Pesticide Interaction Creates Hybrid Residue

Abstract. *When applied in combination, the herbicides* N-*(3,4-dichlorophenyl)-propionamide (propanil) and* N-*(3-chloro-4-methylphenyl)-2-methylpentanamide (solan) were transformed in soil to an unexpected residue—asymmetric 3,3′,4-trichloro-4′-methylazobenzene. Each herbicide contributed one-half of the asymmetric azobenzene molecule.*

In order to control pests and improve production of food and fiber, several pesticides are required for each crop. Such chemicals or their degradation products may interact in complex and sometimes unexpected manners. As an example, the herbicide *N*-(3,4-dichlorophenyl)-propionamide (propanil) and certain insecticides, harmless to rice individually, become destructive in combination. The insecticides inhibit the rice enzyme that would normally detoxify the herbicide and protect the plant (*1*). I now describe a new type of pesticide interaction—combined residue formation.

The herbicides propanil and *N*-(3-chloro-4-methylphenyl)-2-methylpentanamide (solan) both undergo biochemical transformation in soil to the respective symmetric azobenzene residues (*2–4*). Both herbicides are licensed for use on the same crop (*5*). Some substituted anilines, when incubated together in soil, are transformed to asymmetric azobenzenes, in addition to the expected symmetric ones (*6*). The initial step of propanil and solan degradation liberates the respective aniline moieties (*2–4*). In the case of simultaneous or successive herbicide applications, 3,4-dichloroaniline (DCA) and 3-chloro-4-methylaniline (CMA) released from propanil and solan respectively, might combine to form an asymmetric azobenzene, each herbicide supplying one half of the molecule. As such an unexpected residue could be overlooked or mistaken as the degradation product of symmetric azobenzenes, both theoretical interest and practical considerations prompted this investigation.

3,3′-Dichloro-4,4′-dimethylazobenzene (DCDMAB) and 3,3′,4,4′-tetrachloroazobenzene (TCAB) standards were synthesized as described (*2*, *4*). Asymmetric 3,3′,4-trichloro-4′-methylazobenzene (TCMAB) was synthesized by reacting equimolar amounts of 3,4-dichloronitrosobenzene and CMA in glacial acetic acid. The product was separated by filtration and recrystallized from acetone (melting point, 133° to 134°C).

For transformation studies, analytically pure samples of propanil and solan (50 mg each) were mixed into 100 g (dry weight) of fresh Nixon sandy loam (*p*H, 6.0). A similar soil sample received analytically pure DCA (50 mg) and CMA (50 mg). Both soil samples were moistened to 60 percent of their water-holding capacity and were incubated at 27°C in beakers covered with polyethylene film. The samples were aerated daily, and their moisture content was maintained throughout the experiment.

The aniline- and the herbicide-treated samples were extracted and analyzed by quantitative gas chromatography (*3*, *4*) after 1 and 3 weeks, respectively. To achieve increased resolution and sensitivity, the liquid phase of the column packing was increased from 5 to 10 percent and the soil extracts were concentrated 100-fold by evaporation before injection. Residue quantities were calculated by comparing their peak heights to those of appropriate standards. All compounds of interest were quantitatively recovered with the exception of the two anilines. These were partially lost during concentration of the extracts and, therefore, were not measured quantitatively in these experiments.

For mass spectrographic analysis azobenzenes from the soil extracts were purified by two-dimensional preparative thin-layer chromatography on silica gel (Merck F-254) plates. The chromatogram was developed by benzene, and all three azobenzenes moved with an R_F of 0.65. After being dried, the plate was developed in the second direction by hexane. The azobenzenes moved as a single spot with an R_F of 0.28 and were completely separated from the other components of the soil extracts.

Solan and propanil incubated in soil individually produced only symmetric azobenzenes. When the two herbicides were incubated in soil together (Fig. 1), in addition to the former azo peaks (Nos. 5 and 7) a new azo peak (No. 6) appeared. Retention time of this new peak was identical to that of the synthetic TCMAB. An azo compound with identical retention time was produced also in the aniline-treated soil.

The mass spectogram of the azobenzene mixture revealed a pattern with mass numbers 298, 300, 302, and 304 that was characteristic for TCMAB, and could not be confused with the patterns of either of the two other azobenzenes (DCDMAB, 278/280/282; TCAB, 318/320/322/324/326). Thus, the identity of the new metabolite was established by two independent methods.

Table 1. Residues in 100 grams of soil treated with (A), 50 mg CMA + 50 mg DCA, 1 week incubation; (B) 50 mg solan + 50 mg propanil, 3 weeks incubation. Numbers in parentheses refer to Fig. 1.

Compound	A (mg/sample)	B (mg/sample)
Propanil (3)		0.80
Solan (4)		35.00
DCDMAB (5)	4.75	0.50
TCMAB (6)	3.15	1.43
TCAB (7)	3.82	4.86

All three azobenzenes were produced in nearly equal quantities from the anilines (Table 1). The aniline moieties were liberated from the herbicides at different rates, and this influenced the proportions of the azobenzene residues produced. As propanil was degraded

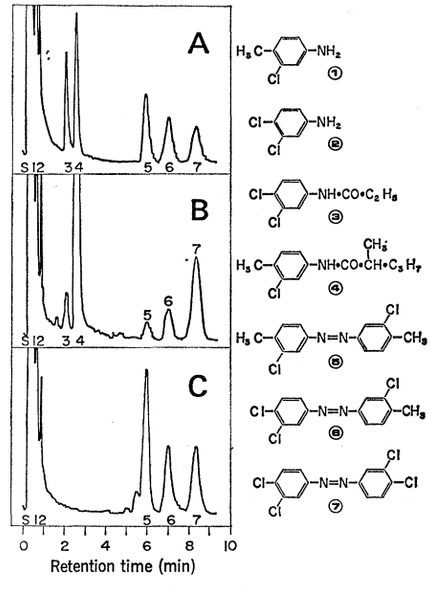

Fig. 1. Asymmetric azobenzene formation from propanil and solan in soil. Left: (A) Gas chromatogram of a reference solution containing per 1 μl injection volume 80 nanograms each of CMA (1) and DCA (2), 400 nanograms each of propanil (3), solan (4), DCDMAB (5), TCMAB (6) and TCAB (7); (B) an extract of soil that was incubated for 3 weeks with 500 ppm each of propanil and solan; (C) an extract of soil that was incubated for 1 week with 500 ppm each of CMA and DCA. *S*, solvent response. Right: Chemical structures of compounds causing the gas chromatograph responses.

more rapidly than solan, TCAB was the dominant azo residue. Second ranking was the asymmetric TCMAB and lowest was DCDMAB. This was to be expected because, in view of the rapid rate of propanil degradation, an excess of DCA molecules competed for CMA molecules released from the more slowly decomposing solan. Consequently, probability of asymmetric azobenzene formation is high if the two herbicides are applied simultaneously or if solan treatment precedes propanil treatment. Asymmetric azobenzene formation is unlikely if propanil treatment precedes solan treatment.

In conclusion, I have established that degradation products of two or more pesticides may combine in soil to form unexpected hybrid products. Insofar as similar reactions may occur in the field, the complexity of pesticide residue problems is increased.

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Vascular Smooth Muscle Reactivity in Normotensive and Hypertensive Rats

Abstract. *Aortic strips from spontaneously hypertensive rats were less responsive than normal animals to the contractile effects of norepinephrine, serotonin, and potassium chloride but more reactive to the relaxant effects of the stimulant of beta receptors, isoproterenol. Thus, hypertension is not the result of an absence of beta receptor or a hypersensitivity of the vascular smooth muscle.*

Increased peripheral resistance of the vascular system is characteristic of hypertension in humans (*1*). We have compared the reactivity in vitro of thoracic aortas from normotensive rats with those from a strain of spontaneously hypertensive rats (SHR) (*2*). The thoracic aorta was used as an indicator of vascular reactivity because it can be readily prepared for the recording of pharmacologic responses, although the aorta exerts little, if any, effect on total peripheral response. The hypertension exhibited by SHR may be analogous to essential hypertension in humans because histopathological changes are absent, especially in the cardiovascular-renal system before the development of hypertension (*3*). Aortas from hypertensive rats are less reactive to norepinephrine, serotonin, and KCl than those from normotensive animals. In addition, thoracic aortas from both the hypertensive and normotensive rats have beta adrenergic as well as alpha adrenergic receptor sites.

The blood pressures of unanesthetized male Wistar rats of the spontaneously hypertensive strain and normotensive Wistar rats (Carworth Farms), 7 to 10 weeks old, were measured by the tail-plethysmographic method (*4*). The animals were killed by a blow on the head, and spirally cut thoracic aortic strips were prepared by the method of Furchgott and Bhadrakom (*5*). Each strip was suspended in an isolated-organ bath (10 ml) containing a modified Krebs bicarbonate solution of the following composition (in grams per liter): KCl, 0.35; $CaCl_2 \cdot H_2O$, 0.37; KH_2PO_4, 0.16; $MgSO_4 \cdot 7H_2O$, 0.29; NaCl, 6.9; $NaHCO_3$, 2.08; and glucose, 1.8. The temperature of the baths was maintained at 37.5°C, and the Krebs bicarbonate solution was oxygenated with a mixture of 95 percent oxygen and 5 percent carbon dioxide. The strips were subjected to an initial tension of 1 g and were kept in the organ baths for approximately 1 hour before drugs were tested. Responses of the strips to drugs were measured isometrically with a force-displacement transducer and recorded on a polygraph as changes in tension in grams. The following drugs were used: L-norepinephrine bitartrate (Calbiochem), serotonin creatinine sulfate (Sigma Chemical), L-isoproterenol-D-bitartrate (Sterling-Winthrop Labs), and KCl (Merck and Co., reagent grade). The concentrations of norepinephrine, serotonin, and isoproterenol are expressed in terms of the free base.

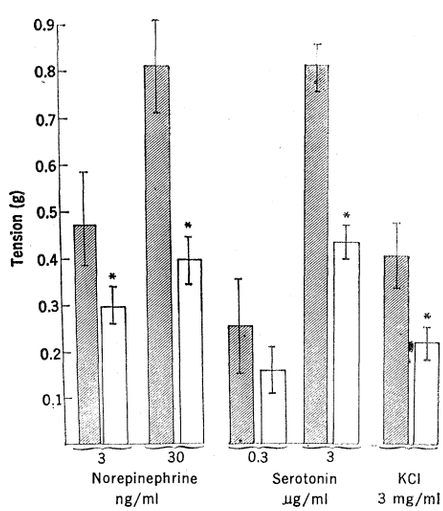

Fig. 1. A comparison of the contractile responses of thoracic aortic strips from five normotensive and nine spontaneously hypertensive rats to norepinephrine, serotonin, and KCl. Data given as means ± standard errors. The asterisks indicate statistically significant differences, $P < .05$. Shaded bars, normotensive animals; open bars, SHR.

The mean systolic blood pressure of nine SHR rats was 174 ± 6 mm-Hg, compared with 122 ± 6 mm-Hg in five normotensive rats. Contractile responses of aortic strips from the SHR to norepinephrine, serotonin, and KCl were less than corresponding responses of aortic strips from the normotensive rats (Fig. 1). The difference was observed not only at the concentrations shown, but over the entire dose-response range. In fact, the maximum contractions of the aortas from normotensive animals were about twice those of the aortas from the SHR (tension, 0.9 compared with 0.5 g).

Thoracic aortas of young rats and rabbits relax in response to stimulation of beta adrenergic receptors whereas aortas from old rats and rabbits lose this response (*6*). Since stimulation of beta adrenergic receptors is in part responsible for vasodilatation in certain vascular beds, we considered that loss of activity of beta adrenergic receptors might be a factor in the production of hypertension in SHR. We compared responses to stimulation of beta receptors of thoracic aortas from SHR and normotensive rats. These tissues were first made to contract with serotonin, and then the relaxation produced by isoproterenol, a beta adrenergic stimulant, was measured. Activity of beta receptors was present in the aortas from each group of rats (Fig. 2). In fact, the responses of aortas from the SHR were greater than those from the normotensive animals.