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DATA EVALUATION REPORT

STUDY TYPE: Metabolism Study 85-1

ACCESSION NUMBER: 073217

TEST MATERIAL: Cyhalothrin

SYNONYMS: (R,S)alpha-cyano-3-phenoxybenzyl (±)-cis-3-(Z-2-chloro-3,3,3-trifluoropropyl-enyl)-2,2-dimethylcyclopropane carboxylate; ICI 146,814; ^{14}CHCN; ^{14}C-cyclopropyl- and ^{14}C-benzyl-ICI; benzyl: batch 1R4; cyclopropyl: batches 2R3, 2R2 and 2R4

STUDY NUMBER(S): ICI Study Number 146814 KMD 005

REPORT NUMBER: Quality Assurance Unit (ICI) RA84174Q

SPONSOR: Imperial Chemical Industries PLC

TESTING FACILITY: ICI Pharmaceuticals Division, Safety of Medicines Dept.

TITLE OF REPORT: Cyhalothrin (ICI): The Disposition and Metabolism of (^{14})-ICI 146,814 in The Dog

AUTHOR(S): A. G. Fowkes, M. P. Harrison, T. R. Marten

REPORT ISSUED: September 17, 1984

IDENTIFYING VOLUME: Volume II, Book 15 of 16 (Tab Reference 20C)

CONCLUSION: This study is classified as CORE MINIMUM because distribution studies were not conducted and a repeated dose absorption, metabolism, distribution and excretion study was not done.

Classification: CORE MINIMUM

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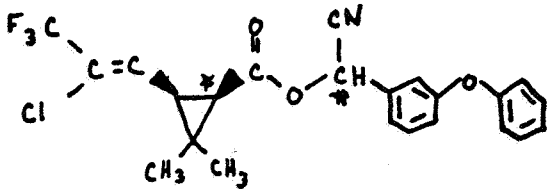
MATERIALS AND METHODS:

Chemical Formulations

Two radiolabelled forms of cyhalothrin were used for these studies. The positions of radiolabelling are shown in the following figure:



The abbreviations ^{14}C-benzyl and ^{14}C-cyclopropyl are used to refer to the compound labelled at positions marked # or * respectively, as shown above. The labelled forms were synthesized by the Radiochemical Unit of the Drug Metabolism Section at ICI Pharmaceuticals Division. For the oral formulations, the radiolabelled compounds were diluted with hexane and corn oil and then the hexane was removed under N_2 at 37°C. For the intravenous studies, the hexane was removed first and the material was re-dissolved in absolute ethanol and diluted with saline. For the individual doses, the radiolabelled ICI 146,814 was diluted with non-labelled cyhalothrin from batch ADM 46156/80 (greater than 99% pure cis Z). The radiochemical dose to each animal was approximately 100 microCi for the oral studies and 50 microCi for the intravenous studies.

Animals

The same three male and three female Alderly Park Beagle dogs were used for all the single dose excretion studies. The dogs weighed approximately 15 kg each.

Single Dose Excretion Studies

The oral studies were conducted at dose levels of 1 and 10 mg/kg and the intravenous studies were conducted at a dose level of 0.1 mg/kg. Since the same animals were used for all of the studies, three weeks were allowed to elapse between each dosing. The studies were conducted in the following order: 1 mg/kg oral benzyl label, 1 mg/kg oral cyclopropyl label, 10 mg/kg oral benzyl label, 10 mg/kg oral cyclopropyl label, 0.1 mg/kg i.v. cyclopropyl label and 0.1 mg/kg i.v. benzyl label. The specific activities of each formulation were as follows: 1 mg/kg benzyl (7.78 microCi/mg), 10 mg/kg benzyl (0.64 microCi/mg), 0.1 mg/kg benzyl (30.5 microCi/mg), 1 mg/kg cyclopropyl (7.07 microCi/mg for males and 6.28 microCi/mg for females), 10 mg/kg cyclopropyl (0.69 microCi/mg) and 0.1 mg/kg cyclopropyl (30.8 microCi/mg). The animals were housed in individual metabolism cages. Urine, feces and cage washes were collected at 24-hour intervals from the time of dosing up to seven days. For the oral 10 mg/kg cyclopropyl label study, urine was collected at 0-8 and 8-24 hours in addition to the 24-hour intervals. Blood samples were collected at pre-dose, 1, 2, 4, 6, 12, 24 and

every 24 hours thereafter for up to 168 hours post dosing. For the intravenous studies, additional samples were taken at 0.5 and 8 hours. Samples were stored at -20°C until analyzed.

Determination of Total Radioactivity in Urine, Feces, Cage Washes, Plasma and Whole Blood

Samples were prepared for liquid scintillation counting. Feces and whole blood were prepared by sample oxidation. The CO_2 produced during oxidation was absorbed in 2-methoxyethylamine and mixed with a toluene based scintillant.

Analysis of Sample Radioactivity

Urine samples were either treated with various enzyme preparations; acidified to pH 1 or basified to greater than pH 10 and heated at 80°C for 30 minutes; or left untreated in pH 5 acetate buffer and analyzed further. The enzyme preparations consisted of combined beta-glucuronidase and sulfatase type H-1 (with and without 1,4-saccharolactone which inhibits beta-glucuronidase activity), sulphatase type V with 1,4-saccharolactone, and beta-glucuronidase type IX. Test incubations were conducted using phenolphthalein glucuronide and p-nitrocatechol as substrates. Feces homogenates were extracted with methanol.

The patterns of radioactivity in the urine and feces samples were analyzed by thin layer chromatography (tlc) using one of the following solvent systems: chloroform:acetic acid (95:5 v/v); ethyl acetate:formic acid (98%):water (70:4:4 v/v); toluene:n-hexane:acetonitrile:chloroform (200:100:2:5 v/v) or toluene:ethanol (2:1 v/v). Radioactive areas were located by autoradiography and scanned.

The ^{14}C-benzyl metabolites were extracted from urine samples from one male and one female dog from the 10 mg/kg oral study using n-hexane (male dog only) and ethyl acetate (both dogs) as extraction solvents. The metabolites were then analyzed by tlc using the second solvent system in the list above. Radioactive areas were excised and further purified by preparative tlc using the first solvent system followed by a third tlc in either ethyl acetate:methanol:water (13:2:1 v/v) or chloroform (saturated with 90% formic acid):diethyl ether (10:3 v/v). Samples were then further analyzed by mass spectrometry.

The ^{14}C-cyclopropyl metabolites were extracted from male urine from the 10 mg/kg oral study using ethyl acetate as the extraction solvent. The samples were analyzed by chromatographing and re-chromatographing with tlc using the second solvent system. Samples selected for further clean up were first chromatographed in chloroform:methanol:acetic acid (10:5:2 v/v) followed by preparative tlc in ethyl acetate:methanol:water (13:2:1 v/v) and rechromatographed again in the second solvent system. For the mass spectrometry, metabolites were compared with known reference materials where possible.

RESULTS:

Disposition of ^{14}C-Benzyl-ICI in the Dog

1 Mg/Kg Oral Dose

The diluted ^{14}C-labelled compound used was greater than 97% pure ^{14}C-ICI. Most of the radioactivity was excreted during the first 48 hours after dosing, mainly via the feces (in both males and females). The mean values at 48 hours were: 75.6% of total dose excreted (excluding cage washes), 24.8% in urine and 50.8% in feces. After 7 days the total excretion of radioactivity including cage washes amounted to 86.0 $\pm$ 4.5% (54.2 $\pm$ 3.9% in feces and 29.7 $\pm$ 7.3% in urine).

The radioactivity in whole blood was found to be attributable to the radioactivity in plasma. Plasma concentrations of radioactivity rose rapidly and peaked between 2 and 12 hours post dose. Three dogs gave secondary peaks at 12 hours while others showed a delayed fall in levels. The half-life of the decline in plasma levels was calculated to be 28 hours.

10 Mg/Kg Oral Dose

Excretion rates were similar to the 1 mg/kg group. 68.8% of the radioactivity was excreted in the first 48 hours. Mean plasma levels peaked at 2 hours post dosing and again at 12 hours post dosing. The half-life of the decline in plasma levels was calculated to be 32 hours.

0.1 Mg/Kg Intravenous Dose

The diluted ^{14}C-labelled compound used was greater than 96% pure ^{14}C-ICI. Excretion patterns were different from those in the oral studies in that significant amounts of radioactivity were excreted over the first three days (as opposed to the first 48 hours) and that radioactivity was more evenly distributed between urine and feces in both males and females. The mean values at 72 hours for males and females combined were: 32.7% of the total dose in urine and 37.1% of the total dose in the feces. Approximately 83% of the dose was recovered in urine, feces and cage washes after 7 days.

Plasma concentrations fell rapidly until 4 hours after dosing and then rose to a peak at 12 hours. Thereafter levels fell again with a half-life of 33.6 hours.

Analysis of Radioactivity in the Urine

TLC analysis of 0-24 hour urine samples indicate that ^{14}C-benzyl-ICI is extensively metabolized in the dog. No parent compound was found in the urine. The following metabolites were identified by TLC and mass spectrometry: 3-phenoxybenzoic acid (3-PBA) and glucuronic acid conjugate, 3-(4-hydroxyphenoxy)benzoic acid and sulphate, N-(3-phenoxybenzoyl)-glycine and two unknowns.

Analysis of Radioactivity in Feces

TLC of methanol extracts of feces samples indicated that for both dose levels 1 mg/kg and 10 mg/kg (oral), the main component excreted within the first 24 hours was unchanged cyhalothrin (74.4% of applied radioactivity for a male dog at 1.0 mg/kg and 93% for a female dog at 10 mg/kg). The sample from the male dog also contained three other components, two bands with similar R_F's to 3-PBA, one which was more polar, and one which was less polar than 3-PBA and may have been a metabolite of the intact ester. The female dog also had a component with a similar R_F to 3-PBA. Fecal samples taken from a female dog between 24 and 48 hours post dosing with 1.0 mg/kg contained only 8.5% unchanged compound and 5 or 6 other components. Samples taken from another female dog between 0 and 24 hours post dosing with 0.1 mg/kg ^{14}C-benzyl-ICI intravenously showed a pattern very similar to the 24-48 hour samples from the 1.0 mg/kg dosed dog. Only 1.5% of the radioactivity present was from unchanged cyhalothrin. Five or six other components were present in similar amounts as the 1.0 mg/kg dog, one of which had a similar R_F to 3-PBA (39.9% of the dose).

Disposition of ^{14}C-Cyclopropyl-ICI in the Dog

1 Mg/kg Oral Dose

The diluted ^{14}C-labelled compound used was greater than 98% pure ^{14}C-ICI. Excretion patterns were similar to those with ^{14}C-benzyl-ICI in that most of the dose was excreted during the first 48 hours, mainly via the feces. There were no significant differences between males and females.

Again, the radioactivity in whole blood was found to be attributable to the radioactivity in plasma. Concentrations in plasma peaked at four hours post dose and then fell, rapidly at first and then more slowly.

10 Mg/kg Oral Dose

Oral administration at this dose had an emetic effect on several dogs, which were subsequently excluded from the data. Two of the dogs lost greater than 10% of the dosed radioactivity. There was some difficulty in obtaining fecal samples; however, excretion of radioactivity still appeared to occur predominantly within the first 24 hours after dosing. In females, 2/3 dogs failed to produce feces, which delayed excretion somewhat. Concentrations in plasma peaked at 12 hours and subsequently declined.

0.1 Mg/kg Intravenous Dose

Radioactivity was excreted rapidly via both urine and feces in approximately equal amounts. The mean total recovery over 7 days was 81.9% with 40.0% in the urine and 38.7% in the feces. The balance was in the cage wash. Concentrations in plasma fell rapidly after dosing.

Analysis of Radioactivity in the Urine

Analysis by TLC and mass spectrometry indicate that this part of the molecule is extensively metabolized. At least twelve metabolites were identified in the urine, some present in both the free form and the conjugated form. There was a variation in the pattern of the metabolites which was dependent upon dose level, route or sex.

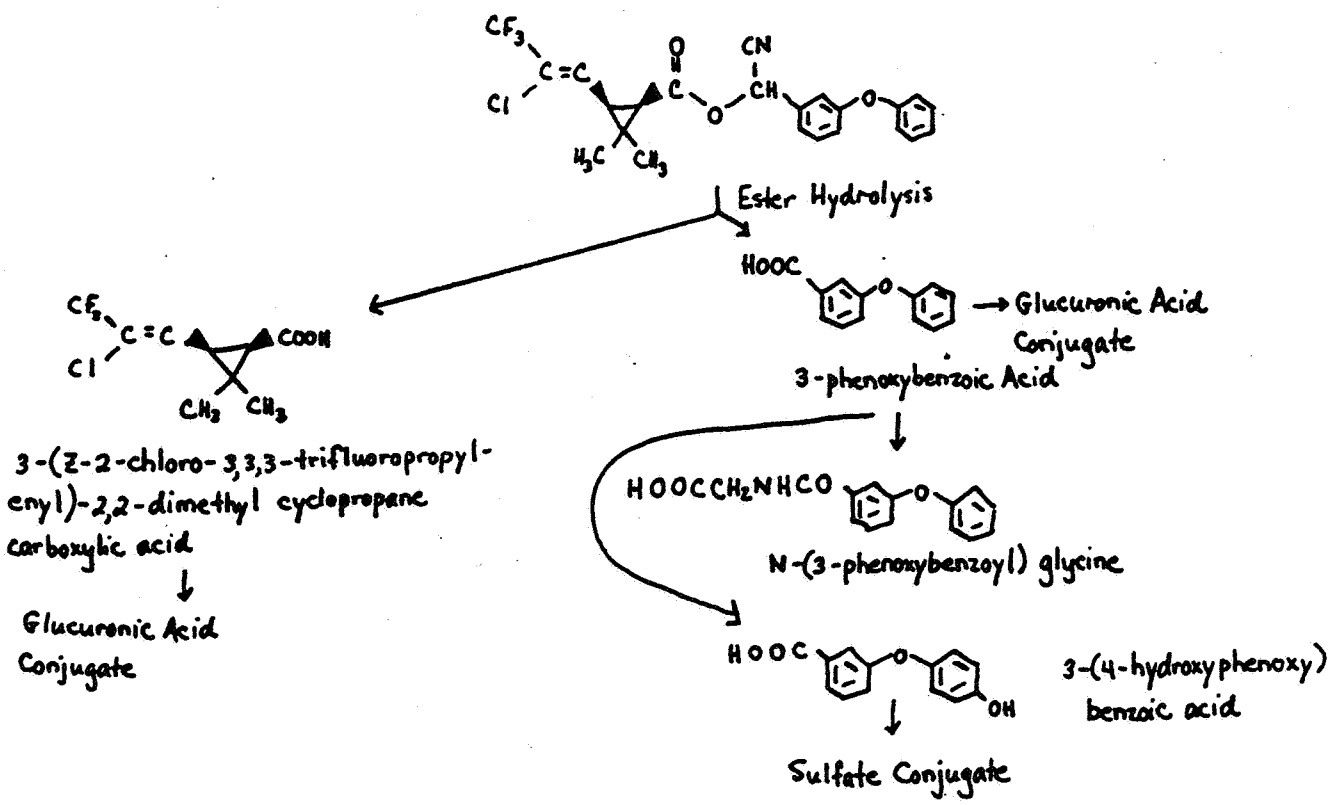
Analysis of Radioactivity in the Feces

At both dose levels 1 mg/kg (oral) and 10 mg/kg (oral), the major component was unchanged cyhalothrin which was mostly excreted during the first 24 hours. Between 24 and 48 hours, 3-5 other components were observed as well, two chromatographing at R_F 0.56 and two more polar components chromatographing at R_F 0.25 and at the origin. Samples were not taken for the 10 mg/kg dose level beyond 24 hours. When ^{14}C-cyclopropyl-ICI was administered intravenously at a dose level of 0.1 mg/kg, the pattern was similar to the pattern observed with 1.0 mg/kg (orally) between 24 and 48 hours. Even less unchanged cyhalothrin was observed in the feces when the compound was administered intravenously (1.4% of the administered dose within the first 24 hours).

DISCUSSION:

Using the urinary excretion data from the intravenous studies and from the lower dose oral studies, the authors concluded that for the ^{14}C-benzyl label the absorption was 80% and for the ^{14}C-cyclopropyl label the absorption was 48%. The high dose oral studies could not be used for this purpose because of fecal contamination of the urine. The authors stated that the discrepancy in absorption rates was probably due to inter-animal variation. This plausible, but is not definitively proven in the study.

The metabolite patterns from each of the two radiolabelled cyhalothrin compounds were quite different from each other indicating extensive cleavage of the ester bond. Urinary metabolites from the ^{14}C-benzyl studies are listed in the results section of this review. There were up to seven metabolites isolated. Twelve metabolites were isolated from the ^{14}C-isopropyl studies. In the feces, a large proportion of the radioactivity was due to unchanged cyhalothrin. One metabolite was found to be common to both labelled studies. Because of its properties, it is thought to be a metabolite of the intact ester. The following figure depicts the identified metabolites of cyhalothrin in the dog:



Excretion in all studies was rapid in both urine and feces, nearly all of it within 48 hours. The difference between the amount of unchanged compound found in the feces in the oral studies versus the intravenous studies was so pronounced that it appears that absorption of the compound is incomplete.

The rat studies indicate that some of the compound is retained in the fat and released slowly. If this is the case with the dog study, then it would partly explain the lack of complete recovery of radioactivity from the initial dose.