ 367 of phosalone observed from earlier GC/MS/MS studies (Figure 4A). Daughter ions of $m/z$ 306, which were expected from the loss of the *S*-ethyl group of isophosalone (Figure 4B), were not observed in the solid probe MS/MS experiment. This finding, in combination with the results of studies of the effects of column length on isophosalone formation, strongly suggests that isophosalone resulted as an artifact of gas chromatography and was not initially present as an impurity of the Zolone EC insecticide formulation.

This study illustrates the applicability of GC/MS and MS/MS for the structural determination and confirmation of unknown compounds in complex mixtures. In this example concerning the identification of isophosalone, a tentative structure was proposed on the basis of its mass spectral fragmentation pathways and fragmentation pathway similarities that existed between the unknown compound and two known components of the mixture, phosalone and phosoxalone. Confirmation of structure was possible through high-resolution mass spectrometry studies in combination with MS/MS experiments.

Findings also illustrate that caution must be used in the interpretation of results of gas chromatography experiments investigating the presence of impurities of organophosphate insecticide products. Although initial results indicated the presence of isophosalone in the formulation, further studies determined that isophosalone was produced as a gas chromatography artifact and probably resulted from reactions in the column. It is recommended that studies investigating the presence of organophosphate insecticide impurities include methods for the impurity isolation and purification that do not involve high temperatures and minimize contact with potentially reactive surfaces. This rationale has been used successfully for the thin-layer chromatography isolation and identification of impurities of the organophosphate insecticides malathion, fenthion, acephate, and methyl parathion (Umetsu et al., 1977; Toia et al., 1980; Chukwudebe et al., 1989).

The mechanism for this type of organophosphate insecticide isomerization reaction has been reviewed in detail by Fest and Schmidt (1983) and involves phosphorothioate anions, formed through dealkylation, serving as nucleophilic agents preferentially alkylating at a sulfur atom, forming a thiolate. It appears that isomerization may be catalyzed by nucleophiles but may also occur via self-alkylation. This isomerization reaction takes place at around 120–180 °C. The fact that isophosalone can result from reactions of phosalone at high temperatures inside the gas chromatography column suggests that reactive surfaces may be involved. It is proposed that similar isomerization reactions could occur in the environment and might possibly be catalyzed by the presence of photosensitizing chemicals or other compounds present on plant surfaces. Isophosalone was not detected in a study by Walia et al. (1989), who investigated the photodegradation of phosalone under ultraviolet and simulated solar light conditions performed in solvents and under solid-state conditions using a thin film on a glass surface. The possibility exists, however, for isophosalone formation under the more complicated environmental conditions involving sunlight, environmental photosensitizers, and plant surfaces. Additional studies are needed to determine the potential for, and possible significance of, isophosa-

lone formation in the environment following phosalone insecticide applications.

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