major peak at δ 29.6 in the $^{13}C$ NMR spectrum. A peak at δ 29.6 is characteristic of a high molecular weight straight-chain hydrocarbon. From these spectra, the percent rubber and percent hydrocarbon were calculated and are reported in Table I.

The benzene-extracted rubber fraction from six of these ten species was found to be contaminated with relatively large quantities of hydrocarbon. However, the four species (*H. radula*, *H. californicus*, *H. resinosus*, and *H. annuus*) giving the highest rubber content by the gravimetric method were relatively pure as analyzed by $^{13}C$ NMR spectroscopy. Our study further indicates that the rubber found in the aerial parts of the plant is concentrated in the leaves rather than in the stems. With two exceptions (*H. arizonensis* and *H. nuttallii nuttallii*), the benzene extract from stems was composed entirely of the straight-chain hydrocarbons.

We earlier reported that cultivated hybrid varieties "894" and "896" had a foliar rubber content of 0.49% and 0.74%, respectively (Stipanovic et al., 1980). Since all cultivated sunflower hybrids are derived from *H. annuus*, it is especially noteworthy that the *H. annuus* collected from Winton, OK, had a rubber content of 1.45%. Bruehrer and Benson (1945) reported a rubber content of 0.55% from *H. annuus* collected at Tucson, AZ, while Minshall (1957) reported that *H. annuus* plants collected at Ontario, Canada, contained 0.26% rubber. The reasons for these differences are uncertain and will require additional study. Our results indicate that there is a high potential for increasing the rubber content of cultivated sunflower. In view of the current interest in a domestic source of natural rubber, the potential of sunflower deserves additional study.

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**Supplementary Material Available:** Method and equation used to calculate the percent hydrocarbon present in the benzene extracts (1 page). Ordering information is given on any current masthead page.

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# Chemical Ionization Mass Spectra of Aldicarb and Metabolites

The isobutane chemical ionization mass spectra of aldicarb [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] and of its metabolites aldicarb sulfoxide and aldicarb sulfone show strong $(M + H)^+$ signals and also large peaks due to the corresponding ions of the protonated oxime and nitrile moieties. These results are of distinct diagnostic and analytical value.

Aldicarb is a widely used systemic pesticide with combined insecticidal, nematicidal, and miticidal properties (U.S. Environmental Protection Agency, 1975). Trace analysis of environmental samples of aldicarb and its toxic metabolites (sulfoxide and sulfone) represents an important but complicated problem. This is due to the considerable reactivity and thermal instability of these molecules, which in the case of gas chromatography (GC) results in decomposition (Hall and Harris, 1979) of the measured molecules. In the case of mass spectrometry (MS) the lability of these compounds prevents the observation of their molecular ions in electron impact mass spectrometric (EI-MS) assays. Thus in the EI-MS studies of aldicarb and of its two oxidation products (Benson and Damico, 1968), no molecular ions $M^+$ were reported. The major fragmentation pathway in the EI-MS is the C–S bond cleavage rather than that giving rise to the methyl isocyanate fragment.

Although not observable by electron ionization, the molecular ion of aldicarb sulfone (only) could be observed by field ionization (Damico et al., 1969) and that of aldicarb itself was seen by field desorption measurements (Fales et al., 1975). Earlier CI studies of aldicarb (Fales et al., 1975; Holmstead and Casida, 1975) gave no evidence for the protonated molecular $(M + H)^+$ ion. In the present communication we report the results of an isobutane CI-MS study of aldicarb and its metabolites. This ionization method provides abundant $(M + H)^+$ ions as well as several diagnostic ions which may prove useful for residue and metabolism studies.

## EXPERIMENTAL SECTION

**Reagents.** Analytically pure samples of aldicarb, aldicarb sulfoxide, and aldicarb sulfone, supplied by the Union Carbide Corp., were provided by Dr. I. Adato (Ministry of Agriculture, Jaffa) and continuously stored at −20 °C.

**Mass Spectrometry.** Mass spectra were recorded on our low-resolution single-focusing Du Pont 21-490B instrument and repeated on the Du Pont 21-490B instrument of Dr. S. Zitrin (Israeli Police Laboratory, Jerusalem). These instruments are equipped with a dual EI/CI ion

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Table I. Chemical Ionization Ions of Aldicarb and Its Metabolites

| name | | m/z (relative intensity) (M + H)+ | (RCH=NOH + H)+ [a] | (RC=N)+ | other ions |
|---|---|---|---|---|---|
| aldicarb (1a), X = S | A | 191 (14) | 134 (10) | 116 (100) | 89 (31), 144 (10) |
| | B | 191 (14) | | 116 (100) | 89 (31), 144 (10) |
| sulfoxide (1b), X = SO | A | 207 (21) | 150 (11) | 132 (100) | 91 (23), 86 (21), 70 (45) |
| | B | 207 (82) | 150 (15) | 132 (100) | 86 (28) |
| sulfone (1c), X = $SO_2$ | A | 223 (81) | 166 (20) | 148 (25) | 145 (38), 85 (38), 121 (70), 81 (100) |
| | B | 223 (100) | 166 (12) | 148 (15) | 145 (12), 85 (10), 121 (12), 81 (22) |

[a] R = $CH_3XC(CH_3)_2$-.

source. The samples were introduced via an unheated direct inlet probe. Source temperature was 150 °C, the emission current was 0.3 mA, and the ionization voltage was 70 V. Under our experimental conditions the reagent gas pressure at the ion source was in the range of 0.5–1 torr.

## RESULTS AND DISCUSSION

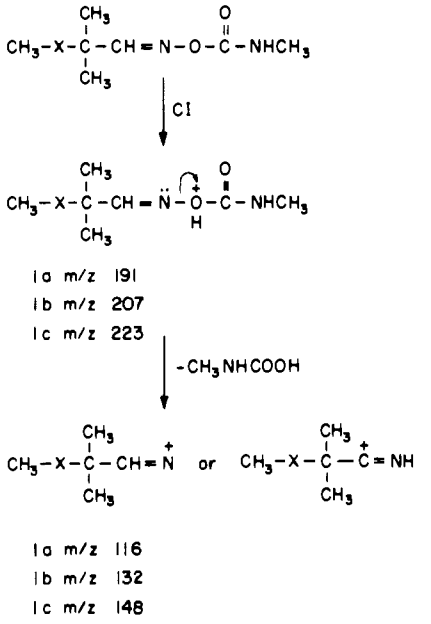
The mass spectral data for aldicarb (**1a**), its sulfoxide (**1b**), and sulfone (**1c**) are summarized in Table I. Two sets of results were obtained under different extents of CI to EI ionization. In the first set (set A in Table I) the ratio of $C_4H_9^+$ to $C_3H_7^+$ is 2.5. Under these conditions EI is still important. In the other set (set B in Table I) the ratio of $C_4H_9^+$ to $C_3H_7^+$ is 4. Here the contribution of EI is lower, leading to smaller extent of fragmentation. The $(M + H)^+$ ion appears in each of the measured compounds, accompanied by two main fragmentation products, one corresponding to the protonated nitrile and the other to the protonated oxime. As expected, the abundance of the protonated molecular ion is larger when CI conditions predominate (set B). The $(M + H)^+$ ions, as well as the protonated oxime and nitrile ions, are diagnostic of aldicarb and its metabolites and can be used to support the identification and trace analysis of these compounds.

Table I illustrates the simplicity of the fragmentation due to chemical ionization in contrast with the complicated spectra and extensive fragmentation resulting from electron impact. Hence, the EI-MS of aldicarb, aldicarb sulfoxide, and aldicarb sulfone are markedly similar (Benson and Damico, 1968), and in none of them could be detected the signals due to the parent ions ($m/z$ 190, 206, and 222), the nitrile ions ($m/z$ 115, 131, and 147), or the oxime derivative ions ($m/z$ 133, 149, and 165).

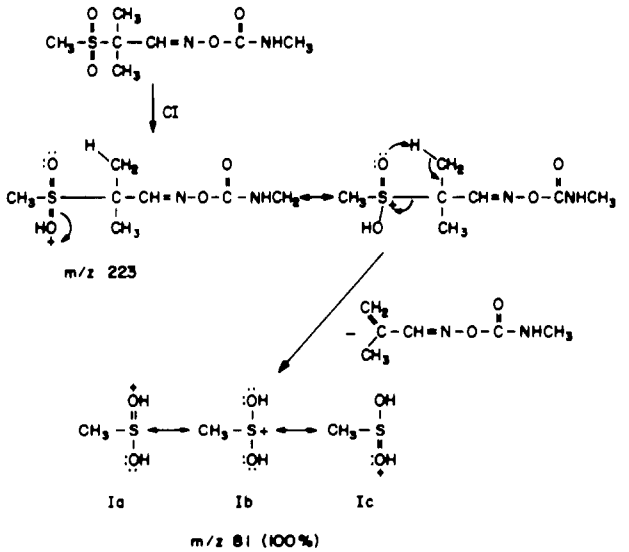
The mechanism proposed for the isobutane CI fragmentation of aldicarb and its metabolites is shown in Schemes I and II. The protonation pattern suggested here is assumed on the basis of the main fragments observed in the mass spectra. Scheme I illustrates the dominant mode of fragmentation for aldicarb and for its sulfoxide and also to a minor extent for aldicarb sulfone. The appearance of the (nitrile + H)+ ion as the most abundant ion in the first two compounds, accompanied by an abundant (oxime + H)+ ion, indicates that in these compounds the N–O bond is more easily cleaved than the S–C bond. These results differ from those of the EI fragmentation (Benson and Damico, 1968). The dissimilarity can be traced to the different ionization modes. In the CI process the oxime oxygen is protonated and this leads to destabilization of the N–O bond. In the EI, however, the ionization occurs at the sulfur atom, which is the element of lowest ionization potential in the aldicarb molecule. The sulfur ionization leads to subsequent cleavage of the C–S bond.

Going from aldicarb sulfoxide to the sulfone, under conditions of set A, fragmentation shifts toward the loss of the sulfur moiety (Scheme II). Presumably, the second

Scheme I. Fragmentation Pattern of Aldicarb and Metabolites (See the Text): 1a, X = S; 1b, X = SO; 1c, X = $SO_2$



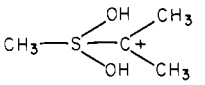

Scheme II. Main Pattern for Fragmentation of Aldicarb Sulfone




oxygen increases the partial positive charge on the sulfur atom. This leads to loss of stability at the C–S bond in the $m/z$ 223 ion formed by CI. It follows that the $(M + H)^+$ ion can undergo a hydrogen rearrangement via a five membered ring transition state with subsequent cleavage of the C–S bond and formation of the $m/z$ 81 (100%) ion. This process is favored by the extensive stabilization

possible in the $m/z$ 81 ion produced (Ia–c, Scheme II). A fragment ($m/z$ 145, 40%) complementary to the sulfur moiety is also observed in the mass spectra.

Hence, unlike in aldicarb and aldicarb sulfoxide, the main CI fragmentation process in aldicarb sulfone parallels that found by pure EI (Benson and Damico, 1968). An additional characteristic cleavage observed in the CI-MS of aldicarb sulfone is that leading to the stable tertiary carbonium ion ($m/z$ 123)



obtained in 70% abundance under set A conditions and in 12% abundance under the conditions of set B. The possibility of applying isobutane CI to the GC–MS of these compounds under mild conditions is of considerable practical importance and is presently being explored in this laboratory. At the same time, however, another simple possibility would be to introduce cleanup samples into the mass spectrometer via a liquid chromatography interface (LC–MS), which could obviate the sample heating and thermal decomposition inherent in the use of GC–MS.

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# Effect of Sesamex on the in Vivo Metabolism of Diflubenzuron in Larvae of Susceptible and Resistant Strains of the Housefly, *Musca domestica* L.

The effect of sesamex [5-[1-[2-(2-ethoxyethoxy)ethoxy]ethoxy]-1,3-benzodioxole] on the in vivo metabolism of diflubenzuron [1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl)urea] was investigated on a susceptible (NAIDM) and two resistant (R-diflubenzuron and R-OMS-12) strains of the housefly. Sesamex reduced significantly the in vivo metabolism of radiolabeled diflubenzuron in all strains. Recovery of internal unmetabolized diflubenzuron was 10–30 times higher in the mature larvae of the R strains when the compound was applied jointly with sesamex. Similarly, sesamex reduced markedly the recovery of polar and nonpolar metabolites of water-soluble conjugates of diflubenzuron both in the external and in the internal fractions of these strains. The recovery of radiolabel in the unextractable residue increased with sesamex treatment in all three strains. These results indicate that MFO enzymes play an important role in the resistance of houseflies to diflubenzuron.

Insecticides are metabolized within the insect system to less toxic or nontoxic polar products by three major types of enzyme systems, e.g., mixed-function oxidase (MFO), hydrolase, and glutathione-dependent transferase (GSAT) (Matsumura, 1976). Biochemical studies have demonstrated that resistant (R) strains usually possess increased ability to metabolize insecticides [reviews by Georghiou (1972), Oppenoorth (1976), and Plapp (1976)]. Since synergists act by inhibiting specific detoxication enzymes, they are frequently utilized as indicators of the possible biochemical mechanisms involved in resistance (Casida, 1970; Wilkinson, 1972).

In an earlier study we reported on the mechanisms of resistance to diflubenzuron [1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl)urea] in a diflubenzuron-selected strain of the housefly (Pimprikar and Georghiou, 1979). It was shown that sesamex [5-[1-[2-(2-ethoxyethoxy)ethoxy]ethoxy]-1,3-benzodioxole] and piperonyl butoxide significantly increased the toxicity of diflubenzuron in the resistant strain, suggesting the involvement of MFO systems in resistance. Sesamex consistently produced higher synergism with diflubenzuron than did piperonyl butoxide. Here we report tests intended to show if the synergistic activity of sesamex was due to suppression of metabolism of diflubenzuron and the type of metabolic pathway affected by the synergist.

## MATERIALS AND METHODS

**Chemicals.** [$^{14}$C]Diflubenzuron (aniline-U-$^{14}$C; specific activity 2.3 mCi/mmol) and [$^{3}$H]diflubenzuron (benzoyl-U-$^{3}$H; specific activity 68 mCi/mmol) were provided by Thompson-Hayward Chemical Co., Kansas City, KS. Unlabeled diflubenzuron and authentic standards of diflubenzuron metabolites were also supplied by the same company. Piperonyl butoxide, technical grade, was provided by FMC Corp., Philadelphia, PA, and sesamex by

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