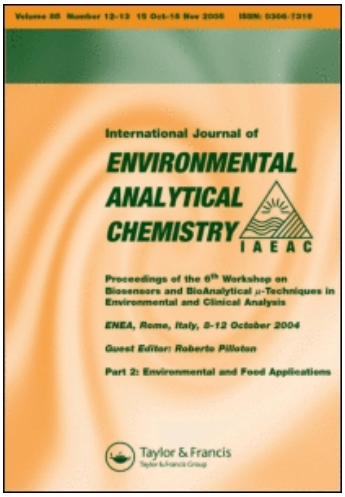

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AMES MUTAGENICITY TESTS OF THREE ACETANILIDE HERBICIDES DURING THEIR ALKALINE DEGRADATION

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Three acetanilide herbicides—propanil, propachlor and pronamide—were studied for alkaline degradation using the HPLC technique and found to be relatively stable for chemical degradation with the highest hydrolysis rate for pronamide ($k_b = 0.4966\ M^{-1}\ hr^{-1}$), followed by propachlor ($k_b = 0.1466\ M^{-1}\ hr^{-1}$) and propanil $k_b = 0.0154\ M^{-1}\ hr^{-1}$). Simultaneously, the Ames mutagenicity test for non-degraded and half-degraded acetanilides have been performed. Testing two types of samples (primary chemicals and mixtures of half-degraded primary chemicals with their degradation products, both in 10%-acetonitrile buffer; the ranges of concentrations were 2–400 mg l^{-1} for propanil and propachlor and 2–50 mg l^{-1} (for pronamide) have shown the increase in mutagenicity for propanil, the decrease for propachlor and almost the same dose-response curve for pronamide during their degradation. The preliminary results of the research indicate that some environmental problems related to the impact of pesticide degradation products can appear, not only to a pesticide itself.

KEY WORDS: Herbicides, acetanilides, HPLC, alkaline degradation, Ames mutagenicity test.

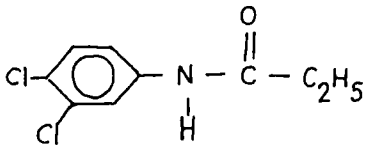
INTRODUCTION

Worldwide use of pesticides in agriculture causes the necessity to study their migration into soil and groundwater, accumulation in living organisms, as well as toxicity and mutagenicity. Application of pesticides is usually regulated on the basis of their degradation rates, relying upon idea that degradation products have no (or at least less) negative effects on biota. However, there are findings that some degradation products can be more active than the primary chemical. Thus, we have decided to try to monitor how the mutagenicity of three acetanilides is being changed during their degradation, when a complex mixture of the primary chemical with its degradation products is formed, as it is common in nature.

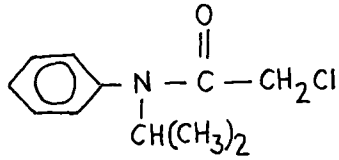
EXPERIMENTAL

Materials

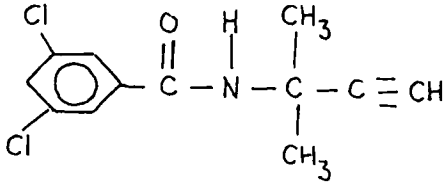
Three acetanilides—propanil, propachlor and pronamide—have been studied. Their structural formulae are given in Figure 1. These chemicals were obtained from the



PROPANIL



PROPACHLOR



PRONAMIDE

Figure 1 Chemical structures of the tested compounds.

Athens Environmental Research Laboratory (Georgia, USA), their purity was about 99.9%. The acetonitrile (HPLC/Spectro Grade) has been obtained from "Pierce" (USA).

Degradation rate studies

The alkaline degradation of three acetanilides mentioned above has been studied using the HPLC technique. A typical hydrolysis experiment consisted of preparing standard solution of the compound of interest, preparing buffer solution of the compound and transferring the latter to individual "rate point tubes" (2 ml sealed ampules), then determining the remaining amount of the compound for each individual tube. The degradation products have been neither determined nor identified. Initial screening hydrolyses runs were performed at three [OH^-] levels: 0.1 N (in case of pronamide it was 0.025 N), 0.01 N and 0.001 N NaOH, and at two temperatures: for propachlor and pronamide $70 \pm 1°C$ and $52 \pm 1°C$, and for propanil $106.5 \pm 1°C$ and $85 \pm 1°C$.

Hydrolysis rate constants were calculated according to J. Ellington *et al.*[1] and found to be rather stable for chemical degradation with the highest hydrolysis rate for pronamide ($k_b = 0.4966\ M^{-1}\ hr^{-1}$), followed by propachlor ($k_b = 0.1466\ M^{-1}\ hr^{-1}$) and propanil ($k_b = 0.0154\ M^{-1}\ hr^{-1}$).

Determination of mutagenicity

Methods. The Ames test (without metabolic activation)—both the standard plate-incorporation and the modified pre-incubation techniques—with three strains of *Salmonella typhimurium* (TA 97, TA 98 and TA 100) has been applied. The standard test[2–4] was used to study water solutions of acetanilides. However, the solubility of the compounds studied is rather low. Therefore, we were able to evaluate the effect of rather low concentrations. To test compounds in higher concentrations, acetonitrile was used as a solvent. However, acetonitrile in concentrations exceeding 15% was toxic for bacteria. Therefore we had to apply modified pre-incubation test.

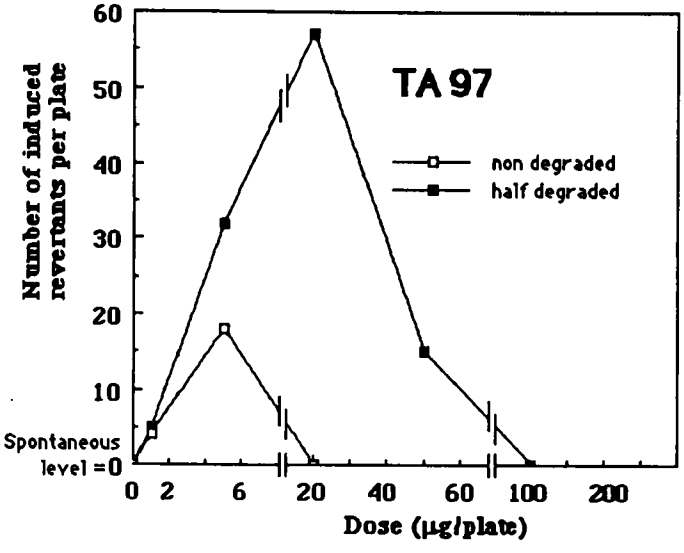
The procedure of pre-incubation was standard[2] except that at first the following mixture was prepared: 2.5 ml of Vogel–Bonner minimal medium, 6.4 ml of distilled water and 1 ml of acetonitrile with a test chemical diluted in it. The result was a 10% acetonitrile solution. 0.5 ml of this solution with 0.1 ml of overnight culture was incubated at 37°C for 20 minutes. After this, the procedure was the same as in the standard plate-incorporation test with two layers of agar media: the mixture was poured into tubes with 2 ml of 0.5% agar (held at 43°C in a water bath) and then into Petri plates.

Such a modification allowed us to study mutagenic effect of the following concentrations: 400, 100, 40, 10 and 2 mg l^{-1} for propanil and propachlor, and 50, 30, 10 and 2 mg l^{-1} for pronamide. Here it should be mentioned that the solubility of these compounds in water is as follows: 580, 225 and 15 mg l^{-1} (at 25°C) for propachlor, propanil and pronamide, correspondingly (although propachlor is more soluble -580 mg l^{-1}, we have not reached this concentration in water practically).

Preparation of half-degraded samples. Two glass ampules (5 ml each) with alkaline solutions of propanil (400 mg l^{-1}) were held at 106.5°C, whereas ampules with propachlor (400 mg l^{-1}) and pronamide (50 mg l^{-1}) were held at 70°C. The corresponding exposure times were 23.73, 8.83 and 1.92 hrs, i.e. until the compound was degraded to 50% of its primary amount. These degradation half-times were determined in hydrolysis rate experiments (see above). Then approximately 0.15 ml of 0.5 M K_2HPO_4 solution (to make pH 7), 1 ml of acetonitrile, 2.5 ml of Vogel–Bonner medium and distilled water (to 5 ml) mixed with the volume of each ampule were added. The result was a 10% solution of the tested compound in acetonitrile. Then 0.5 ml of such mixture with 0.1 ml of bacterial culture was tested for mutagenicity applying pre-incubation technique as described above.

RESULTS AND DISCUSSION

Although there was a joint research on chemical degradation and mutagenicity changes during degradation of three acetanilides, in this paper the attention was



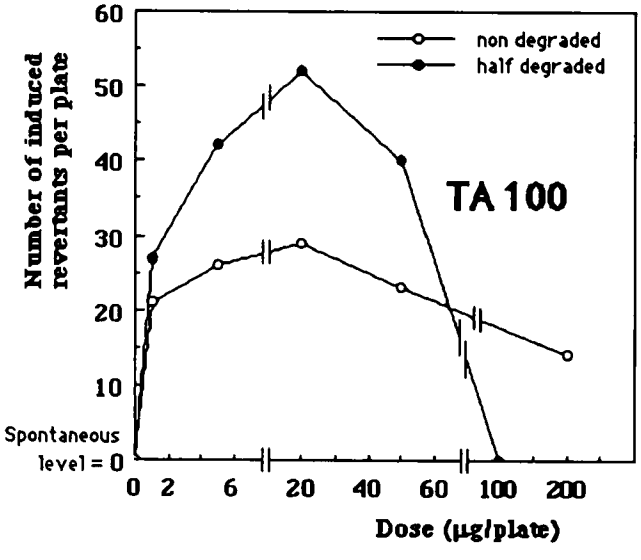


Figure 2 Dose-response curves of non-degraded propanil (—○—) and mixture of half-degraded propanil with its degradation products (—●—). The levels of spontaneous revertants per plate were as follows: for propanil 154 ± 3 (TA 97), 28 ± 2 (TA 98) and 149 ± 4 (TA 100), and for mixture 131 ± 3 (TA 97), 30 ± 1 (TA 98) and 174 ± 2 (TA 100). No significant mutagenic effect was observed for strain TA 98. The blank had no mutagenic effect.

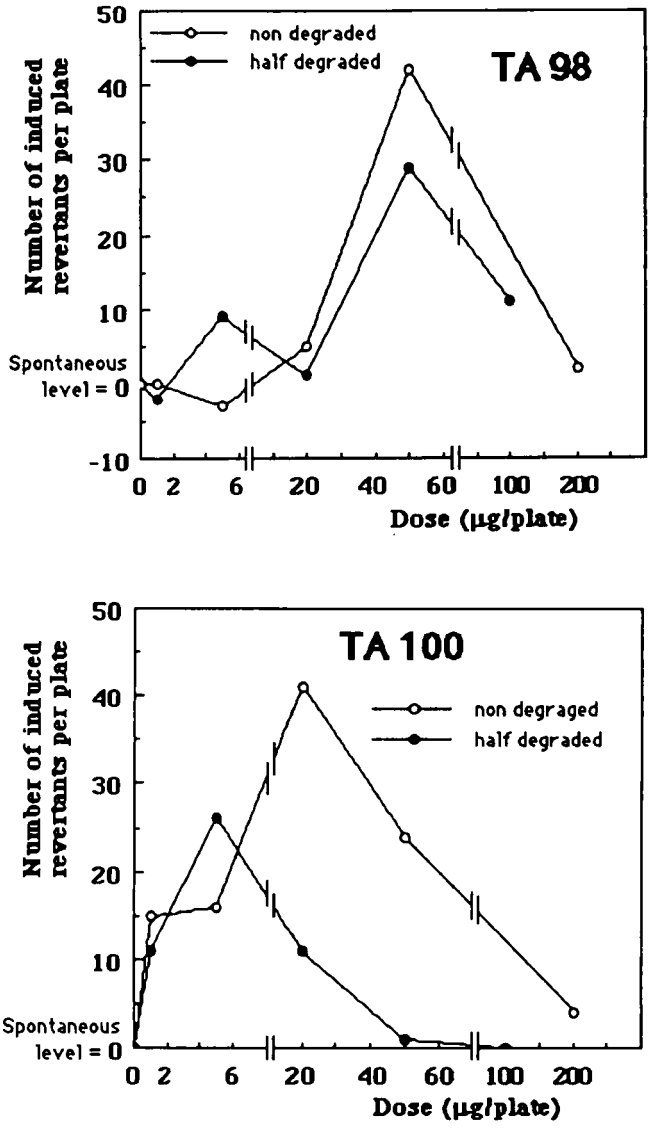


Figure 3 Dose-response curves of non-degraded propachlor (—○—) and mixture of half-degraded propachlor with its degradation products (—●—). The levels of spontaneous revertants per plate were as follows: for propachlor 157 ± 2 (TA 97), 30 ± 2 (TA 98) and 174 ± 3 (TA 100), and for mixture 163 ± 2 (TA 97), 31 ± 1 (TA 98) and 144 ± 6 (TA 100). No significant mutagenic effect was observed for strain TA 97. The blank had no mutagenic effect.

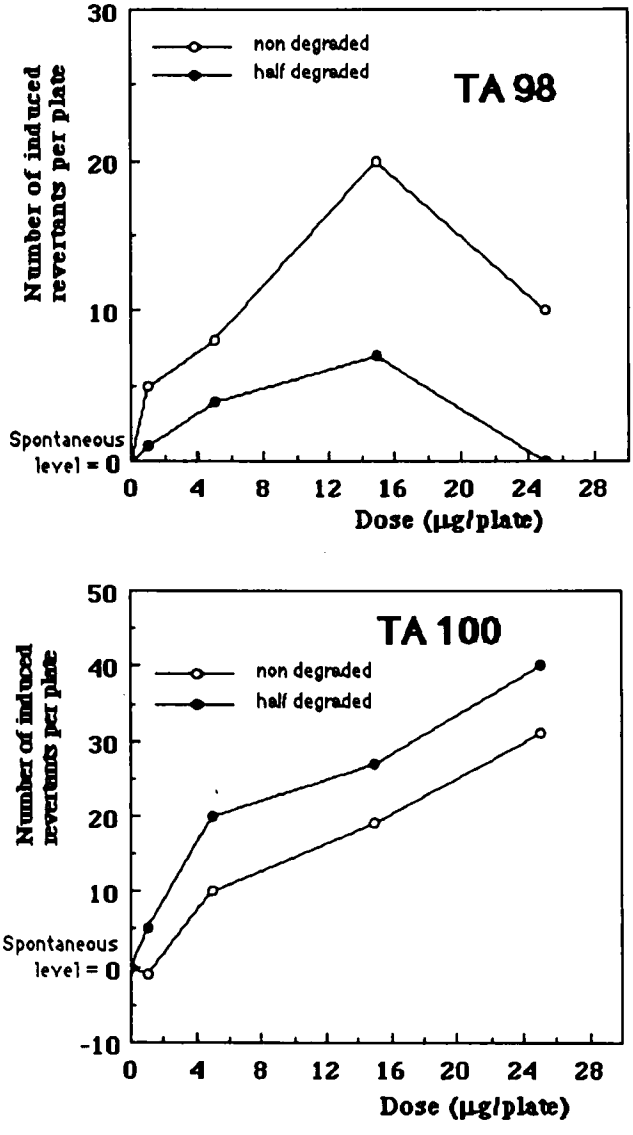


Figure 4 Dose-response curves of non-degraded pronamide (—○—) and mixture of half-degraded pronamide with its degradation products (—●—). The levels of spontaneous revertants per plate were as follows: for pronamide 144 ± 6 (TA 97), 25 ± 1 (TA 98) and 165 ± 4 (TA 100), and for mixture 124 ± 8 (TA 97), 29 ± 2 (TA 98) and 159 ± 4 (TA 100). No significant mutagenic effect was observed for strain TA 97. The blank had no mutagenic effect.

concentrated on their mutagenic effect, presenting here only hydrolysis rate constants. Detailed information about chemical degradation experiments carried out together with our colleagues from the Athens Environmental Research Laboratory (US EPA) will be published separately.

Also we do not present in this paper the results of mutagenicity studies of the above-mentioned acetanilides dissolved in water, because they did not induce mutations in our experiments.

The Figures 2, 3 and 4 show the mutagenicity of propanil, propachlor and pronamide before degradation and after half-degradation. In the latter case, summarized mutagenicity of given acetanilide and its degradation products has been obtained.

Perhaps most interesting results were obtained for propanil (Figure 2). The mutagenicity of propanil during degradation increased for strains TA 97 and TA 100, inducing both frameshift and base pair substitution mutations. However, strain TA 98 did not respond to the action of propanil. Thus, we can conclude that degradation products are more active than pure propanil, and that different features of bacterial strain influence the expression of the mutagenicity.

The opposite effect was obtained with propachlor (Figure 3): its mutagenicity decreased with degradation. Most prominent decrease was with TA 100, less with TA 98, whereas TA 97 did not show statistically significant effect. So in this case, degradation products are less mutagenic than propachlor itself.

The pronamide's mutagenic effect was less elevated than that of the above two compounds, most likely due to the lower concentrations tested (Figure 4).

So, we think that the preliminary results (we are expanding now such studies both in number of compounds and in experiment conditions) show that there can be some environmental (toxicological, carcinological etc.) problems not only with e.g. soil residues of a pesticide, but also with their degradation products.

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