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Note

Improved clean-up procedure for the determination of small residues of carbophenothion in mice

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Carbophenothion is an acaricide and insecticide with a long residual action used as a wheat seed treatment to control the wheat bulb fly *(Leptohylemia coarctata)*. In order to determine small residues of carbophenothion in the body tissues of wood mice *(Apodemus sylvaticus)* trapped on a field sown with carbophenothion treated wheat seed, the analytical method of Jennings *et al.*[1] has been improved by the addition of a better clean-up step which allows greater sensitivity.

EXPERIMENTAL

Apparatus

Gas chromatograph (Series 104, Pye-Unicam, Cambridge, Great Britain) was fitted with a twin (phosphorus and sulphur) flame-photometric detector (Tracor, Austin, Texas, U.S.A.). The column (1.5 m × 4 mm I.D. glass) was packed with 3% SP-2100 on 100–120 mesh Supelcoport (Supelco via Chromatography Services, Hoylake, Great Britain).

Chemicals

n-Hexane and acetone were glass-distilled (Rathburn Chemicals, Walkerburn, Great Britain). Diethyl ether and anhydrous sodium sulphate were AnalaR grade (BDH, Poole, Great Britain), the sodium sulphate was further purified by heating at 400° for 18 h before use. Sand, purified by acid, 40–100 mesh was also from BDH. Acid alumina, activity I for column chromatography, was manufactured by Woelm Pharma (Eschwege, G.F.R.) and the Desaga Guilini dye-test kit by Desaga (Heidelberg, G.F.R.). Sunflower oil was from Alfonal (Byfleet, Great Britain). The carbophenothion used as a reference standard was > 98% pure, a gift from Stauffer (Westport, Conn., U.S.A.).

Procedure

Cut the sample (5 g) into small pieces and grind with anhydrous sodium sulphate (25 g) and sand (5 g) using a pestle and mortar to give a fine dry powder. Load this powder into a pre-extracted Soxhlet thimble and extract it for 16 h with diethyl ether in Soxhlet apparatus. Allow the extract to cool and remove the solvent using a rotary evaporator under reduced pressure.

Prepare acid aluminium oxide of Brockmann activity III by addition of water (4%, w/w) to activity I alumina followed by thorough mixing and storage in a tightly-capped bottle. Check the activity of each batch of deactivated alumina using a Desaga–Guilini dye test kit[2] or by determining the "fat capacity" of the prepared adsorbent[3] (10 g adsorbent in a 10-mm I.D. column retains 0.5 ± 0.05 g sunflower oil when eluted with 200 ml hexane).

Fill chromatography columns (20 mm I.D.) with activity III acid alumina to a height of 50 mm with gentle tapping. Dissolve the sample extract residue in *n*-hexane (2 ml) and apply this to the top of an alumina column.

Rinse the flask twice with 4-ml portions of *n*-hexane and transfer these to the top of the column. When the liquid meniscus touches the top of the adsorbent, add a further 10 ml *n*-hexane and discard the eluate. Elute with 5% (v/v) acetone in hexane (50 ml), collect the eluate and adjust its volume to 50 ml with the same solvent mixture.

Take a 5-μl aliquot for gas chromatography to detect residues above 0.1 ppm carbophenothion in the original tissue sample. For the detection of lower residues, the extract may be concentrated to 1 g original tissue per ml; further concentration results in slight broadening of the carbophenothion peak. A lower limit of detection of 0.002 μg carbophenothion per g tissue was obtained under these conditions.

RESULTS AND DISCUSSION

Extracts of half mice weighing up to 7 g and containing 10% (by weight) ether-extractable fat were cleaned up satisfactorily by the method described. Mouse bodies fortified with carbophenothion by multiple injection of an acetone solution 2 h before extraction showed a carbophenothion recovery of 85–90% over the entire analysis. Fortification of control mouse extracts concentrated to 1 ml per 1 g tissue weight showed no significant deviation in response from that of the corresponding standard carbophenothion solutions in 5% acetone in *n*-hexane. This alumina clean-up method enables carbophenothion to be measured to a lower limit of 0.002 ppm in animal tissues with a high fat content and has been used to demonstrate the magnitude of carbophenothion residues in a large number of mice trapped in a field trial.

REFERENCES

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