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Insecticide Solvents: Interference with Insecticidal Action

Abstract. *Several commercial solvent mixtures commonly used as insecticide carriers in spray formulations increase by more than threefold the microsomal* N-*demethylation of* p-*chloro* N-*methylaniline in midgut preparations of southern armyworm* (Spodoptera eridania) *larvae exposed orally to the test solvents. Under laboratory conditions, the same solvent mixtures exhibit a protective action against the in vivo toxicity of the insecticide carbaryl to the larvae. The data are discussed with respect to possible solvent-insecticide interactions occurring under field conditions and, more broadly, to potential toxicological hazards of these solvents to humans.*

Studies in recent years have established that insects have active microsomal mixed-function oxidase (MFO) systems which are mediated by cytochrome P450; these systems appear similar in all important characteristics measured to date to those occurring in mammals (*1*). The oxidase systems take part in the metabolism of insecticides and of other foreign lipophilic compounds (*2*), and as a consequence they often dictate the duration and intensity of action of many toxicants and other biologically active materials.

One important characteristic of the MFO system is its ability to become rapidly induced after the exposure of organisms to any of a large number of drugs, insecticides, and other chemicals, many of which are MFO substrates (*3*). The induction process, which enhances enzyme activity through an increase in de novo protein synthesis (*3*), often has a marked effect on the susceptibility of an organism to a given toxicant.

There have been numerous reports of MFO induction in insects (*1, 4*), and studies with the southern armyworm (*Spodoptera eridania*, Cramer) have shown that MFO activity in the midgut tissues, the major site of localization of the enzymes in Lepidoptera (*1*), is highly responsive to the inducing action of various alkylbenzenes administered in the diet (*5*).

In that xylenes and other aromatic hydrocarbon compounds are frequently employed as solvents in concentrates and other formulations, it is of interest to investigate the potential inducing capacity of such materials.

Experiments were conducted with sixth (last) instar larvae of the southern armyworm; the larvae were reared under greenhouse conditions (*6*) and carefully synchronized with respect to age (± 1 hour) at the time of the fifth molt. Groups of 30 newly molted sixth-instar larvae were given free access to the semi-defined diets, which are based on agar (*5*), either with or without (control) the addition of various concentrations of the test compounds (*7*). After 24 hours, the midgut tissues were removed by dissection, cleaned, and homogenized to provide the enzyme preparation employed (*6*). Microsomal enzyme activity was measured by the *N*-demethylation of *p*-chloro *N*-methylaniline as described (*5*). Although our data were obtained only with crude midgut homogenates, *N*-demethylase activity has been shown to be located in the microsomal fraction of these homogenates (*5*).

The inducing capacities of the 13 commercial solvents evaluated (*7*) and of several standard materials included for comparison are shown in Table 1. At a dietary concentration of 0.2 percent (weight to volume), several of the solvent mixtures were potent inducers of microsomal *N*-demethylation, the most potent ones Amsco-Solv E-98, Mentor 28, and HAN increasing the level of enzyme activity to 449, 399, and 380 percent of the controls, respectively. Panasols AN-2 and AN-2K and Hess odorless spray base were only slightly less potent, the two former causing a greater than 2.5-fold increase at a dietary concentration of 0.05 percent. Under identical test conditions, phenobarbital, which induces hepatic MFO activity in mammals, caused a threefold increase in *N*-demethylase activity. Although detailed analyses of these solvent mixtures are not available, all such mixtures are known to contain high percentages (83 percent for HAN) of alkylated benzenes and naphthalenes, along with lesser amounts of other aromatic hydrocarbons. The data for the two simple alkylnaphthalenes included in Table 1 demonstrate the high inducing capacity of such compounds, which might constitute major components in the solvent mixtures. Neither unsubstituted benzene nor naphthalene had any effect on enzyme activity.

In an attempt to assess the possible in vivo effects of the solvents on the toxicity of insecticide to insects, groups of 20 sixth-instar armyworms that had been denied access to food for 4 hours after molting were allowed to feed on the leaves of "two-leaf-stage" kidney bean plants that had been sprayed (less than 10 minutes before) as evenly as possible with a Chromatosprayer (Applied Science Laboratories) with 5-ml portions either of acetone solutions of carbaryl (1-

Table 1. Effect of insecticide solvents on the *N*-demethylation of *p*-chloro *N*-methylaniline in midgut tissues of the southern armyworm. Newly molted sixth-instar armyworms were given free access for 24 hours to semidefined artificial diets containing the compounds. Armyworms of the same age fed diets with no added chemicals were used as controls. The *N*-demethylase specific activity in midguts from control armyworms was 0.97 ± 0.10 nmole per milligram of protein per minute (± S.E.). Experiments were replicated two or three times.

Compound	Percent in diet	Specific activity (nmole mg^{-1} min^{-1})	Percent of control activity
Phenobarbital	0.25	2.96 ± 0.18	305
Benzene	0.20	0.99 ± 0.11	102
Naphthalene	0.20	1.45 ± 0.12	149
1-Methylnaphthalene	0.20	3.35 ± 0.20	346
2-Ethylnaphthalene	0.20	3.29 ± 0.25	340
Solvesso xylene	0.20	2.21 ± 0.17	228
HAN	0.20	3.69 ± 0.27	380
Amsco-Solv E-98	0.20	4.35 ± 0.30	449
Panasol AN-2	0.05	2.58 ± 0.20	267
	0.10	2.75 ± 0.21	283
	0.20	2.74 ± 0.21	282
Panasol AN-2K	0.05	2.58 ± 0.19	267
	0.10	3.34 ± 0.22	345
	0.20	3.15 ± 0.23	325
Mentor 28	0.20	3.87 ± 0.28	399
Hess odorless spray base	0.20	2.51 ± 0.20	259
Diisooctylphthalate	0.20	2.07 ± 0.20	214
Benzoflex 9-88	0.20	1.49 ± 0.15	154
Amsco odorless spray base	0.20	1.25 ± 0.10	129
60-second spray oil	0.20	1.21 ± 0.12	125
70-second spray oil	0.20	0.99 ± 0.10	102
100-second spray oil	0.20	0.99 ± 0.11	102

Table 2. Twenty-four-hour mortalities of armyworms feeding on bean leaves sprayed with carbaryl alone or in admixture with a solvent. Percent mortality data are the mean values of at least two experiments run on different days. The median lethal dose (LD_{50}) of carbaryl alone under these experimental conditions was 0.0396 ± 0.002 percent; in admixture with 3 percent Amsco-Solv E-98, the LD_{50} of carbaryl was 0.201 ± 0.012 percent. Abbreviation: Amount, Amt.

Carbaryl (%)	Solvent	Amt. (%)	Mortality (%)
0.000*	None		0
0.010	None		0
0.025	None		15
0.050	None		75
0.10	None		100
0.10	Amsco-Solv E-98	0.5	85
0.10	Amsco-Solv E-98	1.0	75
0.10	Amsco-Solv E-98	2.0	65
0.10	Amsco-Solv E-98	3.0	10
0.10	60-second spray oil	3.0	90
0.10	Solvesso xylene	3.0	85
0.10	Amsco odorless spray base	3.0	65
0.10	HAN	3.0	20
0.10	Panasol AN-2K	3.0	10
0.10	Panasol AN-2	3.0	5

*Acetone control.

naphthyl *N*-methylcarbamate), of the solvent alone, or in admixture with carbaryl. Carbaryl was selected because it is rapidly detoxified by the MFO system (*8*). The 24-hour mortalities (Table 2) indicate that the toxicity of carbaryl is reduced when the insecticide is applied in admixture with several of the solvents that have been demonstrated to be potent inducing agents. In fact, several of the materials afforded the larvae almost complete protection from a dose of carbaryl (0.1 percent), which alone caused 100 percent mortality. None of the solvents themselves caused any overt effects on feeding or on other behaviors at the concentrations used.

The relevance of these findings to the field situation is still unclear. Many insecticide formulations contain 25 to 75 percent of solvent mixtures, and application solutions used with low-volume spray equipment often contain 0.25 to 1.0 part of concentrate to about 20 parts of water; ultra-low-volume application solutions may consist of nearly equal parts of the spray concentrate and water (*9*). If we assume that the average concentrate solvent content is 50 percent, solvent concentrations in the final spray formulation could therefore range from 2.5 to 25 percent, although with high-volume equipment the lower range of solvent concentration approaches 0.002 percent (*9*). The solvent concentrations used in our laboratory experiments range up to 3 percent. Another important consideration in determining whether the observed laboratory effects could occur in the field is whether the solvents remain on the treated surface for a sufficiently long time and at sufficiently high concentration to cause induction. Although this depends to a large extent on ambient weather conditions, most of the solvents used are fairly nonvolatile, as indicated by their boiling points (*7*), and it is probable that they remain on the treated surface for several hours after application. It is also possible that they may to some extent penetrate the leaf cuticle, thus further reducing their rate of loss. Since *N*-demethylase activity in armyworm midgut increases severalfold during the first few hours of exposure to an inducing agent (*5*), and, since subsequent studies have shown that an effect can be observed within 1 hour (*10*), it seems plausible that induction could occur under field conditions.

We are therefore led to the paradoxical conclusion that the carrier solvent in which an insecticide is formulated and applied might aid, under certain circumstances, in the survival of the target pest. The solvent carriers should not be regarded, as is often the case, as "inert" ingredients with little or no biological activity; their effects could provide a partial explanation for the variations in efficacy of different formulations of a given material. In view of our results, and in view of the widespread distribution of these solvents in many agricultural, industrial, and household products, it seems appropriate that the toxicology of some of these solvents to mammals and the potential toxicological interactions with other materials be more fully investigated.

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Stability of the Individual Globin Genes During Erythroid Differentiation

Abstract. *The genes for sheep β^A, β^C, and γ globin were all present in DNA from erythroid cells which synthesized only β^C globin. Similarly, selective excision of nonexpressed genes was shown not to occur during human erythroid differentiation. In contrast, evolutionary deletion of the β^C gene accounts for the inability of many sheep to make this globin.*

Many species have several globin genes which are differentially expressed during ontogeny (*1*). For example, in humans the expression of the gene for γ globin results in the production of hemoglobin F (HbF) ($\alpha_2\gamma_2$) during gestation. Synthesis of γ globin decreases to low levels after birth, when expression of the β globin gene results in the synthesis of hemoglobin A (HbA) ($\alpha_2\beta_2$). An analogous perinatal change from HbF to HbA occurs in sheep. In addition, sheep having the gene for β^A globin exhibit a second change, which occurs during anemia or other erythropoietic stress (*2*); the synthesis of β^A globin ceases and β^C globin is produced, resulting in the appearance of hemoglobin C (HbC) ($\alpha_2\beta_2{}^C$). The change from HbA to HbC is reversible; for example, after termination of erythropoietic stress, the β^A globin gene is again expressed and β^C globin synthesis stops.

Kabat (*3*) proposed that selective intrastrand excision of particular globin genes might occur early during erythropoiesis and be the mechanism whereby terminally differentiated cells become committed to synthesize the individual globins. Erythropoiesis is a continual process, whereby undifferentiated but committed precursor cells enter the erythron and actively synthesize hemoglobin for only a few days before maturing into circulating red cells (*4*). We have obtained experimental evidence which suggests that sheep erythroid cells become irreversibly committed to the synthesis of β^A or β^C globin (or a mixture of the two) during the transition from the undifferentiated precursors to recognizable erythroid cells (*5*). Kabat's model predicts that erythroid cells from those sheep homozygous for β^A globin might, during severe erythropoietic stress, contain the β^C gene but lack the γ gene or, possibly, both γ and β^A genes. Similarly, an adult human making only β globin would lack the gene for γ globin in his erythroid cells. The γ and β genes are closely linked in humans (*6*) and probably are also in other species. Thus, the formation of intrastrand loops by base pairing between homologous DNA sequences adjacent to each gene is a reasonable possibility and would provide a site for nuclease excision of particular genes. Nonetheless, this interesting hypothesis has never been tested directly. To do so we used complementary DNA's (cDNA's) specific for the nucleic acid sequences of the individual human and sheep globins as hybridization probes to determine the complement of genes present in differentiated erythroid cells.

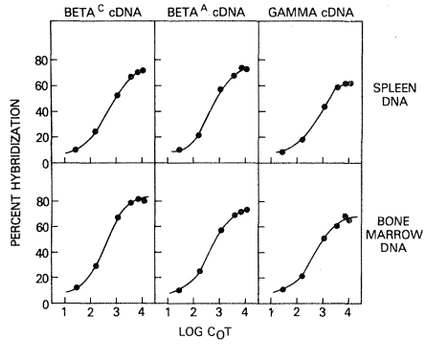

Fig. 1. Annealing of sheep bone marrow and spleen DNA to β^C, β^A, and γ cDNA's. Each hybridization reaction contained 2 mg of DNA and 0.2 ng of one of the cDNA's. Each probe was labeled with ^{3}H-labeled deoxycytidine triphosphate to give a specific activity of approximately 15,000 count/min per nanogram. The specific activities of all of the probes were identical. The reaction volume was 132 μl. Six individual portions (22 μl) were sealed in capillary tubes, incubated at 95°C for 5 minutes, and then placed in a constant temperature bath at 58°C. The capillary tubes were removed at times ranging from 10 minutes to 66 hours, and the contents were expelled into 0.5 ml of 0.05*M* phosphate buffer and stored in liquid nitrogen. At the completion of the hybridization period, each individual reaction was analyzed by batch elution of the single- and double-stranded fractions from hydroxyapatite, as described (*13*).

Messenger RNA's (mRNA's) were prepared from reticulocytes of anemic adult and normal fetal sheep. Full-length cDNA's containing sequences for α and one of the non-α globins (either β^A, β^B, β^C, or γ) were synthesized with RNA-dependent DNA polymerase (*7*). The non-α globin cDNA's were then purified by thermal denaturation of heterologous nucleic acid duplexes followed by chromatography on hydroxyapatite (*8*). For example, HbB cDNA (α,β^B) was annealed to HbF mRNA (α,γ) at 50°C, a permissive temperature that allows formation of γ-β^B duplexes. The temperature was then raised to 68°C, a temperature above the melting temperature of the heterologous β-γ duplexes but below the melting temperature of the homologous α-α duplexes. The single-stranded β^B cDNA was separated from α cDNA–α mRNA duplexes by batch chromatography on hydroxyapatite. Using these methods, we have prepared cDNA's that are specific for each of the sheep globins and are contaminated with only 5 to 15 percent α globin cDNA. Human β globin cDNA was prepared as described with mRNA from reticulocytes of a patient with α thalassemia (Hb H disease) (*9*). A cDNA specific for human γ globin sequences was obtained by using fetal reticulocyte mRNA (α,β,γ) to prepare mixed cDNA. The γ cDNA sequences were recovered by hybridization of this mixed probe to adult reticulocyte mRNA (α,β), followed by recovery of the single-stranded γ cDNA by hydroxyapatite chromatography (*10*).

We initially tested Kabat's gene excision hypothesis using DNA from the bone marrow of an anemic sheep. The animal was homozygous for HbA ($\alpha_2\beta_2{}^A$). After 15 days of phenylhydrazine injection, it was severely anemic (hematocrit, 10 to 12 percent), and mRNA prepared from its reticulocytes directed the synthesis only of β^C and α globin when translated in Xenopus oocytes (*11*). Because of anemia the ratio of myeloid to erythroid cells (M : E) in the