of the mylecule. Indeed, as pointed out, demethylation of the aromatic amine has occurred as shown by the detection of the corresponding hydroquinone derivative.

No unchanged 4-dimethylamino-3,5-xylyl methylcarbamate was found in the urine. The only unconjugated metabolite identified was 4-dimethylamino-3,5-xylenol.

Other ether-soluble radioactive substances were detected but they remain unidentified. Fractions [1*D*] and [1*F*] of Figure 1 could be identical, and chromatographic evidence (Table II) strongly suggests this to be the case. The compound represented by these two fractions is not a carboxylic acid or conjugated sulfate ester since it had an apparent distribution coefficient between ether and 10% sodium bicarbonate solution of 5.3. Any acidic material would have had a coefficient of zero. Fraction [1*E*], an acidic material, might possibly be 6-dimethylamino-3,5-cresotic acid.

In the animal body, phenols undergo two main reactions: conjugation of the hydroxyl group to form both glucuronides and ethereal sulfates. Since hydrolysis of the water-soluble radioactive material resulted in the identification of 4-dimethylamino-3,5-xylenol and 2,6-dimethylhydroquinone, both of these phenols may be present as these conjugated forms. Electrophoretic mobilities of the water-soluble compounds are consistent with the above interpretation.

There is no evidence for the presence of 4 - dimethylamino - 3,5 - dimethylpyrocatechol, a metabolite found in broccoli as a result of Zectran treatment (*7*), in the urine. Apparently, there is no tendency for the dog to hydroxylate this highly substituted aromatic ring to any significant extent. If this had occurred, it should have been possible to find it as a conjugate in the urine.

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INSECTICIDE RESIDUES

The Colorimetric Determination of o-Isopropoxyphenyl-*N*-methylcarbamate

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A method for the determination of o-isopropoxyphenyl-*N*-methylcarbamate residues on various surfaces immediately after spraying and at intervals thereafter has been developed. The insecticide was diazotized with 3-nitroaniline-4-sulfonic acid and determined spectrophotometrically at 490 mμ. The color that developed was very stable in water and obeyed the Beer-Lambert law over the range tested. The method was adapted for the estimation of Sevin, Isolan, Pyrolan, Dimetilan, and Hercules AC-5727.

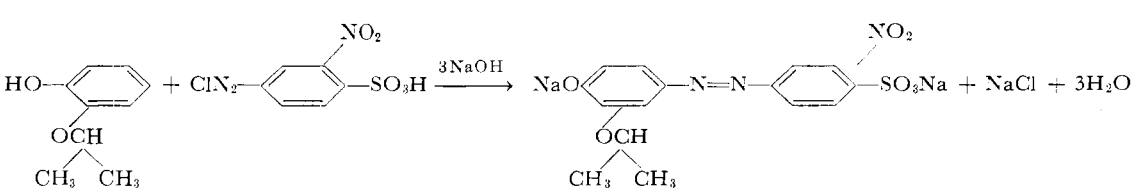
Since the introduction of the first commercial carbamic acid ester, Sevin, considerable interest has been aroused in the use of compounds belonging to this group for pest control. Bayer 39007 (*o*-isopropoxyphenyl-*N*-methylcarbamate) has shown a high degree of activity against mosquitoes and flies. During trials with this compound as a possible insecticide in an antimalarial campaign, it became necessary to estimate residues of the substance on various surfaces. No method was recorded in the literature for the determination of this compound, but other carbamate insecticides were usually determined by the estimation of the phenol obtained upon hydrolysis, by coupling it with *p*-nitrobenzenediazonium fluoroborate (*5*), or 4-amino antipyrine (*2*); by the determination of the aliphatic or aromatic amine found upon hydrolysis (*6*); and by cholinesterase-inhibition methods (*1*).

A simplified procedure has been found in this laboratory for the estimation of microgram quantities of *o*-isopropoxyphenyl-*N*-methylcarbamate, and its possible application to the determination of other compounds is now suggested.

o - Isopropoxyphenyl - *N* - methylcarbamate was dissolved in methanol and hydrolyzed with dilute aqueous sodium hydroxide solution. The *o*-isopropoxyphenol obtained was coupled with 3-nitroaniline-4-sulfonic acid (*4*). In alkaline solution, a red dye (I) was obtained in accordance with the equation.

(I) was stable in water and very soluble, due to its double sodium salt character. Thus, a great advantage was achieved over the colorimetric methods which used organic solvents in the color development stage. Results showed good agreement with the Beer-Lambert law, and were accurate and reproducible.

Five other carbamic acid esters were tested—Sevin, Dimetilan (dimethyl 3-



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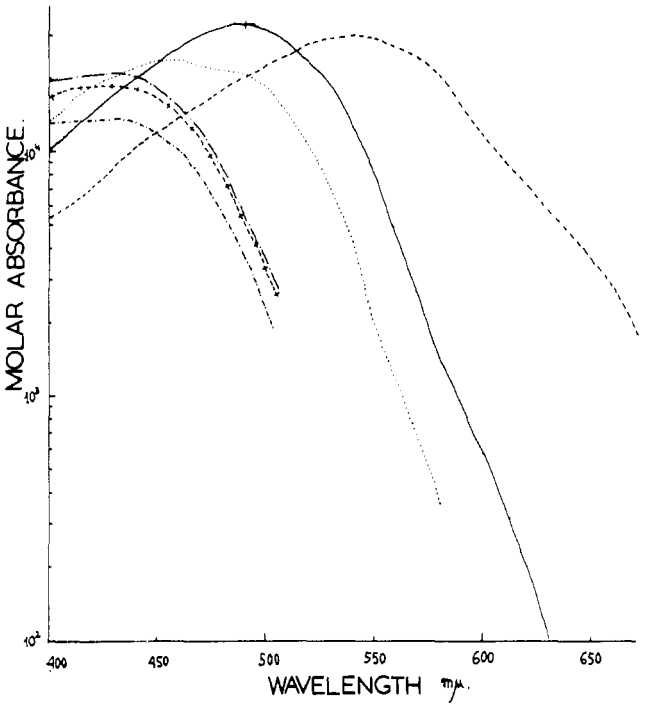

Figure 1. Absorption curves of carbamates

Diazotizing agent: 3-nitroaniline-4-sulfonic acid

——— Bayer 39007
– – – – Sevin
..... Hercules 5727
–·–·– Dimetilan
—·—·— Pyrolan
+—+ Isolan

Table I. Maximum Absorbance Values for Colored Intermediates of Carbamate Insecticides

Compound	Max. Absorbance (Molar Absorptivity), Sulfonated Compound	Max. Absorbance (Molar Absorptivity), Diazo Fluoroborated Compound
Bayer 39007	490 (3.3×10^4)	530 (2.7×10^4)
Sevin	540 (3.0×10^4)	590 (3.8×10^4)
Hercules 5727	460 (2.4×10^4)	500 (3.0×10^4)
Dimetilan	430 (1.4×10^4)	485[a]
Pyrolan	430 (2.1×10^4)	475[a]
Isolan	430 (1.9×10^4)	485[a]

[a] See (5).

Table II. Color Development Rate of o-Isopropoxyphenol Coupling Product

Concentration, μG. per Ml.	Absorbance at Time 5 min.	10 min.	20 min.	30 min.	120 min.	24 hours
0.5	0.055	0.065	0.070	0.070	0.069	0.065
2.0	0.279	0.288	0.294	0.294	0.290	0.285
3.2	0.453	0.470	0.477	0.476	0.471	0.462
4.8	0.664	0.677	0.683	0.683	0.680	0.661

Table III. Color Development Rate with 3-Nitroaniline-4-Sulfonic Acid as Coupling Reagent

Time Lapse after Reaction	Sevin, 540 mμ	Hercules 5727, 460 mμ	Dimetilan, 430 mμ	Isolan, 430 mμ	Pyrolan, 430 mμ
5 Min.	0.698	0.556	0.323	0.372	0.306
10 Min.	0.705	0.556	0.326	0.374	0.310
30 Min.	0.705	0.562	0.329	0.378	0.314
60 Min.	0.705	0.567	0.336	0.385	0.317
120 Min.	0.706	0.573	0.337	0.387	0.318
24 Hours	0.700	0.563	0.329	0.383	0.318

methylpyrazol-5-yl carbamate), Pyrolan (dimethyl 3-methyl-1-phenylpyrazol-5-yl carbamate), Isolan (dimethyl 1-isopropyl-3-methylpyrazol-5-yl carbamate), and Hercules 5727 (*m*-isopropylphenyl *N*-methylcarbamate). All these compounds were hydrolyzed according to the procedures outlined by Miskus *et al.* (5) to give the appropriate coupling suitable moieties. As an example, Bayer 37344 (3,5-dimethyl-4-methylthiophenyl-*N*-methylcarbamate) was hydrolyzed too, but the phenol obtained had a blocked *p*-position and therefore did not react with the diazotizing reagent. As expected, no ortho coupling occurred either.

In all cases, a stable, water soluble, colored compound was obtained. Absorption peaks data for the different products are summarized in Table I. For comparison, the maximal absorption data for the *p*-nitrobenzenediazonium-fluoroborate are given. An expected decrease of 40 to 50 mμ was observed in the spectrum of the new sulfonated-colored compounds.

Experimental

Reagents. Diazotizing Reagent. 3-Nitroaniline-4-sulfonic acid (1200 mg.), 600 mg. of anhydrous sodium carbonate, and 400 mg. of sodium nitrite were dissolved in 1 liter of distilled water. This reagent could be stored indefinitely. The diazotizing reagent was always prepared immediately before use by adding 45 ml. of the above resulting solution to 5 ml. of concentrated hydrochloric acid (analytical grade). The color of the diazotizing solution should be very pale yellow, and should be discarded if it acquires a deep yellow coloration.

Bayer 39007, Sevin, Dimetilan, Pyrolan, Isolan, and Hercules 5727. Pure samples.

Apparatus. Visible region spectrophotometer.

Procedure. Absorption Spectrum. Studies were first carried out on the absorption spectrum of the red azo dye (*I*). Twenty-five milligrams of the carbamate were dissolved in 250 ml. of methanol. Four milliliters of the resulting solution was pipetted into a 25-ml. volumetric flask and 2 ml. of 0.5*N* solution of sodium hydroxide added. After the addition of 3 ml. of distilled water, the solution was kept for 20 minutes at 80° C. on a water bath. After cooling, 1 ml. of the diazotizing reagent, followed by one drop of 5*N* sodium hydroxide, was added. The solution was held at room temperature for 20 minutes to allow the color to develop fully. A study of this aqueous solution revealed a peak at 490 mμ [molar absorptivity $\epsilon = 3.3 \times 10^4$ (Figure 1)]. All further absorption measurements were carried out at 490 mμ.

Color Development Rate. The absorbance of the colored solution reached its optimal value after 20 minutes. The color was stable, and only a slight change occurred in its absorbance thereafter. In Table II absorbance values for different concentrations are given. Although most of the reaction was completed within the first 5 minutes, the color was allowed to develop for 20 minutes in all following experiments.

Calibration Curve. Twenty-five milligrams of pure *o*-isopropoxyphenyl-*N*-methylcarbamate was dissolved in absolute methanol and diluted in a 250-ml. volumetric flask to the mark (solution A). Twenty milliliters of solution A was diluted to 100 ml. with methanol (solution B). Aliquots of 0.5, 1.0, 2.0, 2.5, 4.0, 6.0, and 7.0 ml. of solution B were transferred to 25-ml. volumetric flasks. Two milliliters of a 0.5*N* sodium hydroxide solution and 3 ml. of distilled water were added, and the resulting mixture was kept on a water bath of 80° C. for 20 minutes. After mixture cooled, 1 ml. of diazotizing reagent and 1 drop of 5*N* sodium hydroxide solution were added. The red-colored azo-dye

solutions obtained were diluted to the 25-ml. mark with distilled water, and the final concentrations thus obtained corresponded to 0.4, 0.8, 1.6, 2.0, 3.2, 4.8, and 5.6 μg/ml. of the original carbamate. After the solutions were kept for 20 minutes at room temperature, the absorbance at 490 mμ was recorded. The results were plotted against concentrations and showed a linear relationship over the range tested.

TREATMENT OF ANALOGOUS COMPOUNDS. Sevin, Hercules 5727, Dimetilan, Isolan, and Pyrolan were hydrolyzed (*5*) to give α-naphthol, *m*-isopropylphenol, 3-methylpyrazol, 3-methyl-1-isopropylpyrazol, and 3-methyl-1-phenylpyrazol, respectively. These compounds were coupled with 3-nitroaniline-4-sulfonic acid as described above.

Spectrophotometric data are summarized in Table I. The color developed fully for Sevin during 10 to 15 minutes, while an hour was required for the other compounds (Table III).

Recovery Tests with *o*-Isopropoxyphenyl Carbamate. *o*-Isopropoxyphenyl-*N*-methylcarbamate was sprayed in the native huts in the vicinity of Lagos. The surfaces involved were mainly mud walls, thatch, and wood. Samples were collected only from the first two types. (Sampling from wood by washing the sprayed surface with a solvent gave very low results. These were in contradiction with biological methods used in parallel to the chemical methods, probably because the solvent impregnated the insecticide into the wood texture rather than extracting it.)

Initial insecticide concentration was determined by the filter paper method (*3*), while mud samples were taken by scraping. Thatch samples were taken by cutting out a known area. Preliminary tests have shown that by extraction with methanol the insecticide could be recovered completely from filter papers, mud, and thatch. For these tests, filter papers (Whatman No. 2), thatch cuts, and small mud bricks (specially prepared out of the same mud used in building the native huts) were treated with known amounts of the compound and subsequently subjected to the analysis method as follows.

MUD BRICKS. A methanolic stock solution containing 20 mg. per ml. of *o*-isopropoxyphenyl-*N*-methylcarbamate was prepared. Aliquots of 0.1, 0.25, 0.5, 1.0, and 2.0 ml. of this solution corresponding to 2, 5, 10, 20, and 40 mg. of the insecticide were added to a series of previously prepared mud bricks.

Table IV. *o*-Isopropoxyphenyl-*N*-methylcarbamate from Mud, Thatch, and Filter Papers

Amount Added, μG.	Amount Found, μG.	Difference, μG.	Error, %
	MUD		
2.0	2.05	+0.05	+2.5
5.0	5.00	0.0	0.0
10.0	10.2	+0.2	+2.0
20.0	20.4	+0.4	+2.0
40.0	41.0	+1.0	+2.5
	THATCH		
5.0	4.95	−0.05	−1.0
10.0	10.1	+0.1	+1.0
20.0	19.9	−0.1	−0.5
40.0	39.0	−1.0	−2.5
	FILTER PAPERS		
2.0	1.93	−0.07	−3.5
5.0	5.12	+0.12	+2.4
10.0	10.1	+0.1	+1.0
20.0	20.0	0.0	0.0
40.0	39.1	−0.9	−2.1

After the solvent had dried, the bricks were crushed and quantitatively transferred with the aid of 150 ml. of methanol, into an extraction thimble placed in a Soxhlet apparatus. After boiling for 1 hour, the extract was cooled and transferred to a 250-ml. volumetric flask and diluted to the mark with methanol. Some mud samples gave a distinct coloration to the extract. However, after diluting as described, this coloration did not interfere with the azo dye color. Two milliliters of the resulting solution was transferred to a 25-ml. volumetric flask and hydrolyzed with 2 ml. of 0.5*N* sodium hydroxide solution and subsequently treated as described under "Calibration Curve." In the case of samples containing 40 mg., only 1 ml. was necessary.

FILTER PAPERS. Aliquots of 0.1, 0.25, 0.5, 1.0, and 2.0 ml. of the stock solution were added to a series of filter papers. After the solvent had evaporated, these papers were extracted twice with boiling methanol, and the extracts transferred to a 250-ml. volumetric flask and diluted to the mark. Two milliliters of the resulting solution was subjected to hydrolysis as described for "Mud Bricks," and subsequently treated in the same way.

THATCH SAMPLES. These were treated exactly as the filter papers. Recovery results are summarized in Table IV.

Field samples were treated as described under recovery tests. The method was not suitable for estimating traces of *o*-isopropoxyphenol obtained by degradation of the insecticide prior to the normal analytical procedure, and the quantities recovered included these small amounts. Some of the thatch samples were covered with a carbon deposit due to the use of open fires inside the dwellings. This gave some coloration to the extracts, but, as in the case of the mud samples, it was hardly perceptible after dilution and had no influence on the absorbance of the azo dye.

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