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Metabolic Conversion of N'-(2,4-dimethylphenyl)-N-methylformamidine Pesticide and the Analysis of the Metabolites

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N'-(2,4-dimethylphenyl)-N-methylformamidine is a relatively new pesticide developed in China. The pesticide is similar to amitraz or N'-(2,4-dimethylphenyl)-N-[(2,4-dimethylphenyl) imino]methyl-N-methylmethanimidamide. The metabolic fate of amitraz has been studied by Knowles (1981,1989). The present study describes the identification of N'- (2,4-dimethylphenyl)-N-methylformamidine and its metabolites in rat livers by GC/FTIR and GUMS, and the quantitative analysis of the metabolites in the urine, blood, liver, kidney and spleen by GC-ECD at various times following the oral treatment with N'- (2,4-dimethylphenyl)-N-methylformamidine. The absorption, distribution, degradation of the pesticide in rat body and excretion along with urine were discussed.

MATERIALS AND METHODS

N'- (2,4-dimethylphenyl)-N-methylformamidine and 2,4-dimethylaniline (purity >98%) were obtained from the Laboratory of Pesticide, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The other chemical agents were all of AR-grade, and petroleum ether was distilled again before use.

Male Wistar rats (180-220 g) were obtained from The Center for Experimental Animals, Institute of Biological Physics, Chinese Academy of Sciences. The rats were treated orally with N'- (2,4-dimethylphenyl)-N-methylformamidine dissolved in corn oil at a dosage of 25 mg/kg body weight. After treatment, urine was collected at the time interval of 0-2, 2-4, 4-6.5, 6.5-24, 24-48, 48-72, 72-144 hrs. 3 rats were killed and 20ml blood was taken at the times of 0, 2, 4, 6.5, 24, 48, 72, 144hrs respectively. Livers, kidneys and spleens of the rats were removed, weighted, and then frozen (-20°C) rapidly for subsequent analysis.

GC/FTIR: GC 5880A from H/P Co., including a OV-101 capillary column (30 m X 0.32 mm id.) and a flame ionization detector were used in this study. Column temperature was started at 70°C for 2min, then programmed to 225°C at 7°C/ min, and finally hold at 225°C for 5 min. A 0.5 µ l aliquot of sample injected into the injection chamber at 250°C. The temperature of the detector was 280°C, and the interface, 260°C.
FIS-20E with MCT IR detector was from Digilab Co.. A stainless steel tubing (15 cm X 1 mm id.)coated with gold on inside and enclosed by IR transmitting window KBr was installed as interface. The mid IR range of 3200 cm^{-1}-700 cm^{-1} with the spectral resolution of 8 cm^{-1} and

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the scan speed of 64/sec were measured.

GC/MS: GC/MS TRIO-1000 was produced by VG Co.. The energy of the ionizing beam was 70 ev.. The Scanning mass range was 50-650 m/z in 0.9 sec^{-1} with interval of 0.1 sec.. A capillary column HP-5 (12 m×0.25 mm id.) was used. The temperature of the injection chamber was held at 250°C.The initial temperature for the column was 100°C for 1 min., then programmed at 20°C min^{-1} to a final temperature of 250°C. The temperatures of transfer line and of the MS ion source were held at 250°C and 200°C respectively.

GC/ECD: GC SP-501 made in China with a electron capture detector was used. A glass column (150 cm × 0.4 cm) was packed with 2% QF-1 and 5% OV-17/chromosorb W-HP in 80-100 mesh. The temperatures for the column , detector and for gas chamber were held at 135°C, 215°C and 240°C respectively. The flow rate of carrier gas of nitrogen (purity, 99.99%) was 35ml min^{-1}.

The frozen samples were homogenized and then were extracted by the method as described by Du 1995.

RESULTS AND DISCUSSIONS

The peak 4 (7.37 min.), peak 8 (15.18 min.) and peak 13 (32.20 min.) in Fig.1 can be considered as the representatives of 3 main components in GC/FTIR analysis. Using the GC-FTIR data and Sadller standard FTIR spectrogram, their FTIR spectrograms were identified as 2,4-dimethylamine (in Sadller standard FTIR spectrogram 1661), a compound with the structure of H_3C-C_6H_3-CH_3-NHCHO, and N'- (2,4-dimethylphenyl)-N-methylformamidine for a very similar structure of H_3C-C_6H_3-CH_3-N=CH-$NHCH_3$ respectively.

From MS spectrograms of liver sample and standard N'- (2,4-dimethylphenyl)-N-methylformamidine shown in Fig. 2, it was found that 2,4-dimethylaniline is one of the metabolites of N'- (2,4-dimethylphenyl)-N-methylformamidine present in rat liver. The result was proved again by GC/ECD analysis and shown in Fig.3.The all results of GC/FTIR, GC/MS, and GC/ECD have indicated that the path of metabolism of N'- (2,4-dimethylphenyl)-N-methylformamidine in rats was N'-(2,4-dimethylphenyl)-N-methylformamidine → 2,4-dimethylformaniline → 2,4-dimethylaniline → polar compounds. This is the same as Knowles et al (198 1) described (Fig. 4).

The result of analysis for the blood samples by GC/ECD suggested that N'- (2,4-methylphenyl)-N-methylformamidine was absorbed by digestive tract and transferred rapidly into blood. The maximum concentration of N'- (2,4-dimethylphenyl)-N-methylformamidine in blood was observed in 2--4hrs after oral administration, and the concentration then was reduced by 70% during 24--48hrs and by 90% after 48hrs (Fig.5). The detected metabolites 2,4-dimethylaniline in blood at 2hrs after oral administration has suggested that the metabolism of N'- (2,4-dimethylphenyl)-N-methylformamidine in liver (Fig. 6) is rapid. A high concentration of N'- (2,4-dimethylphenyl)-N-methylformamidine in liver still hold during 2--6.5hr after treatment (Fig.7) has indicated that absorption and transformation were essential before 6.5hrs, and then the metabolic conversion after 6.5hrs to 48hrs.

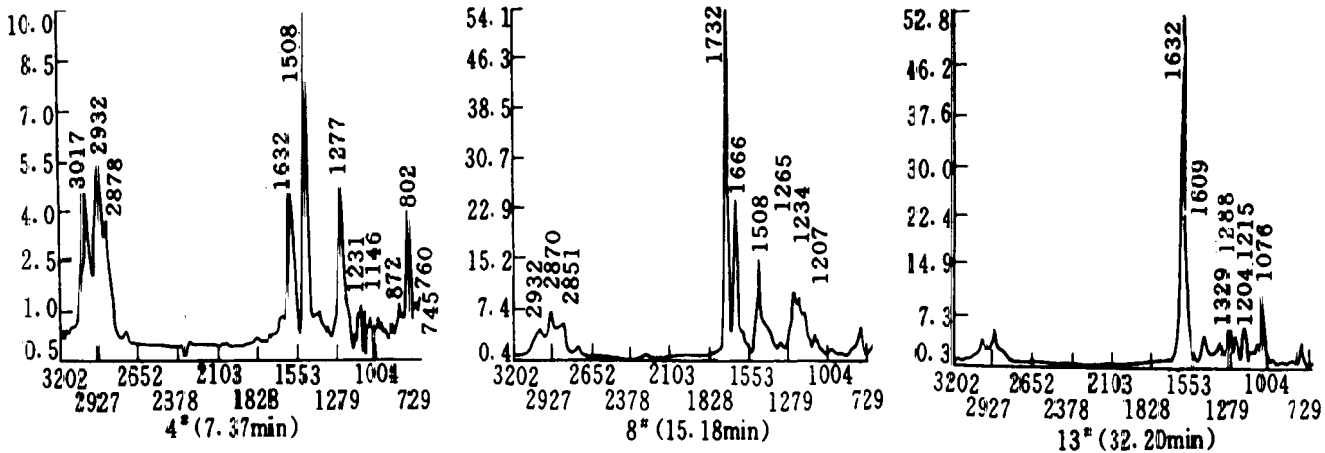

Figure 1. FTIR spectrogram of peak 4,8,13

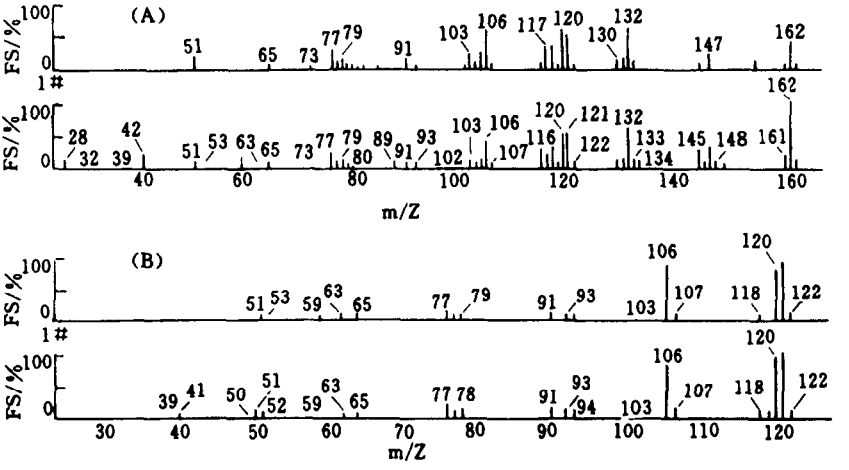

Figure 2. MS spectrogram for liver samples and standard
N'- (2,4-dimethylphenyl)- N-methylformamidine (A) and 2,4-dimethylaniline (B)

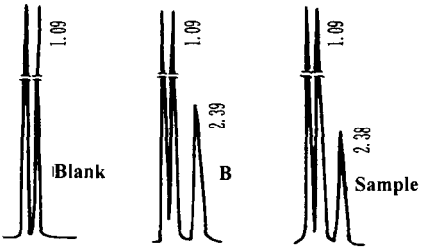

Figure 3. GC/ECD chromatogram for liver sample and standard 2,4-dimethylaniline (B)

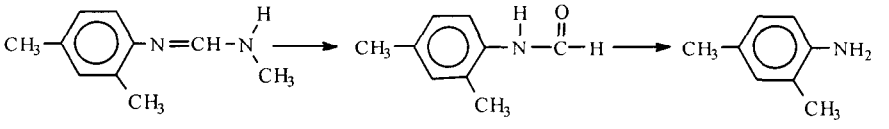

Figure 4. The path of metabolism of N'- (2,4-dimethylphenyl)-N-methylformamidinein rat liver.

The variations of the concentration of N'- (2,4-dimethylphenyl)-N-methylformamidine and 2,4-dimethylaniline in urine (Fig. 8) indicated that the metabolites 2,4-dimethylaniline was predominant in urine after 4 hrs and reached the maximum concentration at 6.5hrs. The average excretion of N'- (2,4-dimethylphenyl)-N-methylformamidine via urine was about 122.6 μ g including 2,4-dimethylaniline within 72 hrs, plus the excretion with fence and the residue in tissues, all together was less than 1/10 of the initial supplement amount (5 mg for

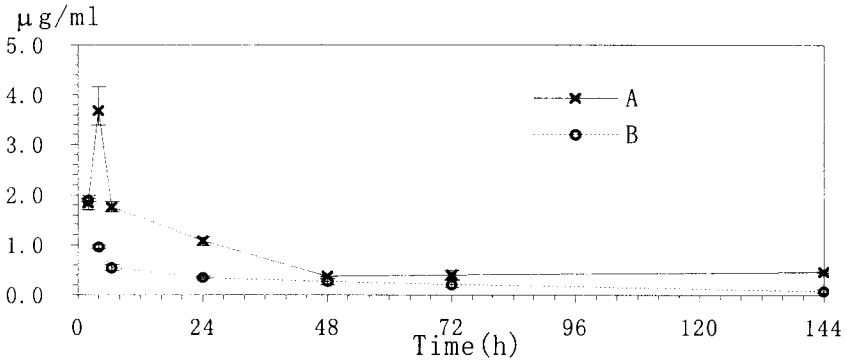

Figure 5. The variation of concentration of N'- (2,4-dimethylphenyl)-N-methylformamidine (A) and 2,4-dimethylaniline (B) in blood

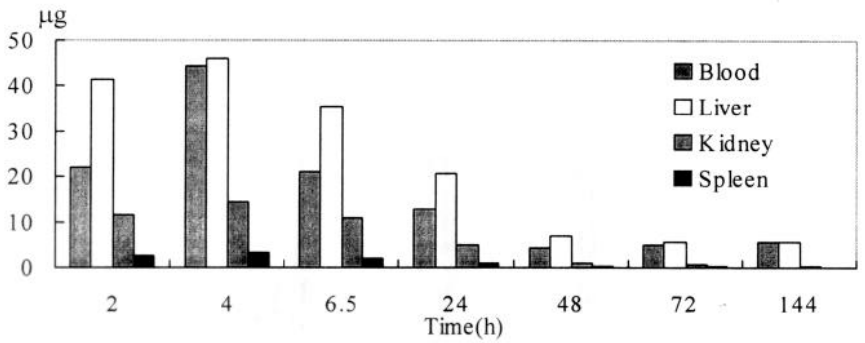

Figure 6. The N'- (2,4-dimethylphenyl)-N-methylformamidine residue in blood and tissues at different times.

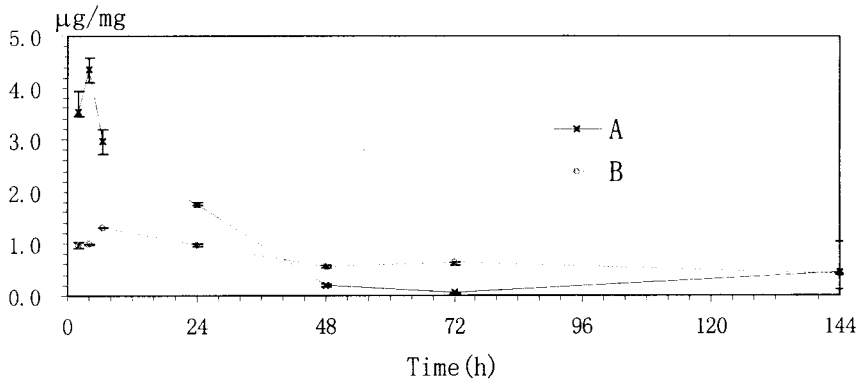

Figure 7. The variation of concentration N'- (2,4-dimethylphenyl)-N-methylformamidine (A) and 2,4-dimethylaniline (B) in liver

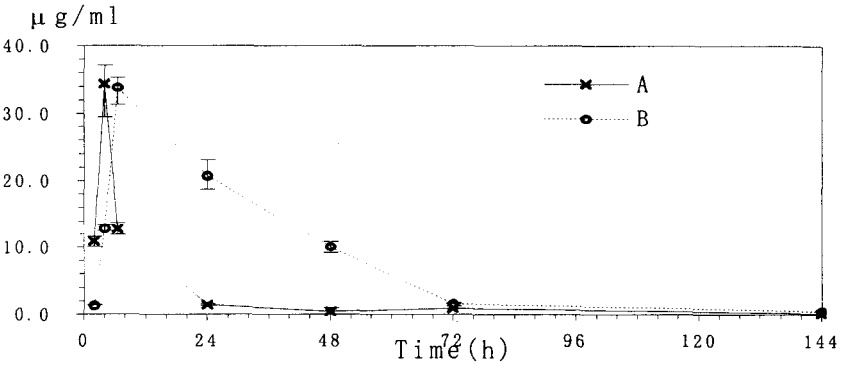

Figure 8. The variation of the concentration of N'-(2,4-dimethylphenyl)-N-methylformamidine (A) and 2,4-dimethylaniline (B) in urine

each rat). The result was similar with the study by Zhu 1994, and consistent with that by Knowles 1981. We infer that metabolites a large quantity are polar compounds with small molecular weight and are hardly extracted with organic solvents, and thus could not be detected.

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