UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

005546

OCT 21 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Assure (NC-302; ethyl 2-[4-(6-chloro-2-quioxalinyloxy)phenoxy]propionate): Evaluation of metabolism studies of NC-302 in rats

Caswell No.: 215D
Project No.: 191/192
Accession No.: 073546

Action Code: 230
Record No. 151063; 152701

SUBMITTER: E. I. Du Pont De Nemours & Co.

TO: Robert J. Taylor
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Registration Division (TS-767C)

FROM: Whang Phang, Ph.D. Whang Phang 5/8/86
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THRU: Marcia van Gemert, Ph.D. Marcia van Gemert 9.26.86
Section Head
and
Theodore M. Farber, Ph.D.
Chief
Toxicology Branch/HED (TS-769C)

Action Requested:
Review metabolism study on NC-302 in rat to complete the data base for registration and tolerance for soybean and cotton.

Results:
The attached DER's(EPA No. 68-02-4225; Dynamac No. 1-011A-1 through 1-011A-8; March 26, 1986) have been approved by Toxicology Branch. The metabolism studies of NC-302 in rats and the core classifications according to the present Toxicology Branch evaluation standards are presented as follows:

1). The study, Distribution, excretion, and metabolism of NC-302 in rats after intravenous administration (10 mg/kg)(unpublished study [No. unavailable]; Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Japan; Feb. 1985), is acceptable.

2). The study, Metabolism of NC-302 in rats (160 mg/kg)(unpublished study by Nissan Chemical Industries, Ltd., Japan. Feb. 1985), is acceptable.

3). The study, Absorption, distribution, and excretion of NC-302 in rats after single oral administration (160 mg/kg)(unpublished study by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Japan. Feb. 1985), is acceptable.

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4). The study, Absorption, distribution, excretion, and metabolic transformation of NC-302 in rats after single oral adminitratin (1.5 mg/kg) (unpublished study by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Japan. Feb. 1985), is acceptable.

5). The study, The influence of repeated administration of NC-302 on metabolic fate in rats (1.5mg/kg)(unpublished study by Biological Research Laboratory, Nissan Chemical Industries, Ltd., Japan. Feb. 1985), is acceptable.

6). The study, The accumulation in tissues and metabolites in plasma and liver after repeated oral administration of NC-302 in rats (1.5 mg/kg) (unpublished study by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Japan. Feb. 1985), provides supplementary data.

7). The study, The biokinectics and metabolism of [^{14}C]NC-302 in the rat (1.5 mg/kg)(unpublished study No. NSA12/8361 by Huntingdon Research Centre, England. Feb. 1984), is acceptable.

8). The study, The accumulation of NC-302 and its metabolites in the tissues of rats during repeated oral administration (1.5 mg/kg)(unpublished study No. HRC/NSA 13/83182 by Huntingdon Research Centre, England. April 15, 1983), provides supplementary data.

9). The study, Dermal absorption study in rats (Haskell Laboratory for Toxicology and Industrial Medicine, Du Pont; 5/1/85) is acceptable.

The EPA guidelines require four metabolism studies which include an intravenous dose, a high oral dose, a low oral dose, and a low dose in pretreated rats. The studies submitted by the petitioner have met these requirements, and the required studies are all acceptable (No's. 1, 2, 3, 4, 5, and 7). Additional studies submitted by the petitioner provide important information to supplement and to confirm certain findings obtained with the required studies.

The results of these studies indicate that NC-302 is readily absorbed from gastrointestinal tract. Highest levels of [^{14}C] are found in the blood, liver, and kidneys, following a single oral dose (either 160 or 1.5 mg/kg). The estimated biological half-lives of [^{14}C] NC-302 in blood and tissues, except fat, are 18-27 hours in both sexes of rats. The average biological half-lives of [^{14}C] NC-302 in fat are 155 and 97 hours in males and females, respectively. The absorbed [^{14}C] NC-302 is excreted rapidly. In males, the major route of elimination is in the feces, whereas in females equal amounts of [^{14}C] are eliminated in the urine and the feces. In fecal samples collected within 48 hours, unchanged NC-302 accounted for approximately 23% of the high oral dose (160 mg/kg) and less than 7% of the low oral dose (1.5 mg/kg). No radioactivity was detected in the expired air. The major metabolite of NC-302 is the corresponding acid, which is metabolized further.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

005546
EPA: 68-02-4225
DYNAMAC No. 1-011
March 27, 1986

OVERVIEW

NC-302

Metabolism of NC-302 in Rats

STUDY IDENTIFICATION: Metabolism of NC-302 in rats.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
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Signature: I. Cecil Felkner

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005546

OVERVIEW

NC-302

STUDY IDENTIFICATION: Metabolism of NC-302 in rats.

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TEST CHEMICAL: NC-302; ethyl 2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]-propionate.

STUDY/ACTION TYPE: Metabolism of NC-302 in rats.

INTRODUCTION: The metabolism of NC-302 in rats is reviewed in this report. Several studies were conducted with [^{14}C-phenyl]- or [^{14}C-quinoxaline]-NC-302 in male and/or female Sprague-Dawley rats. These include all four studies required by EPA guidelines, namely metabolism of NC-302 following an intravenous dose, a high oral dose, a low oral dose, and a low dose in pretreated rats. In addition, a 28-day oral dosing study was conducted. The available data indicate that NC-302 is readily absorbed from the gastrointestinal tract and is then rapidly excreted. The biological half-lives of [^{14}C] in blood and tissues, except fat, were about 18-27 hours in males and females. The average biological half-lives of [^{14}C] in fat were 155 and 97 hours in males and females, respectively. The major route of elimination in males is in the feces, whereas in females equal amounts are eliminated in the urine and feces under nonsaturating conditions. Unchanged NC-302 was found only in the feces. In fecal samples collected during the first 2 days after dosing, NC-302 accounted for 23 percent of the high oral dose (160 mg/kg), whereas less than 7.0 percent was found in the feces of rats receiving the low dose (1.5 mg/kg). The primary metabolite of NC-302 is the corresponding acid, which is then further metabolized.

Absorption:

The available studies indicate that following oral administration, NC-302 is readily absorbed from the gastrointestinal tract. Hirata et al. (1985c) reported that absorption was about 48 and 65 percent of a single oral dose of 160 mg/kg administered to male and female rats, respectively. The calculations were based on comparing elimination of [^{14}C] in the urine of

animals dosed orally with [^{14}C-phenyl]NC-302 at 160 mg/kg with that of animals dosed intravenously at 10 mg/kg. These estimates are based on the assumption that absorption of NC-302 from the gastrointestinal tract and subsequent excretion via the urine was linear. However, this was not demonstrated, and data from other studies indicate that the amount of [^{14}C] eliminated into the urine is dose dependent (see excretion section). Excretion and metabolite identification data suggest that a maximum of 60 percent of the dose may be absorbed. At a single dose of 1.5 mg/kg, absorption is relatively higher. Hawkins et al. (1983a) estimated that approximately 67 and 89 percent of the oral dose of [^{14}C-quinoxaline]NC-302 at 1.5 mg/kg was absorbed by male and female rats, respectively. The estimate was based on the total amount of [^{14}C] found in the urine, bile, liver, and carcass.

Gastrointestinal absorption apparently occurs via the hepatic portal system, as indicated by the high [^{14}C] levels noted in blood, liver, and kidneys following oral dosing at 160 or 1.5 mg/kg (Hirata et al., 1985c,d,e).

Tissue Distribution:

Following a single oral dose of [^{14}C]NC-302 to male and female rats at 160 or 1.5 mg/kg, there was an initial rapid uptake of [^{14}C]. Highest levels of [^{14}C] were found in the blood, liver, and kidneys (Hirata et al., 1985c,d). For the high dose, maximum levels were observed at 6 to 9 hours after dosing except in fat, which showed maximum levels (about 37 ppm) after 24 hours (Hirata et al., 1985c). Residue levels were slightly higher in the high-dose females than in males receiving the same dose. Maximum residues in blood, liver, and kidneys of males receiving 160 mg/kg were 183, 199, and 119 ppm, respectively, and 256, 287, and 168 ppm in females. A significant correlation was found between [^{14}C] levels in blood and those levels found in each tissue, except fat and adrenals (Hirata et al., 1985c). The major metabolite found in plasma, liver, kidney, brain, and fat of male and female rats dosed orally with

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[^{14}C-quinoxaline]- and/or [^{14}C-phenyl]NC-302 at 160 mg/kg was NC-302 acid, comprising between 73 and 93 percent of the total extractable radioactivity for each tissue. Small amounts of NC-302 phenol, 6-chloroquinoxaline-2-one (CQO), 2-(4-hydroxyphenoxy)propionic acid (PPA), and unknowns AM-1 and AM-2 were generally found. In the fat, NC-302 acid was found to form a complex with lipid as indicated by lipase hydrolysis (Hirata et al., 1985b).

Following the initial increase, [^{14}C] levels in blood and various tissues gradually decreased. The biological half-lives of [^{14}C] in blood were 26.7 and 19.4 hours in males and females, respectively. The rates of [^{14}C] disappearance from each of the other tissues were similar to that for blood, except for fat. The average biological half-lives of [^{14}C] in fat were 155 and 97 hours in males and females, respectively (Hirata et al., 1985c).

The distribution of [^{14}C] in tissues following a single oral dose of [^{14}C-phenyl]NC-302 at 1.5 mg/kg to non-pretreated and NC-302 pretreated rats was studied (Hawkins et al., 1983a; Hirata et al., 1985d,e). Pretreated rats were dosed daily for 14 days with unlabeled NC-302 at 1.5 mg/kg prior to [^{14}C-phenyl]NC-302 administration (Hirata et al., 1985e). Tissue [^{14}C] residues were determined at 6 hours and at 1, 2, 4, and 7 days (Hawkins et al., 1983a), or at 1 and 7 days after [^{14}C] dosing (Hirata et al., 1985d,e). The [^{14}C] distribution in male and female rat tissues was similar for both dosing regimens and was also similar to that in rats given a single oral dose at 160 mg/kg. Maximum [^{14}C] residues were found in blood, liver, kidneys, gastrointestinal tract, and thyroid (3-7 ppm) 6 hours after dosing (Hawkins et al., 1983a). [^{14}C] residue levels then decreased to less than 0.13 ppm 7 days after dosing (Hawkins et al., 1983a; Hirata et al., 1985d,e). The biological half-lives of [^{14}C] in blood were about 19-20 hours in both males and females (Hirata et al., 1985d,e). Residue levels in plasma were higher than those in whole blood and the biological half-lives averaged 35.2 and 26.7 hours for male and female rats, respectively (Hawkins et al., 1983a). The major metabolite found in plasma, liver, and kidneys of male and female

rats dosed with [^{14}C-phenyl]- or [^{14}C-quinoxaline]NC-302 was NC-302 acid, which accounted for 81-96 percent of the total extractable radioactivity for each tissue. Other minor metabolites were also noted. There were no significant differences in metabolite distribution with respect to sex or the position of the [^{14}C] label (Hirata et al., 1985d,e).

The distribution of [^{14}C] in the tissues of rats dosed orally with [^{14}C-quinoxaline]NC-302 at 1.5 mg/kg for 28 consecutive days was studied at various intervals during and after the 28-day dosing period (Hawkins et al., 1983b; Hirata et al., 1985f). The patterns of [^{14}C] levels in the whole blood were similar for both males and females during dosing. [^{14}C] residues in blood increased following dosing and reached plateau levels by the third and fifth dose in females (3 ppm) and males (4 ppm), respectively (Hirata et al., 1985f). In another study, residues in tissues of male rats increased following repeated dosing and reached maximum levels (blood, 6 ppm; liver, 5 ppm; kidneys, 4 ppm; muscle, 0.7 ppm; and fat, 1.5 ppm) after 7 days of [^{14}C]NC-302 administration. Tissue residue levels then decreased gradually, and by the 28th dose contained about 55 percent of the maximum radioactivity, except for kidneys and fat, which contained about 82 and 93 percent (Hawkins et al., 1983b). Residue levels in male rat tissues decreased rapidly after the 28-day dosing period and were less than 0.2 ppm, except in fat, where [^{14}C] residues remained constant at about 1-1.5 ppm throughout the 8-day withdrawal period (Hawkins et al., 1983b; Hirata et al., 1985f). The primary metabolite found in plasma and liver of male rats after 28 doses was NC-302 acid, which accounted for 93 and 72 percent of the sample radioactivity, respectively (Hirata et al., 1985f). The distribution of [^{14}C] in the tissues of female rats was not studied.

The metabolism and [^{14}C] tissue distribution of [^{14}C-phenyl]NC-302 administered to male rats in a single intravenous dose was studied by Hirata et al. (1985a). Maximum tissue residues in plasma, whole blood, liver, kidneys, and brain were observed 5 minutes after dosing and were reported to be 79.8, 46.6, 38.7, 31.6, and 9.1 ppm, respectively. Peak residue levels in the testes and fat were observed 3 hours after dosing

and were 5.2 and 4.1 ppm, respectively. Residue levels decreased gradually and by 7 days were less than 2 ppm for all tissues. The biological half-life of [^{14}C] in blood of male rats was 21.1 hours. In females, the biological half-life of [^{14}C] in blood was 16.9 hours

Excretion and Metabolism:

Seven days following a single oral dose of [^{14}C]NC-302 to rats at 160 mg/kg, most of the radioactivity was eliminated in the feces; males eliminated more [^{14}C] in the feces than females. About 85 and 73 percent of the dose was eliminated in the feces by males and females, respectively, whereas 8 and 26 percent was eliminated in the urine of males and females, respectively (Hirata et al., 1985c). The sex difference was more apparent at the low dose of 1.5 mg/kg. About 68-75 percent of the dose was eliminated in feces of male rats and 21-27 percent in the urine, whereas equal amounts (43-57 percent) were eliminated in the feces and urine of female rats (Hawkins et al., 1983a; Hirata et al., 1985d,e). There was no apparent difference in the rates of [^{14}C] elimination in urine and feces of males and females following oral dosing with either [^{14}C-phenyl]- or [^{14}C-quinoxaline]NC-302. Maximum levels of [^{14}C] in urine and feces occurred during the first or second day after dosing. [^{14}C] levels then decreased gradually.

No radioactivity was detected in expired air, and only 1.1-5.6 and 0.7-3.8 percent of the dose remained in the carcasses of male and female rats, respectively, 5-7 days after a single oral dose with either [^{14}C-phenyl]- or [^{14}C-quinoxaline]NC-302 (at 160 or 1.5 mg/kg or a single oral dose at 1.5 mg/kg to pretreated rats) (Hawkins et al., 1983a; Hirata et al., 1985c,d,e).

In animals dosed at 160 mg/kg, approximately 22.5 to 24.1 percent of the dose is eliminated into the feces unchanged within 48 hours with no apparent difference between male and female rats (Hirata et al., 1985b). There was no difference in the amount of unchanged parent compound found

in the feces of male rats dosed with either [^{14}C-phenyl]- or [^{14}C-quinoxaline]NC-302. At least five metabolites were identified in feces of rats dosed with [^{14}C-phenyl]NC-302; NC-302 acid and PPA were the major metabolites comprising 33.6 and 14.0 percent of the total radioactivity (or 24.6 and 10.3 percent of the dose) in male feces, respectively, and 24.7 and 15.1 percent (or 14.5 and 8.9 percent of the dose) in female feces. In addition, the minor metabolites AM-1 and AM-2 as well as traces of NC-302 phenol were found in feces of both males and females.

In urine, NC-302 acid and PPA were also the major metabolites, although their ratios in males and females were different. In males, NC-302 and PPA accounted for 21.6 and 49.4 percent, respectively, of the total extractable radioactivity within 48 hours, whereas in females they accounted for 53.4 and 25.1 percent, respectively. In addition to these five metabolites, glucuronide conjugates of NC-302 acid and PPA were identified as indicated by β-glucuronidase hydrolysis (Hirata et al., 1985b). NC-302 acid was also found to be the major metabolite in urine (20.9 percent) and feces (23.63 percent) of male rats dosed with [^{14}C-quinoxaline]NC-302. In urine, about 11 percent of the extractable radioactivity was unidentified metabolite AM-2 and 17 percent remained in the origin of the chromatogram (Hirata et al., 1985b).

Metabolic patterns noted in urine and feces of male and female rats (both pretreated for 14 days with unlabeled NC-302 or non-pretreated) given a single oral dose of [^{14}C]NC-302 at 1.5 mg/kg were similar to those found in rats given the high dose. However, the amounts of unchanged NC-302 found in the feces were much lower and accounted for 6.6 and 5.3 percent of the dose in non-pretreated males and females, respectively, and 2.8 and 2.0 percent of the dose in NC-302 pretreated rats, respectively. These data indicate increased metabolism in pretreated rats (Hirata et al., 1985d,e).

In rats given a single intravenous dose of [^{14}C]NC-302 at 10 mg/kg, the metabolic patterns were similar to those observed in rats dosed orally, except for the absence of unchanged NC-302 in the feces (Hirata et al., 1985a).

Biliary excretion of [^{14}C] following a single oral dose of [^{14}C-phenyl]NC-302 to males at 160 mg/kg (Hirata et al., 1985c) or [^{14}C-quinoxaline]NC-302 to males and females at 1.5 mg/kg (Hawkins et al., 1983a) occurred at a steady rate during the 24- and 48-hour collection periods, respectively. About 65 percent of the biliary radioactivity excreted during 24 hours after dosing with [^{14}C-phenyl]-NC-302 was identified either as NC-302 acid (31 percent) or its glucuronide conjugate (34 percent). The remaining [^{14}C] was associated with unidentified metabolites (Hirata et al., 1985b).

The metabolic pathway of NC-302 in rats is shown in Figure 1. The data indicate that NC-302 is hydrolyzed to the corresponding acid, which is converted to the other metabolites as indicated in Figure 1.

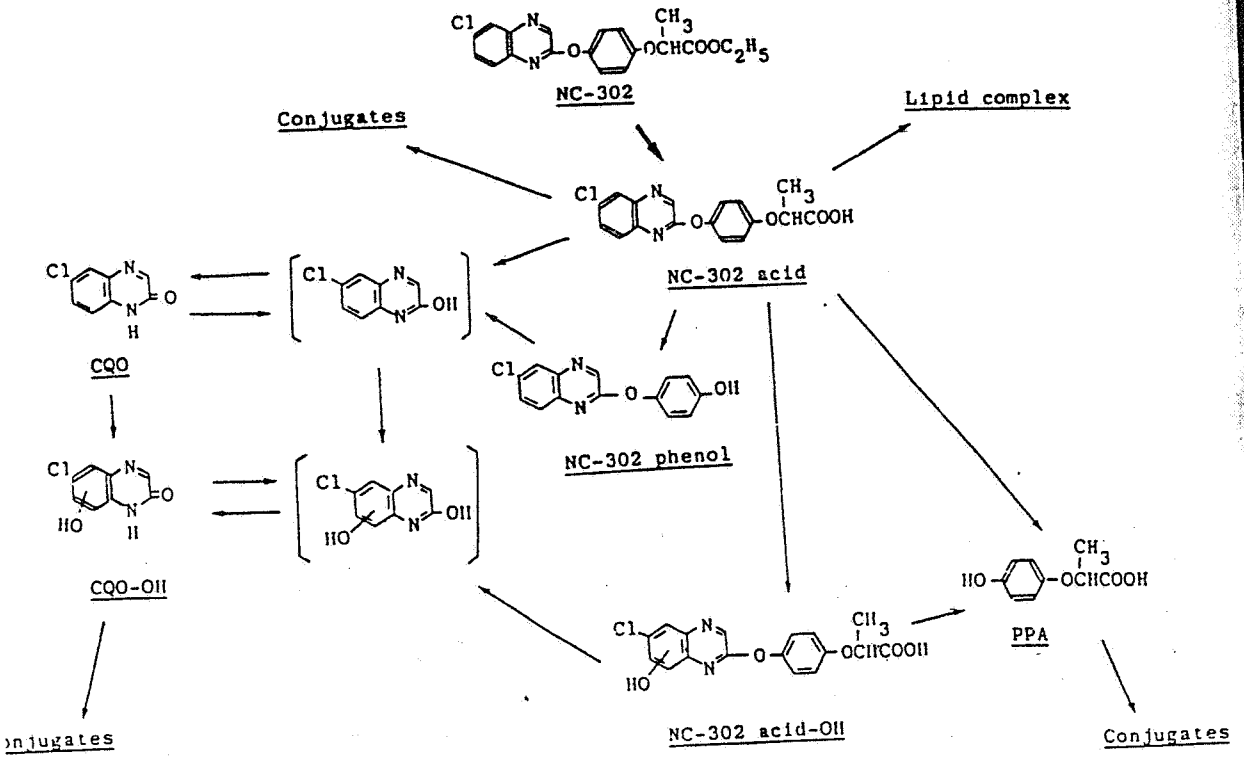

Figure 1. Metabolic Pathways of NC-302 in Rat

Source: Hirata et al. (1985b).

REFERENCES

Hirata, H., Onitsuka, H., Takano, S., Yamaguchi, I., and Misato, T. (1985a) Distribution excretion and metabolism of NC-302 in rats after intravenous administration. (Unpublished study [No. not available] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E. I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.)

Hirata, H., Onitsuka, H., Takano, S., Yamaguchi, I., and Misato, T. (1985b) Metabolism of NC-302 in rats. (Unpublished study [No. not available] prepared by Nissan Chemical Industries, Ltd., Japan, for E. I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.)

Hirata, H., Onitsuka, H., and Takano, S. (1985c). Absorption, distribution and excretion of NC-302 in rats after single oral administration (160 mg/kg). (Unpublished study [No. not available] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E. I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.)

Hirata, H., Onitsuka, H., Takano, S., Yamaguchi, I., and Misato, T. (1985d) Absorption, distribution, excretion and metabolic transformation of NC-302 in rats after single oral administration (1.5 mg/kg). (Unpublished study [No. not available] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E. I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.)

Hirata, H., Onitsuka, H., and Takano, S. (1985e) The influences of repeated administration of NC-302 on metabolic fate in rats. (Unpublished study [No. not available] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E. I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.)

Hirata, H., Onitsuka, H., and Takano, S. (1985f) The accumulation in tissues and metabolites in plasma and liver after repeated oral administration of NC-302 in rats (1.5 mg/kg/day). (Unpublished study [No. not available] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E. I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.)

Hawkins, D. R., Elsom, L. F., Davidson, C., and Roberts, D. (1983a) The biokinetics and metabolism of [^{14}C]NC-302 in the rat. (Unpublished study No. NSA 12/8361 prepared and submitted by Huntingdon Research Centre, Huntingdon, Cambridgeshire, England, for E. I. Dupont de Nemours and Co., Inc., Wilmington, DE; dated February 14, 1983.)

Hawkins, D. R., Down, W. H., Moore, D. H., Ballard, S. A., and Whitby, B. R. (1983b) The accumulation of NC-302 and its metabolites in the tissues of rats during repeated oral administration. (Unpublished study No. HRC/HSA 13/83182 prepared and submitted by the Department of Chemical Metabolism and Radiosynthesis, Huntingdon Research Centre, Huntingdon, Cambridgeshire, England, for E. I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated April 15, 1983.)

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EPA: 68-02-4225
DYNAMAC No. 1-011A-1
March 26, 1986

DATA EVALUATION RECORD

NC-302

Metabolic Study in Rats

STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., Takano, S., Yamaguchi, I., and Misato, T. Distribution, excretion and metabolism of NC-302 in rats after intravenous administration. (Unpublished study [No. unavailable] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E.I. duPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.) Accession No. 073546.

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Signature: I. Cecil Felkner

Date: 3-26-86

1. CHEMICAL: NC-302; ethyl-2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]-propionate.

2. TEST MATERIAL: [^{14}C-phenyl]NC-302 had a specific activity of 8.5 mCi/mmol and a radiochemical purity of greater than 99 percent.

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., Takano, S., Yamaguchi, I., and Misato, T. Distribution, excretion and metabolism of NC-302 in rats after intravenous administration. (Unpublished study [No. unavailable] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E.I. duPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.) Accession No. 073546.

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EPA Section Head

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7. CONCLUSIONS:

A. When male and female Sprague-Dawley rats were given single intravenous doses of [^{14}C-phenyl]NC-302 at 10 mg/kg, the distribution of radioactivity 7 days after administration was as follows: 16.5 and 38.7 percent of the dosed radioactivity was recovered in the urine of male and female rats, respectively; 71 and 51 percent of the radioactivity was eliminated by fecal excretion in male and female animals, respectively; and 2 to 3 percent of the administered radioactivity remained in the carcasses. Radioactivity was not detected in the expired air of the dosed animals. The major route of elimination in both male and female rats was by fecal excretion. Female rats excreted slightly more radiocarbon into the urine and less into the feces than did male animals. The biological half-lives of [^{14}C] in blood were 21 and 17 hours for male and female rats, respectively.

Metabolites of NC-302 in the 0- to 24-hour excreta of male rats were determined. 2-(4-Hydroxyphenoxy)propionic acid (PPA) was the major metabolite identified in the urine, accounting for 47 percent of the total urinary radioactivity. In the feces, 2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]propionic acid (NC-302 acid) and PPA were the main metabolites, accounting for 50 and 24 percent of the fecal radioactivity, respectively. All other metabolites were present at concentrations of less than 2 percent. Unchanged NC-302 was not detected in the feces or urine.

The highest tissue residues of radiocarbon were found in the plasma, whole blood, liver, and kidneys. Whole-body autoradiograms supported the tissue residue levels found; radioactivity was observed in the intestines, kidneys, liver, lungs, and blood from 1 to 24 hours postadministration, and little if any radioactivity was observed 72 hours postadministration.

B. This study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Unlabeled NC-302 of unspecified purity and [^{14}C-phenyl]NC-302 having a specific activity of 8.5 mCi/mmol and a radiochemical purity greater than 99 percent were the test materials used in this study.

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[1] Only items appropriate to this DER have been included.

2. The test animals were 5-week-old CD Sprague-Dawley rats of both sexes.

3. [^{14}C-phenyl]NC-302 was mixed with unlabeled NC-302 in dimethyl acetamide at a final "concentration of 15,000 ppm." The test animals each received a single intravenous dose of 10 mg/kg of [^{14}C-phenyl]NC-302 via the tail vein (approximately 2 to 5 µCi/rat).

4. For the excretion studies, five rats of each sex were individually housed in metabolism cages after dosing. Urine, feces, and expired air were collected separately at 24-hour intervals for 7 days. [^{14}C] in expired air was trapped in a monoethanolamine:methanol (1:1, v/v) mixture. At 7 days postadministration, the test animals were sacrificed, their fur was clipped, and the carcasses were digested in 6 N hydrochloric acid at 100°C. Radioactivity in urine, feces, expired air, carcasses, and fur was radioassayed using standard techniques.

5. To determine radiocarbon distribution in the tissues, 40 male rats each received a single intravenous dose of [^{14}C-phenyl]NC-302. Five rats were sacrificed at each of the following times; 0 (5 minutes), 1, 3, 9, 24, 72, 120, and 168 hours postadministration. Samples of blood, plasma, liver, kidneys, testes, brain, and fat were taken and radioassayed.

6. To determine blood radiocarbon concentrations, blood was taken from the tail vein of five rats of each sex at the following times after dosing: 5 minutes, 0.5, 1, 3, 6, 24, 48, 72, 96, and 120 hours. The blood was radioassayed using standard techniques.

7. To perform whole-body autoradiography, rats were sacrificed (number not specified) at 1, 3, 9, 24, 72, and 168 hours postadministration. Following sacrifice, each animal was frozen in a dry ice:acetone bath for about 30 minutes. The frozen carcasses were placed in 5 percent aqueous (w/v) carboxymethyl cellulose at -70°C and then mounted onto a microtome stage in a cryostat maintained at -20°C. Thin sections (50 µm) were freeze dried and exposed to X-ray film.

8. For metabolite identification, 24-hour urine samples from male rats were diluted fivefold with distilled water, adjusted to pH 2 with concentrated hydrochloric acid, and extracted twice with ethyl acetate. Extracts were concentrated under vacuum at 40°C and separated by thin-layer chromatography (TLC). Twenty-four-hour fecal samples were homogenized and extracted twice with 80 percent aqueous methanol. Methanol extracts were separated from the feces by centrifugation. Residues (centrifuge precipitates) were radioassayed following combustion. Extracts were concentrated under vacuum at 40°C and separated by TLC.

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Metabolites were identified by cochromatography with the following authentic compounds: NC-302, 2-[4(6-chloro-2-quinoxalinyloxy)phenoxy]propionic acid (NC-302 acid), 4-(6-chloro-2-quinoxalinyloxy)phenol (NC-302 phenol), and 2-(4-hydroxyphenoxy)propionic acid (PPA). TLC was performed on precoated silica gel (GF-254, 0.25-mm thickness, Merck) plates. The plates were developed in benzene:ethanol:acetic acid (20:1:1, v/v) and detection was by UV absorption.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Seven days following intravenous dosing, most of the radioactivity was eliminated in the feces, accounting for approximately 71 and 51 percent of the dose in males and females, respectively (Table 1). [^{14}C] elimination in feces and urine was highest on day 1 and decreased gradually during the 7-day observation period (Table 1). The total radioactivities detected in the urine of dosed male and female animals were approximately 17 (range, 10 to 24 percent) and 39 percent (range, 34 to 47 percent), respectively.

Little if any radioactivity was eliminated in expired air (Table 2). The total recovered radioactivities in the urine, feces, fur, and carcasses of dosed male and female rats accounted for approximately 92 (range, 87 to 95 percent) and 93 percent (range, 89 to 95 percent) of the dose, respectively (Table 2).

B. Blood levels of NC-302 equivalents in male and female rats following intravenous dosing of [^{14}C-phenyl]NC-302 are shown in Figure 1 (CBI, p. 17). The biological half-lives of radiocarbon in the blood from 5 minutes to 120 hours following dosing were 21.1 hours in male rats and 16.9 hours in females.

C. Maximum tissue residues of NC-302 equivalents in plasma, whole blood, liver, kidneys, and brain of male rats occurred 5 minutes after administration of [^{14}C-phenyl]NC-302 and were reported to be 79.8, 46.6, 38.7, 31.6, and 9.1 ppm, respectively (Table 3). Peak residue levels in the testes and fat were observed at 3 hours postadministration and were 5.2 and 4.1 ppm, respectively. Residue levels decreased gradually in all tissues by 168 hours to less than 2 ppm.

D. Whole-body autoradiograms demonstrated that at 1 hour after dosing, high concentrations of radioactivity were present in the blood, liver, lung, kidneys, intestines, teeth, and skin. At 3, 9, and 24 hours, radioactivity was observed in the liver, kidneys, lungs, blood, and intestines. By 72 hours, the radioactivity was hardly observed in any tissues.

TABLE 1. Excretion of Radiocarbon into Urine and Feces After Intravenous Administration of [^{14}C-Phenyl]NC-302

Sex	Percent Recovery of Administered Radioactivity[a] at Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	% Total [^{14}C] Recovered
				Urine				
Males	6.72 ±0.93	4.18 ±0.62	2.79 ±0.50	1.45 ±0.28	0.72 ±0.14	0.43 ±0.10	0.24 ±0.04	16.54 ±2.25
Females	16.62 ±1.72	8.52 ±0.86	5.85 ±0.29	3.72 ±0.48	1.97 ±0.33	1.28 ±0.24	0.71 ±0.14	38.68 ±2.54
				Feces				
Males	28.69 ±2.44	20.78 ±0.58	11.77 ±1.14	5.35 ±0.74	2.20 ±0.41	1.16 ±0.24	0.92 ±0.29	70.88 ±1.68
Females	20.64 ±1.84	14.70 ±1.19	7.71 ±1.00	5.08 ±0.67	1.79 ±0.30	0.87 +0.12	0.33 +0.07	51.11 +2.03

[a]Mean value ± S.E. of five rats.

Source: CBI pp. 12 and 13.

TABLE 2. Cumulative Excretion and Retention of Radioactivity by Rats 7 Days After Intravenous Administration of [^{14}C-Phenyl]NC-302

	Percent Recovery of Administered Radioactivity[a]	
	Males	Females
Urine	16.54 ± 2.25	38.68 ± 2.54
Feces	70.88 ± 1.68	51.11 ± 2.03
Expired Air	< 0.01	< 0.01
Fur	0.76 ± 0.11	0.85 ± 0.03
Carcass	3.42 ± 0.35	2.08 ± 0.21
Total	91.59 ± 1.71	92.72 ± 1.22

[a]Mean value ± S.E. of five rats.

Source: CBI p. 14.

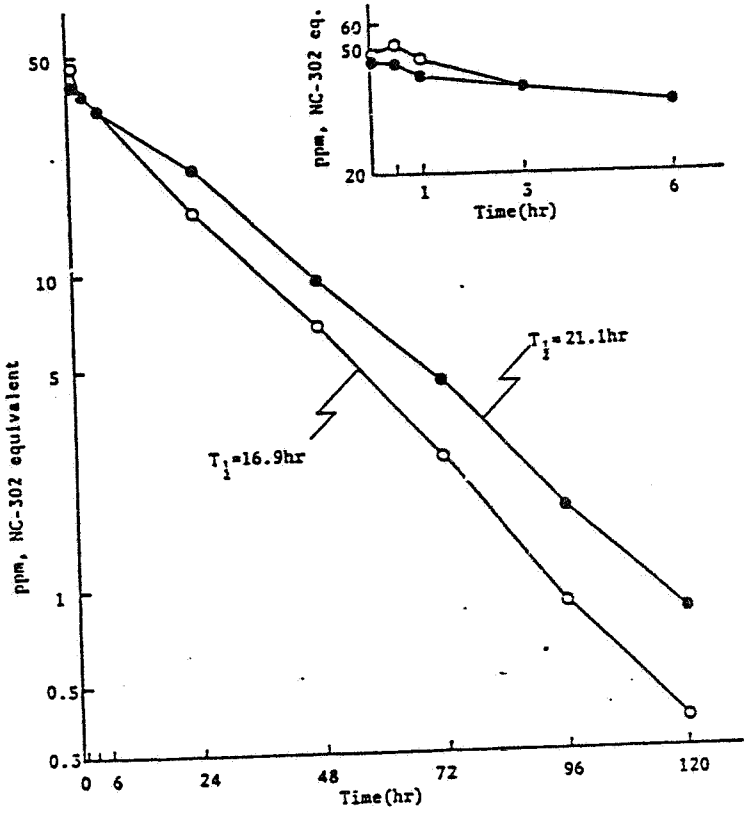

Fig. 1 ^{14}C mean levels in blood of male and female rats after intravenous administration of ^{14}C-NC-302(10 mg/kg).

●——● Male ○——○ Female

SOURCE: CBI p. 17.

TABLE 3. Levels of NC-302 Equivalents in Male Rat Tissue Following Intravenous Administration of [^{14}C-Phenyl]NC-302

	Hours After Administration (ppm)[a]							
	0 Hour[b]	1 Hour	3 Hours	9 Hours	24 Hours	72 Hours	120 Hours	168 Hours
Plasma	79.8±2.4	64.6±3.0	56.8±2.5	43.8±1.4	38.9±2.9	11.8±1.5	2.0±0.5	1.5±0.2
Blood	46.6±2.0	41.3±1.6	38.8±1.5	30.1±1.1	25.5±1.9	7.9±1.0	1.2±0.3	1.0±0.2
Liver	38.7±3.0	23.7±0.5	24.6±1.2	23.2±1.5	11.7±0.6	4.4±0.1	0.9±0.2	0.5±0.1
Kidneys	31.6±2.0	26.9±1.0	26.5±0.8	27.3±1.0	23.3±1.6	11.9±1.3	2.1±0.4	1.4±0.2
Testes	1.8±0.1	4.6±0.2	5.2±0.2	4.4±0.2	4.1±0.3	1.2±0.1	0.2±0.1	0.1±0.0
Brain	9.1±0.8	2.2±0.1	0.7±0.0	0.6±0.0	0.5±0.0	0.2±0.0	< 0.1	< 0.1
White Fat	1.4±0.4	4.0±0.2	4.1±0.5	2.9±0.2	2.8±0.2	1.5±0.1	0.8±0.0	0.8±0.1

[a]Values are means ± S.E. of five male rats and expressed as ppm (NC-302 equivalents).

[b]Five minutes postadministration.

Source: CBI p. 15.

E. Urine and fecal samples collected from male rats during the first 24 hours after dosing were analyzed for metabolites. Following ethyl acetate extraction, 94 percent of the urinary radioactivity was recovered. The main metabolite detected was PPA, accounting for 47 percent of the urinary radioactivity (Table 4). The parent compound was not detected.

Ninety percent of the fecal radioactivity (28.7 percent of the administered dose) was extractable with 80 percent aqueous methanol. The two main metabolites identified by TLC were NC-302 acid and PPA, accounting for 49.6 and 24.0 percent of the fecal radioactivity, respectively. The parent compound again was not detected (Table 4).

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The distribution, excretion, and metabolism of NC-302 in CD(SD) rats after intravenous administration of [^{14}C-phenyl]NC-302 at 10 mg/kg were studied.

All radioactivity was excreted via the urine and feces, especially into the feces, by 7 days postadministration. It appeared that biliary excretion was the primary route of elimination for the radiolabel from the test animals. A sex difference in excretion patterns was apparent with the female rats excreting more radiolabel into the urine than did the males. Radioactive residues remaining in male and female rats 7 days after dose administration were only 2 to 3 percent of the dose.

The biological half-lives of radiolabel in the whole blood from 5 minutes to 120 hours after [^{14}C-phenyl]NC-302 administration were 21 hours in male rats and 17 hours in females.

[^{14}C] distribution levels in plasma, whole blood, liver, kidneys, brain, testes, and fat were determined. The order of radiolabel concentrations was as follows: plasma, whole blood, kidneys, liver > fat, testes > brain. In whole-body autoradiograms of male rats dosed with [^{14}C-phenyl]NC-302, radioactivity was observed in intestines, kidneys, liver, lungs, and blood from 1 to 24 hours postadministration, whereas it was hardly observed on and after 72 hours.

TABLE 4. Metabolite Distribution in Urine and Feces of Male Rats after Intravenous Administration of [^{14}C]NC-302[a]

Compounds	0- to 24-Hour Urine Sample	0- to 24-Hour Fecal Sample	Urine + Feces
NC-302	<0.1(<0.1)[b]	<0.1(<0.1)	<0.1(<0.1)
NC-302 phenol	1.2(0.1)	0.9(0.2)	0.9(0.3)
NC-302 acid	7.9(0.5)	49.6(14.2)	(41.5(14.7)
AM-1 (unknown)	7.7(0.5)	1.6(0.5)	2.8(1.0)
AM-2[c]	13.8(0.9)	4.8(1.4)	6.5(2.3)
PPA	47.0(3.2)	24.0(6.9)	28.5(10.1)
Origin	13.8(0.9)	6.3(1.8)	7.6(2.7)
Others	2.8(0.2)	2.7(0.8)	2.8(1.0)
Aqueous soluble	5.8(0.4)	- (-)	1.2(0.4)
Residues	- (-)	10.1(2.9)	8.2(2.9)
TOTAL	100.0(6.7)	100.0(28.7)	100.0(35.4)

[a] Metabolites were separated by TLC using benzene:ethanol:acetic acid (20:1:1, v/v) as the developing solvent.

[b] Values represent the percent of total urinary or fecal [^{14}C] and (percent of administered dose).

[c] Unknown metabolite AM-2, thought to be hydroxylated NC-302 acid (NC-302 acid-OH).

In 24-hour urine samples from male rats, at least five metabolites were detected; NC-302 acid, PPA, and hydroxylated NC-302 acid (NC-302 acid-OH) were identified. PPA was the primary metabolite in male rat urine. The metabolites identified in 24-hour fecal samples were similar to those found in the urine; however, NC-302 acid was the primary fecal metabolite. The metabolite distribution suggests that the NC-302 administered intravenously was initially hydrolyzed to NC-302 acid, and the NC-302 acid was further degraded to PPA. It was concluded that the primary metabolites in rat excreta (urine and feces) were NC-302 acid followed by PPA. No unchanged NC-302 was identified in the urine and feces.

B. A quality assurance statement was not provided.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: Although the Materials and Methods section could have been presented more thoroughly, this study provides some useful information on the metabolism, tissue distribution, and excretion of NC-302 in rats. The authors' conclusions were supported by individual animal data. The excretion data indicated a sex difference in radiocarbon elimination, with the females excreting more [^{14}C] into the urine and less into feces than the male animals. Elimination of radiocarbon from the blood also showed a sex difference, with the biological half-lives of radioactivity in the blood being 21 hours in male rats and 17 hours in females. Tissue distribution of radiocarbon and metabolite identification in rat excreta were determined only in male rats. Therefore, differences in tissue compartmentalization of [^{14}C-phenyl]NC-302 and metabolite formation among males and females could not be assessed.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 4-7.

APPENDIX A

Materials and Methods

ASSURE 005546

Page_____ is not included in this copy.

Pages 28 through 31 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__X__ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

005546

EPA: 68-02-4225
DYNAMAC No. 1-011-A2
March 26, 1986

DATA EVALUATION RECORD

NC-302

Metabolic Study in Rats

STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., Takano, S., Yamaguchi, I. and Misato, T. Metabolism of NC-302 in rats. (Unpublished study [No. unavailable] by Nissan Chemical Industries, Ltd., Japan, for E. I. Du Pont de Nemours and Co., Wilmington, DE; dated February 1985.) Accession No. 073546.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-26-86

1. CHEMICAL: NC-302; ethyl-2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]-propionate.

2. TEST MATERIAL: [^{14}C-phenyl]NC-302 and [^{14}C-quinoxaline]NC-302 with radiochemical purities > 98 percent and six authentic metabolite standards were used (see Appendix A, CBI Table 2).

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., Takano, S., Yamaguchi, I. and Misato, T. Metabolism of NC-302 in rats. (Unpublished study [No. unavailable] by Nissan Chemical Industries, Ltd., Japan, for E. I. Du Pont de Nemours and Co., Wilmington, DE; dated February 1985.) Accession No. 073546.

5. REVIEWED BY:

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Date: 4/2/86 (See Page 9)

Clint Skinner, Ph.D.
EPA Section Head

Signature: Clint Skinner
Date: 4.18.86

7. CONCLUSIONS:

A. The metabolic pathways of the herbicide NC-302 were studied in 5-week-old Sprague-Dawley rats (number not specified) following administration of single oral doses at 160 mg/kg. Males and females were dosed with [^{14}C-phenyl]NC-302 and another group of males was dosed with [^{14}C-quinoxaline]NC-302 (see Appendix A, CBI Table 1 for chemical structures). Approximately 23 percent of the administered dose was not absorbed, but was excreted in the feces regardless of the position of the [^{14}C] label. The NC-302 that was absorbed was extensively metabolized (Figure 1). The first metabolic step appeared to be the hydrolysis of the ethyl ester group to yield 2-[4-(6-chloro-2-quinoxalinyloxy)-phenoxy]propionic acid (NC-302 acid). NC-302 acid (including conjugates) was the major metabolite present in all tissues and excreta examined except for the urine of male rats, where 2-(4-hydroxyphenoxy)propionic acid (PPA) accounted for 50 percent of the [^{14}C]. It appeared that all other metabolites detected arose from the NC-302 acid. Minor metabolites that were identified included PPA, 6-chloroquinoxaline-2-one (CQO), hydroxylated NC-302 acid (NC-302 acid-OH), hydroxylated CQO (CQO-OH), 4-(6-chloro-2-quinoxalinyloxy)phenol (NC-302 phenol), several conjugates, and a few unidentified compounds.

B. This metabolic study is acceptable assuming that the authors used sufficient numbers of animals per group to ensure that the results represent the metabolism of NC-302 by rats in general and not just by one or two animals.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

Test animals were male and female CD(SD) rats (Charles River Japan) that were 5 weeks old. Animals (numbers not specified) were administered single oral doses of [^{14}C]NC-302 diluted with nonradioactive NC-302 at 160 mg/kg as a suspension in aqueous 1 percent Tween 80. The final specific activities of the administered doses were not given. Groups of male rats were administered either [^{14}C-phenyl]NC-302 or [^{14}C-quinoxaline]-NC-302. Female rats received only [^{14}C-phenyl]NC-302. After dosing the rats were housed in metabolism cages for 48 hours and urine and feces were collected. Bile was collected for 24 hours from an unspecified number of male rats dosed with [^{14}C-phenyl]-NC-302. Plasma, liver, and kidney were obtained from male rats at 0.25, 6, 24, and 48 hours after [^{14}C-phenyl]NC-302

[1]Only items appropriate to this DER have been included.

Proposed Metabolic Pathway for NC-302

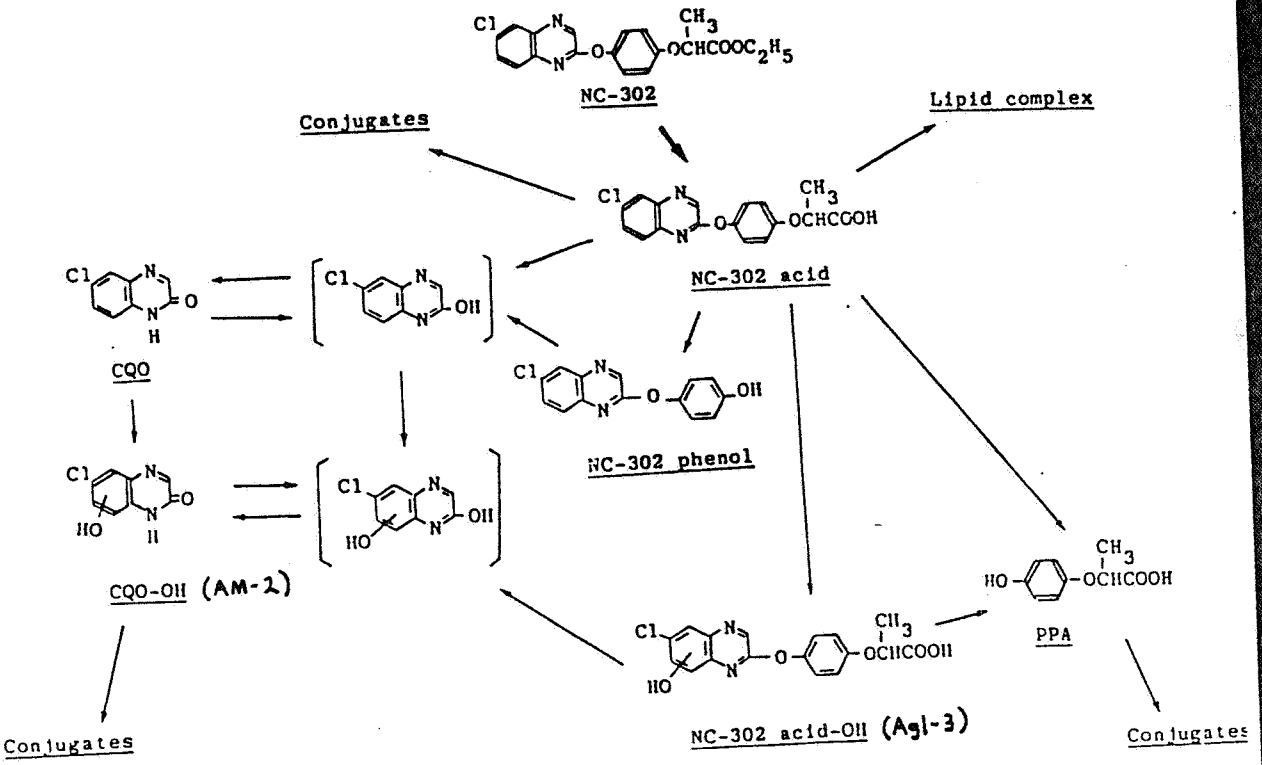

Figure 1. (CBI Figure 25)

administration and at 48 hours after [^{14}C-quinoxaline]NC-302 administration, whereas those from female rats were collected only 6 hours after [^{14}C-phenyl]NC-302 administration. Brain and fat samples were obtained from male and female rats at 6 and 24 hours after [^{14}C-phenyl]NC-302 administration.

Urine was diluted with water, acidified to pH 2.0, and extracted twice with ethyl acetate. The aqueous phase was incubated first with β-glucuronidase, then with aryl sulfatase. Following each enzymatic treatment, the aqueous phases were extracted twice with ethyl acetate. Each ethyl acetate extract was concentrated and analyzed by thin-layer chromatography (TLC). Polar metabolites remaining at the origin were subjected to β-glucuronidase treatment.

Feces, liver, and kidney samples were homogenized in 80 percent methanol and centrifuged, and the solvent was decanted. After a second extraction with fresh methanol, the extracts were pooled and the methanol was evaporated. Distilled water was added to each concentrate, adjusted to pH 2.0, and then extracted twice with ethyl acetate. The ethyl acetate extracts were analyzed by TLC, and the radioactivity remaining at the origin was subjected to β-glucuronidase treatment.

Other tissue samples were analyzed by TLC following these procedures: 1) plasma was diluted with water, adjusted to pH 2.0, and then extracted twice with ethyl acetate; 2) brain samples were fractionated as in CBI Figure 1 (see Appendix A), and ethyl acetate extracts were applied to TLC plates; and 3) fat samples were extracted as in CBI Figure 2 (see Appendix A) before being applied to the TLC plates. Bile was applied directly to the TLC plates.

Metabolites were quantified following TLC analysis by scraping off the radioactive zones identified by autoradiography and counting the [^{14}C] by liquid scintillation spectrometry (LSC). Metabolite identification was achieved by cochromatography with authentic standards (see Appendix A, CBI Table 2) using TLC and three different solvent systems and by gas chromatography-mass spectrometry (GC/MS).

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Forty-eight hours following administration of a single oral dose of [^{14}C-phenyl]NC-302 at 160 mg/kg, 5.3 and 73.2 percent of the dose was recovered in the urine and feces of male rats, respectively, and 16.5 and 58.6 percent in the urine and feces of female rats, respectively. When male rats were dosed with [^{14}C-quinoxaline]NC-302 at 160 mg/kg, 5.5 and 72.6 percent of the dose was recovered in the urine and feces, respectively, after 48 hours.

B. At least five different metabolites were found in the excreta of male and female rats dosed with [^{14}C-phenyl]NC-302 (Table 1). NC-302 acid and PPA were the major metabolites in urine, comprising 21.6 and 49.4 percent, respectively, of male urine and 53.4 and 25.1 percent, respectively, of female urine. Several unidentified minor metabolites (e.g., AM-1 and AM-2) as well as traces of NC-302 phenol were also found in both male and female urine.

Approximately 87 to 89 percent of the radioactivity in the feces was extracted with 80 percent methanol. Unchanged NC-302 comprised 30.7 and 38.6 percent of the 48-hour fecal [^{14}C] in males and females, respectively. In male rats, virtually all of the parent compound was excreted by 24 hours, indicating that it was not absorbed. The fecal metabolite profile was similar to that of urine; NC-302 acid and PPA were the major metabolites, comprising 33.6 and 14 percent of the total radioactivity in males, respectively, and 24.7 and 15.1 percent in females, respectively. As with urine, minor metabolites AM-1 and AM-2 as well as traces of NC-302 phenol were found in feces from both males and females. In addition to cochromatography with known standards on TLC, the identities of NC-302 acid and PPA were confirmed by GC/MS.

C. Metabolites found in the urine and feces of male rats 48 hours following a single oral dose of [^{14}C-quinoxaline]NC-302 are shown in Table 2. Similar metabolic profiles for the urine and feces were observed as when [^{14}C-phenyl]NC-302 was administered, except for the identification of CQO and the presence of larger amounts of aqueous soluble and more polar metabolites. NC-302 was not found in urine, but comprised 33.2 percent of the fecal radioactivity. NC-302 acid was the major metabolite identified in both excreta, comprising 20.9 and 32.6 percent of the [^{14}C] in urine and feces, respectively.

D. The radioactivity remaining at the origin of the developed TLC plates was removed and treated with β-glucuronidase; the resulting aglycones were analyzed again by TLC. Free NC-302 acid and PPA were found in treated samples from urine and feces of both sexes dosed with [^{14}C-phenyl]NC-302, but in very small quantities. In male rats dosed with [^{14}C-quinoxaline]NC-302, NC-302 acid was found in both urine and feces. A few unidentified aglycones were also observed in the feces.

E. The metabolites of [^{14}C-phenyl]NC-302 in the aqueous phases were not investigated because of the small amount of radioactivity in those fractions. Incubation of the aqueous metabolites of [^{14}C-quinoxaline]NC-302 in urine with β-glucuronidase and aryl sulfatase yielded 65 and 29 percent of the radioactivity extractable with ethyl acetate, respectively. TLC analysis of the β-glucuronidase-treated samples showed the release of at least three aglycones. Agl-1, corresponding to the

TABLE 1. NC-302 Metabolites[a] in Urine and Feces of Male and Female Rats 48 Hours after Administration of a Single Oral Dose of [^{14}C-Phenyl]NC-302 at 160 mg/kg

	Male		Female	
Compound	Urine	Feces	Urine	Feces
Organic Soluble (ethyl acetate)	95.8(5.10)[b]	87.3(63.90)	98.5(16.29)	85.2(49.95)
NC-302	< 0.1(<0.01)[c]	30.7(22.48)	<0.1(<0.02)[c]	38.6(22.64)
NC-302 phenol	trace	trace	trace	trace
NC-302 acid	21.6(1.15)	33.6(24.57)	53.4(8.83)	24.7(14.45)
AM-1	3.4(0.18)	0.7(0.49)	2.5(0.41)	< 0.1(<0.01)
AM-2[d]	11.7(0.62)	2.9(2.15)	4.6(0.76)	2.0(1.20)
PPA	49.4(2.63)	14.0(10.25)	25.1(4.14)	15.1(8.87)
Origin	7.1(0.38)	2.3(1.71)	10.3(1.71)	3.1(1.81)
Others	2.6(0.14)	3.1(2.25)	2.6(0.44)	1.7(0.98)
Aqueous soluble	4.2(0.22)	2.1(1.55)	1.5(0.25)	1.5(0.90)
Residues	- (-)	10.6(7.73)	- (-)	13.3(7.77)
Total	100.0(5.32)	100.0(73.18)	100.0(16.54)	100.0(58.62)

[a] Metabolites were separated by TLC using solvent system B (benzene:ethanol:acetic acid, 20:1:1, v/v); see Appendix A, CBI Table 2, for chemical structures.

[b] Values represent the percent of total urinary or fecal [^{14}C] and (percent of administered dose).

[c] Below the assay detection limit.

[d] Metabolite AM-2 was identified by GC/MS to be hydroxylated NC-302 acid; the exact position of the hydroxyl group could not be established.

Source: CBI Tables 4 and 5.

TABLE 2. NC-302 Metabolites[a] in Urine and Feces of Male Rats 48 Hours after Administration of a Single Oral Dose of [^{14}C-Quinoxaline]NC-302 at 160 mg/kg

Compound	Urine	Feces
Organic soluble (ethyl acetate)	72.5(3.96)	84.1(61.05)
NC-302	< 0.1(<0.01)[c]	33.2(24.10)
NC-302 phenol	1 5(0.08)	0.7(0.50)
CQO	1.2(0.07)	1.2(0.88)
NC-302 acid	20.9(1.14)	32.6(23.63)
AM-1	3.3(0.18)	0.7(0.54)
AM-2[d]	11.1(0.61)	3.5(2.54)
Origin	16.9(0.92)	4.2(3.04)
Others	17.6(0.96)	8.0(5.82)
Aqueous soluble	27.5(1.50)	3.0(2.21)
Residues	-- (--)	12.9(9.34)
Total	100.0(5.46)	100.0(72.60)

[a] Metabolites were separated by TLC using solvent system 8 (benzene:ethanol:acetic acid, 20:1:1, v/v), see Appendix A, CBI Table 2, for chemical structures.

[b] Values represent the percent of total urinary or fecal [^{14}C] and (percent of administered dose).

[c] Below the assay detection limit.

[d] Metabolite AM-2 was identified by GC/MS to be hydroxylated NC-302 acid; the exact position of the hydroxyl group could not be established.

Source: CBI Table 6.

NC-302 acid, Agl-2, and Agl-3 comprised 2.1, 4.6, and 51.2 percent of the total radioactivity, respectively. Approximately 41.2 percent of the total [^{14}C] remained at the origin. Agl-3 was also released by sulfatase treatment. Metabolite Agl-3 was shown by GC/MS to be hydroxylated CQO (CQO-OH); the exact position of the hydroxyl group could not be established.

β-Glucuronidase treatment of the aqueous metabolites in feces from rats dosed with [^{14}C-quinoxaline]NC-302 released at least four aglycones, the major one being NC-302 acid. The relative amounts of these fractions were not reported.

F. The authors reported that 65 percent of the biliary radioactivity at 0 to 24 hours after dosing was either NC-302 acid (31 percent) or its glucuronide conjugate (34 percent). Most of the other [^{14}C] was in the form of unidentified polar metabolites (29.7 percent); no NC-302 was detected. Small amounts of NC-302 phenol, AM-1, AM-2, PPA, and CQO were also found. No unchanged NC-302 was found in any tissue.

The major radiolabeled metabolite found in plasma, liver, kidney, brain, and fat was NC-302 acid, comprising between 73 and 93 percent of the total extractable radioactivity for each tissue. In the fat, NC-302 acid was found to form a complex with lipid, as indicated by lipase hydrolysis. The concentration of the complex in fat increased with time.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. After administration of a single oral dose of [^{14}C-phenyl]NC-302 to rats, the major metabolites in urine were NC-302 acid and PPA. Hydroxylated NC-302 acid, NC-302 phenol, and several unidentified compounds were also present as minor metabolites. In urine of male rats, NC-302 acid and PPA accounted for 22 and 50 percent of the radioactivity, respectively, whereas in females they accounted for 53 and 25 percent, respectively. Both compounds were also excreted as conjugates. The parent compound, NC-302, was not detected in the urine.

In male and female rats administered [^{14}C-phenyl]NC-302, unchanged NC-302 accounted for 31 and 39 percent of the total [^{14}C] excreted in the feces, respectively. The fecal metabolites were similar to those found in urine. NC-302 and PPA accounted for 34 and 14 percent of the radioactivity excreted in male feces, respectively, and 25 and ~~125~~ 15 percent of the [^{14}C] in female feces, respectively.

Following oral administration of [^{14}C-quinoxaline]NC-302, the major metabolite detected in the urine and feces of the male rats was NC-302 acid. Minor metabolites included CQO, NC-302 acid-OH, NC-302 phenol, and several unidentified compounds. The parent compound was excreted in the feces (33 percent of fecal [^{14}C]) but not in the urine. Compared with [^{14}C-phenyl]NC-302 administration, there was an increase in the relative proportion of aqueous metabolites; one of which was suggested to be a hydroxylated CQO conjugate.

In bile, NC-302 acid and its β-glucuronide conjugate were the major metabolites; no NC-302 was detected. Practically all the radioactivity in plasma, liver, kidney, and brain was identified as NC-302 acid, with proportions of 93 to 97 percent, 80 to 85 percent, 73 to 91 percent, and 75 to 94 percent, respectively. NC-302 acid-OH, NC-302 phenol, PPA, CQO, and a few unidentified compounds were also detected as minor metabolites. No unchanged NC-302 was found in any tissue. NC-302 acid and its lipid complex accounted for most of the radioactivity detected in fat.

The proposed metabolic pathway for NC-302 in the rat is presented in Figure 1.

B. A quality assurance statement was not provided.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: We agree with the authors' proposed metabolic pathway for NC-302. The data presented support the conclusion that NC-302 acid is the first metabolite formed and is further metabolized to a number of other compounds. The metabolic pathways in males and females are similar although the proportions of NC-302 acid and PPA in the urine following [^{14}C-phenyl]NC-302 administration were different between sexes. However, several deficiencies were noted. The number of animals per dose group was not reported. The authors indicated that they used more than one rat per group, and they stated that five rats per sex per dose were used in other metabolic studies with this compound. Also, the final specific activity of the dosed compound and the weight of the animals were not reported. In addition, there was no explanation why younger than usual rats (5 weeks) were used in this study.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 4-8.

APPENDIX A

Materials and Methods

ASSURE 005546

Page_____ is not included in this copy.

Pages 43 through 51 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

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005546

EPA: 68-02-4225
Dynamac No. 1-11A-3
March 26, 1986

DATA EVALUATION RECORD

NC-302

Metabolic Study in Rats

STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., and Takano, S. Absorption, distribution and excretion of NC-302 in rats after single oral administration (160 mg/kg). (Unpublished study [No. unavailable] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E.I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.) Accession No. 073546.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-26-86

1. CHEMICAL: NC-302; ethyl-2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]-propionate.

2. TEST MATERIAL: 1) [^{14}C-phenyl]NC-302 had a specific activity of 8.5 mCi/mmol and a radiochemical purity greater than 99 percent. 2) [^{14}C-quinoxaline]NC-302 had a specific activity of 7.8 mCi/mmol and a radiochemical purity greater than 98 percent.

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., and Takano, S. Absorption, distribution and excretion of NC-302 in rats after single oral administration (160 mg/kg). (Unpublished study [No. unavailable] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E.I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.) Accession No. 073546.

5. REVIEWED BY:

Lynne L. W. Binari, M.S.
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Date: 3/26/86

Charles E. Rothwell, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Charles E Rothwell
Date: 3-26-86

6. APPROVED BY:

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Metabolism
Technical Quality Control
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Date: March 25, 1986

Whang Phang, Ph.D.
EPA Reviewer

Signature: W Lyle Long
Date: 4/3/86

Clint Skinner, Ph.D.
EPA Section Head

Signature: W D Burnam
Date: 4.18.86

7. CONCLUSIONS:

A. The absorption, distribution, and elimination of radiolabel were investigated following oral administration of either [^{14}C-phenyl]- or [^{14}C-quinoxaline]NC-302 at 160 mg/kg to male and/or female Sprague-Dawley rats.

Most of the radioactivity was eliminated into the urine and feces within 3 days following dose administration. The major route of elimination in both the male and female rats was in the feces. However, the females excreted more radiocarbon into the urine and less into the feces than male rats. The elimination of [^{14}C-phenyl]NC-302 and [^{14}C-quinoxaline]NC-302 in male rats was similar. No radioactivity was detected in the expired air of the treated animals.

Radiolabel was widely distributed throughout the tissues of the male and female rats following [^{14}C]NC-302 administration; the plasma, liver, blood, and kidneys had the highest concentrations of radioactivity at 6 to 9 hours after dosing. Residue levels in the tissues of female rats were higher than those found in the males. The average biological half-lives of [^{14}C] residues in blood were 26.7 and 19.4 hours in male and female rats, respectively. The disappearance rate of radiolabel from each tissue was similar to that of the blood, except for the adipose tissue. Half-lives of 60.5 and 155 hours were seen in brown and white fat, respectively, in males, and a half-life of 96.7 hours was seen in adipose tissue (type not specified) in females. Whole-body autoradiograms from male rats administered [^{14}C]NC-302 supported the tissue [^{14}C]-residue analyses. At 6 hours postadministration, high levels of radioactivity were observed in the gastrointestinal tract, blood, liver, lungs, and kidneys, whereas radioactivity was barely evident by 72 hours postadministration.

A comparison of urinary excretion of radiolabel between rats dosed intravenously and orally with [^{14}C]NC-302 was made to determine the extent of [^{14}C]NC-302 absorption. Over a 7-day test period, female rats absorbed a higher percentage ($\geq$ 65 percent) of radioactivity than did the males ($\geq$ 48 percent).

B. This study is acceptable.

Items 8 through 10--see footnote 1.

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[1] Only items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test animals were 5-week-old male and female CD Sprague-Dawley rats. [^{14}C]NC-302 was mixed with unlabeled NC-302 in aqueous 1 percent Tween 80 and administered to the rats via stomach tube at 160 mg/kg (about 5 µCi/mL/rat).

2. For the excretion studies, five rats of each sex were given a single dose of [^{14}C-phenyl]NC-302, and three male rats each received a single dose of [^{14}C-quinoxaline]NC-302. The test animals were individually housed in metabolism cages following dosing. Urine, feces, and expired air were collected separately at 24-hour intervals and radioassayed by liquid scintillation counting (LSC) using standard techniques. Seven days after [^{14}C]NC-302 was administered, animals were sacrificed, the fur was removed, and carcasses were dissolved in 6 N hydrochloric acid at 100°C. Both the fur and carcass were radioassayed.

   To assess biliary excretion, four male rats received a single dose of [^{14}C-phenyl]NC-302, and bile was collected at 6-hour intervals for 24 hours through a cannula inserted into the bile duct. Radioactivity in bile was assayed by LSC.

3. [^{14}C] tissue distribution was determined in 45 male and 45 female rats dosed with [^{14}C-phenyl]NC-302. Groups of five rats of each sex were sacrificed at each of the following time intervals after dosing: 0.25, 1, 3, 6, 9, 24, 72, 120, and 168 hours. Blood, liver, kidneys, cerebrum, cerebellum, spleen, lungs, adrenals, heart, pancreas, eyes, parotid gland, thymus, skin, fat, testes, salivary gland, muscle, bone, brown fat, lymph node, blood vessel, epididymis, and gastrointestinal tract were taken from the male rats. Blood, liver, kidneys, brain, fat, ovaries, uterus, and adrenals were taken from female rats. To obtain plasma samples, whole blood was centrifuged to remove red blood cells. All tissue samples were radioassayed by LSC following combustion using standard techniques.

4. Whole-body autoradiography was conducted on male rats (number not specified) sacrificed at 0.25, 1, 6, 24, 72, 120, and 168 hours after dose administration. Following sacrifice, the rats were frozen in a dry ice:acetone bath for about 30 minutes. The frozen carcass was placed in 5 percent (w/v) aqueous carboxymethylcellulose at -70°C and then mounted onto a microtome stage (maintained in a cryostat at -20°C). Thin sections (50 µm) were freeze dried and exposed to X-ray film.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Following oral administration of [^{14}C-phenyl]NC-302 at 160 mg/kg to male and female rats, most of the radioactivity was eliminated in the feces (Table 1). The total radioactivities detected in the feces from males and females dosed with [^{14}C-phenyl]NC-302 were approximately 85 (range, 74 to 95 percent) and 73 percent (range, 65 to 78 percent), respectively. The total radioactivities detected in the urine of [^{14}C-phenyl]NC-302-dosed male and female rats were approximately 8 (range, 5 to 10 percent) and 26 percent (range, 21 to 33 percent) of the administered dose, respectively. In the feces, approximately 73 (range, 61 to 86 percent) and 59 percent (range, 55 to 62 percent) of the administered radioactivity was recovered in males and females, respectively, during the initial 48 hours after dosing with [^{14}C-phenyl]NC-302 (Table 1). Elimination of [^{14}C] in the urine was highest during days 1 and 2, and thereafter decreased gradually. Little if any radioactivity was eliminated in expired air. The total recovered radioactivities from the urine, feces, fur, and carcasses of [^{14}C-phenyl]NC-302-dosed male and female rats were approximately 97 (range, 88 to 103 percent) and 102 percent (range, 96 to 109 percent), respectively (Table 2).

Elimination of [^{14}C] in the three male rats dosed with [^{14}C-quinoxaline]NC-302 was similar to male rats dosed with [^{14}C-phenyl]NC-302 (Table 1). The total radioactivity detected in the urine was about 8 percent (range, 7 to 9 percent), while the total radioactivity detected in the feces was about 81 percent (range, 80 to 83 percent) (Table 1). Total radioactivity recovered from the urine, feces, fur, and carcasses of [^{14}C-quinoxaline]NC-302-dosed male rats was approximately 94 percent (range, 92 to 96 percent) (Table 2).

The rate of biliary excretion of radiolabel over a 24-hour period from male rats dosed with [^{14}C-phenyl]NC-302 is shown in Table 3. Biliary excretion of radioactivity occurred at a steady rate of about 1 percent of the administered dose per hour.

B. [^{14}C] residues in tissues of male and female rats at various intervals following a single oral dose of [^{14}C-phenyl]NC-302 are presented in Appendix B (CBI pp. 18, 19, and 22: Tables 7-1 and 7-2 for male rats and Table 10 for females, respectively).

TABLE 1. Percent Recovery of Radioactivity in Rat Urine and Feces Following Oral Administration of [^{14}C]NC-302

Sex/Form of Radiolabel	Percent Recovery of Administered Radioactivity							% Total [^{14}C] Recovered
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
Urine								
Males/ [^{14}C-phenyl]NC-302[a]	2.97 ± 0.35	2.35 ±0.29	1.48 ±0.29	0.63 ±0.10	0.26 ±0.05	0.18 ±0.05	0.09 ±0.02	7.96 ±0.94
Males/ [^{14}C-quinoxaline]-NC-302[b]	2.96 ±0.54	2.50 ±0.27	1.29 ±0.09	0.63 ±0.12	0.37 ±0.07	0.25 ±0.03	0.16 ±0.02	8.16 ±0.59
Females/ [^{14}C-phenyl]NC-302[a]	7.60 ±0.92	8.94 ±0.57	5.43 ±0.47	2.36 ±0.17	1.17 ±0.17	0.41 ±0.10	0.33 ±0.04	26.24 ±2.04
Feces								
Males/ [^{14}C-phenyl]NC-302[a]	52.86 ±5.26	20.32 ±3.77	7.40 ±0.98	2.64 ±0.31	1.24 ±0.30	0.63 ±0.13	0.36 ±0.13	85.46 ±3.47
Males/ [^{14}C-quinoxaline]-NC-302[b]	52.11 ±1.91	20.49 ±3.51	4.93 ±0.68	1.90 ±0.24	1.00 ±0.09	0.52 ±0.07	0.37 ±0.03	81.33 ±0.81
Females/ [^{14}C-phenyl]NC-302[a]	34.67 ±1.67	23.95 ±1.66	8.65 ±0.86	3.55 ±0.81	1.03 ±0.14	0.46 ±0.06	0.30 ±0.04	72.60 ±2.09

[a]Mean values ± S.E. of five rats.

[b]Mean values ± S.E. of three rats.

Source: CBI pp. 13, 14, and 15.

TABLE 2. Cumulative Recovery of Radioactivity in Rats Following Oral Administration of [^{14}C]NC-302[a]

	[^{14}C-phenyl]NC-302		[^{14}C-quinoxaline]NC-302
	Males[b]	Females[b]	Males[c]
Urine	7.96±0.94	26.24±2.04	8.16±0.59
Feces	85.46±3.47	72.60±2.09	81.33±0.81
Expired air	<0.01	<0.01	<0.01
Fur	1.09±0.04	1.22±0.05[c]	1.06±0.06
Carcass	2.66±0.06	1.91±0.17[c]	3.13±0.01
Total	97.16±2.71	102.00±2.10	93.68±1.26

[a]Expressed as percent of administered dose.

[b]Mean values ± S.E. of five rats.

[c]Mean values ± S.E. of three rats.

Source: CBI p. 16.

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TABLE 3. Biliary Excretion of Radioactivity in Rats Following Oral Administration of [^{14}C-phenyl]NC-302

Time (hr)	Percent [^{14}C] Recovered[a]
0- 6	5.56±1.58
6-12	6.51±1.02
12-18	6.24±0.77
18-24	4.04±1.11
0-24	22.35±0.85

[a]Mean values ± S.E. of four male rats.

Source: CBI p. 17.

In the male rats, residue levels in tissues increased after dosing, with the highest [^{14}C] levels noted at 6 to 9 hours postadministration; exceptions were the adrenals and fat which showed their peak [^{14}C] concentrations at 3 and 24 hours, respectively. The major sites of radiolabel accumulation in the male rats were plasma (~234 ppm), liver (~199 ppm), blood (~183 ppm), and kidneys (~119 ppm). High correlation coefficients (greater than 0.91) between blood and tissue concentrations were calculated for all tissues except brown fat (0.8780), adrenals (0.7765), and white fat (0.2323). The disappearance of [^{14}C] from the blood of male rats had an average biological half-life of 26.7 hours (Figure 1). The disappearance rates of radiolabel from each tissue were similar to those of the blood except for brown and white fat, which had average biological half-lives of 60.5 and 155.0 hours, respectively.

Whole-body autoradiograms of male rats dosed with [^{14}C-phenyl]-NC-302 supported the tissue radioanalysis. Initially (15 minutes), high concentrations of radioactivity were observed in the stomach and small intestines. At 6 hours postadministration, high levels of radioactivity were seen in the large intestines, blood, liver, lungs, kidneys, bone marrow, and teeth. Levels of radioactivity decreased thereafter, with little being evident on or after 72 hours following dosing.

In female rats dosed with [^{14}C-phenyl]NC-302, maximum concentrations of [^{14}C] occurred in the tissues at 9 hours postadministration (see Appendix B, CBI Table 10, p. 22). The primary sites of radiolabel accumulation were plasma (~347 ppm), liver (~287 ppm), blood (~256 ppm), kidneys (~169 ppm), adrenals (~122 ppm), and ovaries (~118 ppm). High correlation coefficients (greater than 0.96) between the radiolabel concentrations found in the blood and those found in each tissue occurred, except for adipose tissue (0.7053). The disappearance rate of [^{14}C] from the blood of female rats had an average biological half-life of 19.4 hours (Figure 1). The disappearance rates of radiolabel from each tissue were similar to that of the blood except for adipose tissue, which had an average biological half-life of 96.7 hours.

C. To assess the extent of radiolabel absorbed by male and female rats after oral administration of the test material, total radioactivities recovered in the urine following intravenous (10 mg/kg) or oral dosing (160 mg/kg) of [^{14}C-phenyl]NC-302 were compared at several time intervals over a 7-day test period (Table 4). Male rats absorbed approximately 48 percent of the administered radiocarbon, while female animals absorbed about 65 percent of the dosed radioactivity over the 7-day test period.

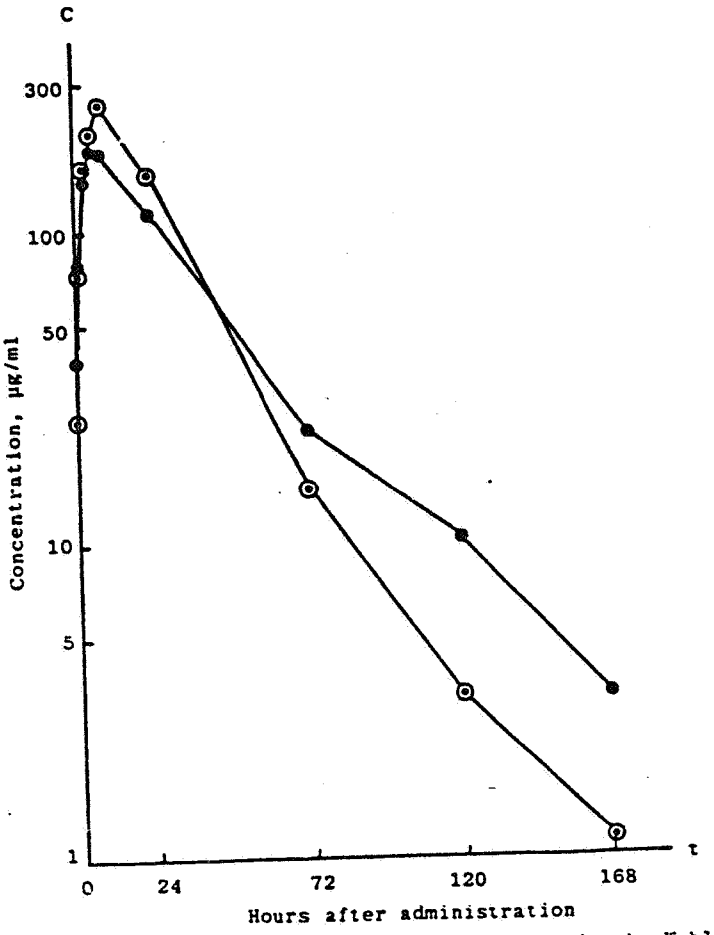

Fig.1 Each concentration of radioactivity in the W.blood of male and female rats after single oral administration of ^{14}C-NC-302 at 160 mg/kg.
●—● :Male rats ⊙—⊙ :Female rats

SOURCE: CBI p. 27.

TABLE 4. Absorption of Radioactivity in Rats After [^{14}C]NC-302 Administration

Period (days)	Percent Radioactivity Excreted in The Urine After Dosing[a]		Percent Absorbed[c]
	Oral	Intravenous[b]	
Males			
0-1	2.97±0.35	6.72±0.93	44.2
0-2	5.31±0.55	10.90±1.54	48.7
0-3	6.80±0.77	13.69±1.92	49.7
0-4	7.43±0.85	15.14±2.04	49.1
0-5	7.69±0.89	15.86±2.15	48.5
0-6	7.87±0.93	16.30±2.22	48.3
0-7	7.96±0.94	16.54±2.25	48.1
Means ± S.E.	-	-	48.1±0.7
Females			
0-1	7.60±0.92	16.62±1.72	45.7
0-2	16.54±1.42	25.14±2.46	65.8
0-3	21.97±1.83	30.99±2.65	70.9
0-4	24.32±1.91	34.71±2.65	70.1
0-5	25.49±1.98	36.69±2.64	69.5
0-6	25.94±2.03	37.96±2.59	68.3
0-7	26.24±2.04	38.68±2.54	67.8
Means ± S.E.	-	-	65.4±3.4

[a] Mean values ± S.E. of five rats.

[b] Values are from another study, "Distribution, Excretion, and Metabolism of NC-302 in Rats after Intravenous Administration" by Hirata et al., February 1985.

[c] Percent absorbed = $\frac{\text{Total excreted urinary radioactivity (p.o.)}}{\text{Total excreted urinary radioactivity (i.v.)}} \times 100$

Source: CBI pp. 25 and 26.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES: 005546

A. The absorption, distribution, and excretion of NC-302 in CD Sprague-Dawley rats after a single oral administration of [^{14}C]NC-302 at 160 mg/kg were studied.

Almost all of the radioactivity was excreted in the urine and feces (mostly in the feces) by 3 days following dose administration. Active biliary excretion was observed, and a large percentage of the administered radioactivity was eliminated via this route. Radiocarbon residues remaining in the rats 7 days after administration were 2 to 3 percent of the dose. No radioactivity was detected in expired air.

Following [^{14}C]NC-302 administration, peak concentrations of radioactivity in male rat tissues occurred at 6 to 9 hours, except for fat and adrenals. Peak concentrations of radioactivity in adipose tissue and adrenals occurred at 24 and 3 hours, respectively. The maximal concentrations of radioactivity in each tissue were as follows: plasma, 233.8 ppm; liver, 199.4 ppm; whole blood, 182.8 ppm; kidneys, 119.2 ppm; blood vessel, 87.5 ppm; adrenals, 82.4 ppm; heart, 74.3 ppm; and lung, 71.6 ppm.

In the female rats, peak radiocarbon concentrations in tissues occurred at 9 hours after [^{14}C]NC-302 administration, except in adipose tissue. The maximal radiocarbon concentrations found were as follows: plasma, 347.1 ppm; liver, 287.3 ppm; whole blood, 256.0 ppm; kidneys, 168.5 ppm; adrenals, 122.3 ppm; ovaries, 117.9 ppm; and uterus, 95.0 ppm. The radiolabel concentrations were lowest in the brain in both the male and female rats (6-7 ppm).

There were good correlations between blood radiocarbon concentrations and tissue concentrations, except for adipose tissue. In addition, the biological half-life of radiocarbon for each tissue was similar to that of the radioactivity in the blood. The biological half-lives of radioactivity in the blood of male and female rats were 26.7 and 19.4 hours, respectively.

Whole-body autoradiograms supported the results obtained from the radiocarbon analysis of the tissues. Apart from the gastrointestinal tract, high concentrations of radioactivity were observed in the blood, liver, kidneys, lungs, bone marrow, and teeth at 6 hours postadministration. On and after 120 hours, hardly any radioactivity was observed in the tissues, indicating that no accumulation of radiocarbon occurred after [^{14}C]NC-302 administration.

Gastrointestinal absorption in male and female rats was calculated to be 48 and 65 percent, respectively, of the single oral dose of 160 mg/kg.

B. A quality assurance statement was not provided.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: In general, the authors' conclusions are supported by individual animal data. This study provides some useful information on the absorption, distribution, and elimination of NC-302 in rats. The results indicate that NC-302 is absorbed from the gastrointestinal tract after oral administration. Radiolabeled material originating from [^{14}C]NC-302 was eliminated into the urine and feces, primarily into the feces. Female rats excreted more radiolabel into the urine and less into the feces than the male animals. Radiolabel was widely distributed throughout the tissues following [^{14}C]NC-302 administration, with blood, liver, and kidneys having the highest levels of radioactivity. Tissues of female rats contained higher [^{14}C]-residue levels than the same tissues of male rats. It should be noted that the calculations for the percentage of the oral dose that was absorbed are only rough estimates. The authors assumed that absorption of NC-302 from the gastrointestinal tract and subsequent excretion via the urine was linear. However, the effect of dose on the percent excretion of [^{14}C] in the urine following either oral or intravenous dosing was not demonstrated.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 4-6, and Appendix B, CBI Table 7, pp. 18-19, and CBI Table 10, p. 22.

APPENDIX A

Materials and Methods

ASSURE 005546

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Pages 66 through 72 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

005546

EPA: 68-02-4225
DYNAMAC No. 1-011A-4
March 26, 1986

DATA EVALUATION RECORD

NC-302

Metabolic Study in Rats

STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., Takano, S., Yamaguchi, I., and Misato, T. Absorption, distribution, excretion and metabolic transformation of NC-302 in rats after single oral administration (1.5 mg/kg). (Unpublished study [No. unavailable] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E. I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.) Accession No. 073546.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-26-86

005546

1. CHEMICAL: NC-302; ethyl-2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]-propionate.

2. TEST MATERIAL: 1) [^{14}C-phenyl]NC-302 had a specific activity of 8.5 mCi/mmol and a radiochemical purity greater than 99 percent. 2) [^{14}C-quinoxaline]NC-302 had a specific activity of 7.8 mCi/mmol and a radiochemical purity greater than 98 percent.

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., Takano, S., Yamaguchi, I., and Misato, T. Absorption, distribution, excretion and metabolic transformation of NC-302 in rats after single oral administration (1.5 mg/kg). (Unpublished study [No. unavailable] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E. I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.) Accession No. 073546.

5. REVIEWED BY:

Lynne L. W. Binari, M.S.
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Charles E. Rothwell, Ph.D.
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6. APPROVED BY:

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Date: March 25, 1986

Whang Phang, Ph.D.
EPA Reviewer

Signature: W Phang
Date: 4/4/86

Clint Skinner, Ph.D.
EPA Section Head

Signature: W J Skinner
Date: 4-8-86

7. CONCLUSIONS:

A. The absorption, distribution, and elimination of radiolabel were investigated following oral administration of [^{14}C]NC-302 at 1.5 mg/kg to male and female Sprague-Dawley rats.

Most of the radioactivity was eliminated in the urine and feces within 3 days following dose administration. The major route of elimination in both sexes was in the feces. However, a sex-related difference in the total elimination of radiolabel was apparent; female rats excreted more radioactivity into the urine than did the male animals. Elimination of [^{14}C-phenyl]NC-302 and [^{14}C-quinoxaline]NC-302 in male rats was similar. No radioactivity was detected in the expired air of the treated animals.

Absorption and distribution of radioactivity were rapid. Maximum blood concentrations of NC-302 equivalents occurred about 6 hours postadministration. The highest concentrations of radioactivity were in plasma, whole blood, kidneys, and liver 24 hours following dosing. Male rat tissues contained higher levels of [^{14}C] residues than did female tissues, with the exception of adipose tissue. The biological half-lives of [^{14}C] in blood were 20.4 and 19.6 hours in male and female rats, respectively.

The major metabolites identified in the rat excreta following administration of [^{14}C-phenyl]NC-302 were 2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]propionic acid (NC-302 acid) and 2-(4-hydroxyphenoxy)propionic acid (PPA). However, females excreted more NC-302 acid and less PPA in the urine than did males. NC-302 acid was also the major metabolite excreted in the feces of male and female rats. Unchanged NC-302 was not detected in the urine but accounted for 5 to 7 percent of the administered radioactivity in the feces.

A comparison of the metabolites in the plasma, kidneys, and liver from male and female rats dosed with [^{14}C-phenyl]NC-302 and male rats dosed with [^{14}C-quinoxaline]NC-302 showed no significant differences with respect to sex or position of [^{14}C] label. The primary metabolite identified in the plasma, kidneys, and liver of male and female rats was NC-302 acid.

B. The study is acceptable.

Items 8-10--see footnote 1.

---

[1] Only items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test animals were 5-week-old male and female CD Sprague-Dawley rats. [^{14}C]NC-302 was mixed with unlabeled NC-302 in 1 percent aqueous Tween 80 and administered to the rats via stomach tube at 1.5 mg/kg (approximately 5 µCi/mL/140 g body weight).

2. For the excretion studies, five rats of each sex were given a single dose of [^{14}C-phenyl]NC-302 and an additional five male rats received a single dose of [^{14}C-quinoxaline]-NC-302. Results were given to indicate that female rats were also dosed with [^{14}C-quinoxaline]NC-302. Test animals were individually housed in metabolism cages following dosing. Urine, feces, and expired air were collected separately at 24-hour intervals for 7 days and radioassayed by liquid scintillation counting (LSC) using standard techniques. [^{14}C] in expired air was trapped in monoethanolamine:methanol (1:1, v/v). Seven days after [^{14}C]NC-302 administration, the animals were sacrificed, and each carcass was dissolved in 6 N hydrochloric acid (HCl) at 100°C and radioassayed.

3. To monitor [^{14}C] levels in blood, blood was taken from the tail vein of five male and five female rats at the following times after administration of [^{14}C-phenyl]NC-302: 0.25, 1, 3, 6, 9, 24, 48, 72, 96, 120, 144, and 168 hours. Blood samples were then weighed and radioassayed.

   [^{14}C] tissue distribution was determined in groups of five rats of each sex after sacrifice at 24 and 168 hours following a single dose of [^{14}C-phenyl]NC-302. Plasma, whole blood, liver, kidneys, heart, lungs, pancreas, spleen, brain, eyes, fat, testes/ovaries, and epididymis/uterus were taken. Whole blood and tissues were radioassayed by LSC following combustion using standard techniques.

4. Urine and feces collected 0 to 48 hours postadministration and plasma, liver, and kidneys obtained 24 hours postadministration were analyzed for metabolites. Plasma and urine samples were diluted 20- and 5-fold with distilled water, respectively. The diluted samples were adjusted to pH 2 with concentrated HCl and then extracted twice with ethyl acetate. Liver, kidneys, and feces were homogenized and extracted twice with 80 percent aqueous methanol. Following each extraction, the methanol extract was separated from particulate material by centrifugation. Residues (centrifuge precipitates) were radioassayed following combustion. Extracts were combined and concentrated by evaporation under vacuum at 40°C. Distilled water was added to the concentrates (10 mL/g sample), and samples were adjusted to pH 2 and then extracted twice with ethyl acetate. Ethyl acetate extracts from plasma, liver, kidneys, urine, and feces were concentrated and subjected to thin-layer chromatography (TLC).

TLC was performed on precoated silica gel plates (GF-254, 0.25 mm thick, Merck). Metabolites were identified by cochromatography with authentic compounds. The developing solvent systems utilized were as follows: a) ethyl ether: n-hexane:acetic acid (10:10:0.3, v/v), b) benzene:ethanol: acetic acid (20:1:1, v/v), and c) benzene:ethyl acetate: acetic acid (16:8:1, v/v). Developed TLC plates were exposed to X-ray film to produce autoradiograms and authentic standards were detected by UV absorption.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Following oral administration of [^{14}C-phenyl]NC-302 at 1.5 mg/kg to male and female rats, most of the radioactivity was eliminated in the feces. The total radioactivities detected in the feces of male and female animals were approximately 69 (range, 55 to 78 percent) and 57 percent (range, 50 to 75 percent), respectively. Most of the radioactivity was eliminated by day 3 postadministration, accounting for approximately 59 (range, 46 to 65 percent) and 52 percent (range, 43 to 70 percent) of the administered radioactivity in males and females, respectively (Table 1). Elimination of [^{14}C] in the urine was highest during days 1 and 2, then decreased gradually (Table 1). The total radioactivities detected in the urine of [^{14}C-phenyl]NC302-dosed male and female rats averaged 27 (range, 21 to 37 percent) and 43 percent (range, 36 to 56 percent) of the administered dose, respectively. No radioactivity was detected in expired air. The total recovered radioactivities from the urine, feces, and carcasses of [^{14}C-phenyl]NC-302-dosed male and female rats were approximately 99 (range, 95 to 102 percent) and 101 percent (range, 86 to 113 percent), respectively (Table 2).

Elimination of [^{14}C] in male rats given a single oral dose of 1.5 mg/kg [^{14}C-quinoxaline]NC-302 was similar to male rats dosed with [^{14}C-phenyl]NC-302 (Table 1). The total radioactivity detected in the urine averaged 25 percent of the administered dose (range, 21 to 29 percent), while the total radioactivity detected in the feces averaged 68 percent (range, 62 to 76 percent) (Table 1). Total radioactivity recovered from the urine, feces, and carcasses of [^{14}C-quinoxaline]NC-302-dosed male rats was approximately 97 percent (range, 93 to 100 percent) (Table 2).

B. Maximum blood concentrations of [^{14}C]NC-302 equivalents were observed at about 6 hours postadministration in male and female rats, 4.6 and 4.2 ppm, respectively (Figure 1, CBI p. 25). [^{14}C] concentrations then decreased steadily to approximately 0.1 ppm by 120 hours postadministration. The biological half-lives of [^{14}C] in the blood (from 9 to 168 hours after dosing) averaged 20.4 and 19.6 hours for male and female rats, respectively.

TABLE 1. Percent Recovery of Radioactivity in Rat Urine and Feces Following Oral Administration of [^{14}C]NC-302

Sex/Form of Radiolabel	Percent Recovery of Administered Radioactivity[a] Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	% Total [^{14}C] Recovered
Urine								
Males/ [^{14}C-phenyl]NC-302	9.06 ±0.71	8.39 ±0.93	5.14 ±1.09	2.49 ±0.38	1.24 ±0.14	0.70 ±0.07	0.35 ±0.05	27.38 ±2.59
Males/ [^{14}C-quinoxaline]NC-302	10.04 ±0.67	7.49 ±0.66	3.63 ±0.30	2.19 ±0.25	1.02 ±0.10	0.64 ±0.09	0.40 ±0.07	25.39 ±1.64
Females/ [^{14}C-phenyl]NC-302	22.92 ±2.10	9.35 ±0.56	5.41 ±0.72	2.90 ±0.45	1.24 ±0.14	0.53 ±0.07	0.19 ±0.03	42.53 ±3.78
Feces								
Males/ [^{14}C-phenyl]NC-302	19.83 ±2.36	25.94 ±1.44	12.96 ±0.70	5.98 ±0.47	2.45 ±0.25	1.20 ±0.17	0.61 ±0.08	68.97 ±3.72
Males/ [^{14}C-quinoxaline]NC-302	26.05 ±1.27	22.02 ±1.29	10.45 ±0.73	4.74 ±0.16	2.60 ±0.31	1.42 ±0.20	0.81 ±0.19	68.08 ±2.40
Females/ [^{14}C-phenyl]NC-302	25.00 ±4.11	17.60 ±2.45	9.25 ±1.34	3.58 ±0.35	1.16 ±0.16	0.46 ±0.07	0.19 ±0.03	57.24 ±4.68

[a]Mean values ± S.E. of five rats.

Source: CBI pp. 15, 16, and 17.

TABLE 2. Cumulative Recovery of Radioactivity in Rats 7 Days Following Oral Administration of [^{14}C]NC-302[a]

	[^{14}C-phenyl]NC-302		[^{14}C-quinoxaline]-NC-302
	Males	Females	Males
Urine	27.38±2.59	42.53±3.78	25.39±1.64
Feces	68.97±3.72	57.24±4.68	68.08±2.40
Expired Air	<0.01	<0.01	<0.01
Carcass	2.95±0.30	1.01±0.22	3.78±0.16
Total	99.29±1.33	100.78±4.60	97.26±1.23

[a]Mean values ± S.E. of five rats expressed as percent of administered dose.

Source: CBI p. 14.

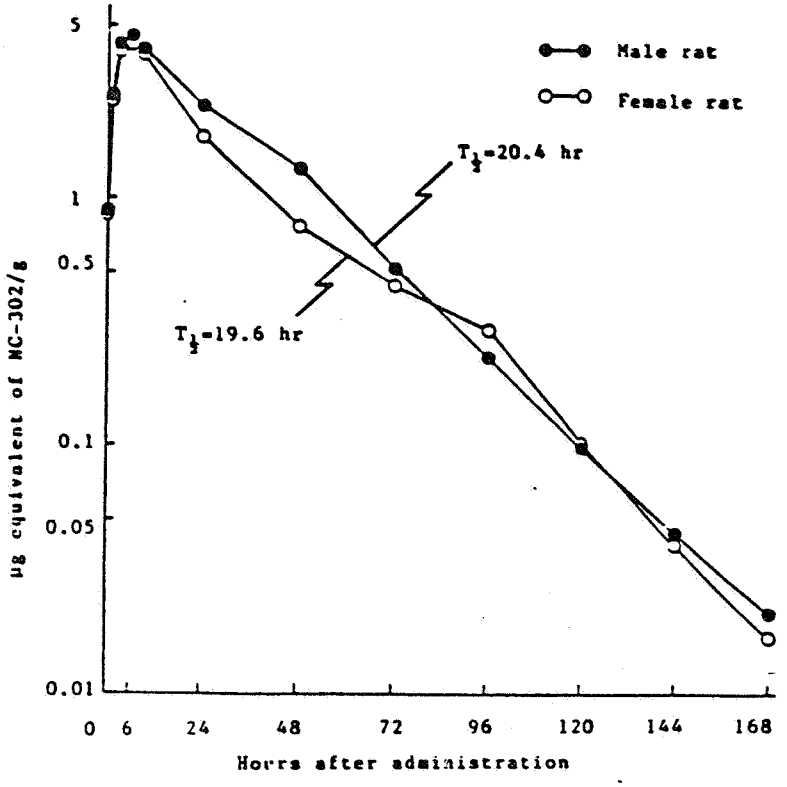


Fig.1 Blood concentrations of radioactivity after single oral administration of ^{14}C-phenyl-NC-302 at 1.5 mg/kg in rats. Each point represents the mean value of 5 rats.

SOURCE: CBI page 25.

Radioactive residue levels in the tissues of male and female rats at 24 and 168 hours following [^{14}C-phenyl]NC-302 administration are presented in Appendix B (CBI Table 8, p. 19). The tissues containing the highest residues of NC-302 equivalents at 24 hours postadministration were plasma (4.3 and 2.6 ppm in males and females, respectively), whole blood (2.6 and 1.6 ppm), kidneys (1.8 and 1.2 ppm), and liver (1.3 and 0.7 ppm). Residue levels in all other tissues were less than 0.75 ppm. At 168 hours postadministration residue levels in the tissues were less than 0.05 ppm.

C. After 2 days, the distribution of metabolites in the urine and feces of males and females dosed with [^{14}C-phenyl]NC-302 are shown in Table 3; chemical structures are presented in Appendix C (CBI pp. 12 and 13). In the urine of male and female rats, more than 93 percent of the sample radioactivity was extractable with ethyl acetate. The primary metabolite detected in the urine of male rats was PPA, which accounted for 48 percent of the urinary radioactivity. In females, NC-302 acid and PPA accounted for 56 and 21 percent of total urinary radioactivity, respectively. Unchanged NC-302 was not detected in the urine of male or female rats. In the feces of male and female rats, more than 85 percent of the radioactivity was extractable with ethyl acetate. The profile of metabolites in feces of males and females was similar. The primary metabolites were NC-302 acid (males, 31 percent; females, 35 percent of fecal radioactivity) and PPA (males and females, 27 and 30 percent, respectively). Unmetabolized NC-302 accounted for 12 and 14 percent of the fecal radioactivity in female and male rats, respectively.

The metabolites identified in plasma, kidneys, and liver of male and female rats 24 hours after administration of [^{14}C-phenyl]-NC-302 or [^{14}C-quinoxaline]NC-302 are shown in Table 4. No significant differences in metabolite distribution in the plasma, kidneys, or liver were observed with respect to sex or position of the [^{14}C] label. More than 93 percent of the radioactivity in the samples was extractable. The primary metabolite identified was NC-302 acid, accounting for 81 to 96 percent of the total radioactivity in the samples.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The absorption, distribution, excretion, and metabolic transformation of NC-302 in CD Sprague-Dawley rats after a single oral administration of [^{14}C]NC-302 at 1.5 mg/kg were studied.

Following administration of [^{14}C-phenyl]NC-302 or [^{14}C-quinoxaline]NC-302 to male and female rats, most of the radioactivity was excreted into the urine and feces within 4 days. Female rats excreted more radioactivity into the urine than did the males.

TABLE 3. Relative Concentrations of NC-302 and Its Metabolites in the Urine and Feces of Male and Female Rats Administered Single Oral Doses of 1.5 mg/kg of [^{14}C-phenyl]NC-302[a]

	Males		Females	
Compound[b]	Urine	Feces	Urine	Feces
Organic soluble	93.2 (16.3)[c]	86.0 (39.3)	98.3 (31.8)	85.3 (36.3)
NC-302	<0.1 (<0.1)	14.4 (6.6)	<0.1 (<0.1)	12.4 (5.3)
NC-302 phenol	0.9 (0.2)	0.7 (0.3)	[illegible] (0.1)	0.7 (0.3)
NC-302 acid	8.0 (1.4)	31.2 (14.3)	[illegible].4 (18.2)	34.6 (14.7)
AM-1 (unknown)	15.3 (2.7)	0.7 (0.3)	8.0 (2.6)	0.7 (0.3)
AM-2[d]	13.5 (2.4)	5.9 (2.7)	3.5 (1.1)	1.9 (0.8)
PPA	47.7 (8.2)	27.4 (12.5)	21.1 (6.8)	30.1 (12.8)
Origin	4.9 (0.9)	2.0 (0.9)	7.5 (2.4)	2.1 (0.9)
Others	2.8 (0.5)	3.7 (1.7)	1.5 (0.5)	2.8 (1.2)
Aqueous soluble	6.8 (1.2)	2.0 (0.9)	1.7 (0.5)	2.3 (1.0)
Residues	--	12.0 (5.5)	--	12.4 (5.3)
Total	100.0 (17.5)	100.0 (45.7)	100.0 (32.3)	100.0 (42.6)

[a]Values are expressed as percent of radioactivity in the sample; see Appendix C for chemical structures.

[b]Metabolites were separated by TLC.

[c]Values in parentheses are percent of administered [^{14}C] dose in combined excreta from days 1 and 2.

[d]Unknown metabolite AM-2, thought to be hydroxylated NC-302 acid.

Source: CBI pp. 20 and 21.

TABLE 4. Residue Levels of NC-302 and Its Metabolites in Plasma, Kidneys, and L of Male and Female Rats Administered Single Oral Doses of [^{14}C-phenyl]NC-302 or [^{14}C-quinoxaline]NC-302 at 1.5 mg/kg[a] 00554

Compounds[b]	Males			Females		
	Plasma	Liver	Kidneys	Plasma	Liver	Kidn
			[^{14}C-phenyl]NC-302			
Organic soluble	99.2	95.4	97.1	99.2	96.0	96.7
NC-302	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NC-302 phenol	0.2	0.9	0.9	0.2	0.3	0.3
NC-302 acid	94.4	87.4	82.1	94.4	88.6	80.9
AM-1 (unknown)	0.9	0.4	0.4	0.5	0.5	0.2
AM-2[c]	2.0	1.1	1.0	2.2	0.9	0.7
PPA	<0.1	1.0	0.7	0.1	1.1	0.8
Origin	0.2	2.1	9.2	0.2	2.6	11.8
Others	1.5	2.5	2.8	1.4	2.0	2.0
Aqueous soluble	0.8	0.9	0.3	1.0	0.3	0.2
Residues	--	3.7	2.5	--	3.7	3.1
			[^{14}C-quinoxaline]NC-302			
Organic soluble	99.0	93.1	93.9	98.8	97.1	94.2
NC-302	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NC-302 phenol	<0.1	0.4	0.4	<0.1	0.3	<0.1
CQO	<0.1	0.4	<0.1	<0.1	0.1	<0.1
NC-302 acid	95.8	87.3	81.2	95.6	91.0	81.9
AM-1 (unknown)	0.5	<0.1	<0.1	0.4	0.1	0.3
AM-2[c]	1.5	0.8	1.0	1.8	0.9	0.6
Origin	0.2	0.8	1.2	<0.1	1.0	1.3
Others	1.0	3.4	10.1	1.0	3.7	10.1
Aqueous soluble	1.0	0.7	0.8	1.2	0.4	0.8
Residues	--	6.2	5.3	--	2.5	5.0

[a] Values are expressed as percent of [^{14}C] in the sample.

[b] Metabolites were separated by TLC.

[c] Unknown metabolite AM-2, thought to be hydroxylated NC-302 acid.

Source: CBI pp. 22 and 23.

Peak [^{14}C] concentrations in the blood of male and female rats occurred at 6 hours postadministration, and these concentrations were 4.600 µg equivalents of NC-302/g and 4.189 µg/g, respectively. Blood radiolabel concentrations decreased to less than 0.5 percent of maximum levels by 168 hours postadministration. The biological half-lives for [^{14}C] in the blood were 20.4 and 19.6 hours in male and female rats, respectively.

The highest [^{14}C]NC-302 residue levels in male rat tissues at 24 hours postadministration were found in plasma (4.30 µg/mL), whole blood (2.55 µg/mL), kidneys (1.79 µg/g), and liver (1.30 µg/g). Radiolabel concentrations in other tissues (lungs, heart, brain, spleen, pancreas, fat, eyes, and gonads) were less than 0.75 µg/g, and the lowest was observed in the brain (0.05 µg/g). Tissue levels of [^{14}C] decreased to less than 0.05 µg/g by 168 hours postadministration. Radiolabel concentrations in the tissues of female rats at 24 and 168 hours were lower than those found in the males, except for the adipose tissue.

At least five metabolites were detected in the 0- to 48-hour urine and fecal samples after administration of [^{14}C-phenyl]NC-302. The primary metabolites in rat excreta were NC-302 acid and PPA. Minor metabolites, AM-1 (unknown) and AM-2 (hydroxylated NC-302 acid), were observed. A small amount of unchanged NC-302 was also detected in the feces.

No significant differences in the metabolic profile in plasma, liver, and kidneys were observed with respect to sex or position of [^{14}C] label. The main metabolite in each tissue was NC-302 acid.

B. A quality assurance statement was not provided.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: In general, the authors' conclusions are supported by the results presented. However, the Materials and Methods section could have been presented more thoroughly to indicate the origin of the results of tissue residues of female rats dosed with [^{14}C-quinoxaline]-NC-302. This study provides some useful information on the absorption, distribution, elimination, and metabolism of NC-302 in rats following administration at the low dose of 1.5 mg/kg. The results of this study and a similar study (DER Dynamac No. 1-11A-3) conducted with a higher dose of 150 mg/kg can be used to determine the effect of dose on the metabolism of NC-302 in rats.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 4-7; Appendix B, CBI Table 8, p. 19; and Appendix C, CBI Tables 1 and 2, pp. 12 and 13.

APPENDIX A

Materials and Methods

ASSURE 005546

Page_____ is not included in this copy.

Pages 86 through 89 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

CBI Table 8

Table 8 Mean concentrations of radioactivity in tissues of male and female rats after single oral administration of ^{14}C-phenyl-NC-302 at 1.5 mg/kg.

Tissues	Male		Female	
	24hr	168hr	24hr	168hr
W.blood*	2.55 ± 0.13	0.02 ± 0.01	1.60 ± 0.12	0.02 ± 0.01
Plasma*	4.30 ± 0.35	0.04 ± 0.01	2.58 ± 0.19	0.03 ± 0.01
Liver	1.30 ± 0.15	0.02 ± 0.00	0.68 ± 0.01	<0.01
Kidney	1.79 ± 0.13	0.02 ± 0.01	1.22 ± 0.04	0.03 ± 0.02
Heart	0.59 ± 0.04	<0.01	0.41 ± 0.03	<0.01
Lung	0.74 ± 0.08	<0.01	0.47 ± 0.04	<0.01
Brain	0.05 ± 0.01	<0.01	0.03 ± 0.00	<0.01
Pancreas	0.46 ± 0.03	<0.01	0.32 ± 0.03	<0.01
Spleen	0.27 ± 0.02	<0.01	0.18 ± 0.01	<0.01
Eye	0.15 ± 0.01	<0.01	0.13 ± 0.01	<0.01
Testis	0.48 ± 0.04	<0.01	–	–
Epididymis	0.75 ± 0.06	0.01 ± 0.00	–	–
Ovary	–	–	0.46 ± 0.06	0.01 ± 0.00
Uterus	–	–	0.66 ± 0.05	<0.01
Fat	0.42 ± 0.04	0.05 ± 0.00	0.58 ± 0.08	0.03 ± 0.00

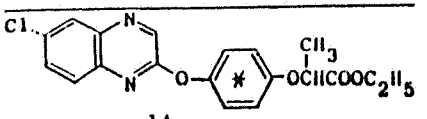
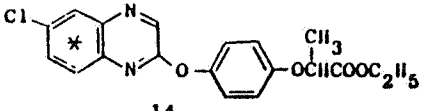
Values are expressed as ppm (μg equivalent of NC-302/g) and mean ± S.E of 5 rats.

* ; μg / ml.

APPENDIX C

CBI Tables 1 and 2

Table 1 Radiochemical properties of ^{14}C-NC-302.

Structure	Specific radioactivity	Radiochemical purity
 ^{14}C-phenyl-NC-302	8.5 mCi/mmole	More than 99%
 ^{14}C-quinoxaline-NC-302	7.8 mCi/mmole	More than 98%

*; Indicate the positions labelled uniformly with ^{14}C.

Table 2 A list of authentic compounds.

Designation	Chemical structure
NC-302	R-$OC_6H_4OCH(CH_3)COOC_2H_5$
NC-302 acid	R-$OC_6H_4OCH(CH_3)COOH$
NC-302 phenol	R-OC_6H_4OH
PPA	$HOC_6H_4OCH(CH_3)COOH$
CQO	
R:	

005546

EPA: 68-02-4225
DYNAMAC No. 1-11A-5
March 25, 1986

DATA EVALUATION RECORD

NC-302

Metabolic Study in Rats

STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., and Takano, S. The influences of repeated administration of NC-302 on metabolic fate in rats. (Unpublished study [No. unavailable] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E.I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.) Accession No. 073546.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-25-86

1. CHEMICAL: MC-302; ethyl-2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]-propionate.

2. TEST MATERIAL: [^{14}C-phenyl]MC-302 had a specific activity of 8.5 mCi/mmol and a radiochemical purity greater than 99 percent.

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., and Takano, S. The influences of repeated administration of MC-302 on metabolic fate in rats. (Unpublished study [No. unavailable] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E.I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.) Accession No. 073546.

5. REVIEWED BY:

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Date: 3/26/86

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EPA Reviewer

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Clint Skinner, Ph.D.
EPA Section Head

Signature: W C Skinner
Date: 4-18-86

7. CONCLUSIONS:

A. The metabolism of a single oral dose of [^{14}C-phenyl]NC-302 at 1.5 mg/kg to CD (SD) rats following repeated daily dosing with unlabeled material at 1.5 mg/kg for 14 days was studied. About 23 and 73 percent of the radioactivity was eliminated in the urine and feces, respectively, of males and about 49 and 45 percent in the urine and feces of females. No radioactivity was found in exhaled air. Distribution of administered radioactivity was similar between males and females. Maximum blood concentrations of radioactivity occurred at 9 hours postadministration. Blood [^{14}C] levels then decreased at estimated biological half-lives of 18.9 and 19.8 hours in males and females, respectively. Plasma, blood, kidneys, and liver contained the highest levels of radioactivity at 24 hours postadministration.

2-[4-(6-Chloro-2-quinoxalinyloxy)phenoxy]propionic acid (NC-302 acid) and 2-(4-hydroxyphenoxy)propionic acid (PPA) were the major metabolites present in the excreta. Females excreted a larger proportion of NC-302 acid in the urine than the males. About 5 to 6 percent of the administered radioactivity was excreted unchanged in the feces of the rats. NC-302 acid was the major metabolite detected in the plasma, liver, and kidneys of both sexes.

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Groups of five male and five female Sprague-Dawley rats (5 weeks old) were administered a single oral dose of unlabeled NC-302 at 1.5 mg/kg, daily for 14 days, followed by one dose of [^{14}C-phenyl]NC-302 at 1.5 mg/kg. Both compounds were dissolved in aqueous 1 percent Tween 80.

2. For the excretion studies, five rats/sex were placed separately in metabolism cages. Urine, feces, and expired air were collected at 24-hour intervals for 7 days and radioassayed by liquid scintillation counting using standard methods. The animals were then sacrificed and the carcasses were dissolved in hydrochloric acid and radioassayed.

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[1]Only items appropriate to this DER have been included.

3. Radioactivity levels in blood were monitored in a second set of five rats/sex. Blood was taken through the tail vein at the following times after [^{14}C-phenyl]NC-302 administration: 0.25, 1, 3, 6, 9, 12, 24, 48, 72, 96, 120, 144, and 168 hours. Blood samples were weighed and radioassayed by LSC following combustion. Additionally, five rats of each sex were sacrificed at 24 and 168 hours after dosing. Whole blood, plasma, liver, kidneys, heart, lungs, pancreas, spleen, brain, eyes, fat, testes/ovaries, and epididymis/uterus were taken and radioassayed by LSC following combustion.

4. Urine and feces collected 0-48 hours after dose administration and plasma, liver, and kidneys collected 24 hours after administration were analyzed for metabolites. Plasma and urine samples were diluted with water, adjusted to pH 2 with concentrated hydrochloric acid, and extracted with ethyl acetate. After extraction, the aqueous phase from the urine of males was adjusted to pH 5 with 0.10 M acetate buffer and incubated with bovine liver β-glucuronidase. Following incubation, the aqueous phase was adjusted to pH 2 and extracted twice with ethyl acetate.

   Liver, kidneys, and feces were homogenized and extracted twice with 80 percent aqueous methanol. Methanol extracts were diluted with water, adjusted to pH 2, and extracted with ethyl acetate. Ethyl acetate extracts from plasma, liver, kidneys, urine, and feces were analyzed by thin-layer chromatography (TLC). NC-302 and its metabolites were identified by cochromatography with the following authentic compounds: 2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]propionic acid (NC-302 acid), 4-(6-chloro-2-quinoxalinyloxy)phenol (NC-302 phenol), and 2-(4-hydroxyphenoxy)propionic acid (PPA).

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Following the administration of [^{14}C]NC-302 to males, most of the radioactivity was eliminated in the feces, whereas in dosed females more radioactivity was eliminated in the urine (Table I). The total radioactivities detected in the excreta of male and female rats 7 days after dosing with [^{14}C]NC-302 were 23 and 49 percent, respectively, in the urine and 73 and 45 percent in the feces. No radioactivity was detected in exhaled air, and only 1.08 and 0.68 percent of the administered dose remained in the male and female carcasses, respectively, at the end of the study. The total recovery of [^{14}C] 168 hours postadministration was 97.1 and 94.8 percent of the administered dose for male and female rats, respectively.

TABLE 1. Radioactivity in Urine and Feces Following Oral Administration of [^{14}C-phenyl]NC-302 to Rats[a]

Sex	Percent Recovery of Administered Radioactivity[b] Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Percent Total [^{14}C] Recovered
				Urine				
Males	10.78 ±0.53	7.25 ±0.73	3.07 ±0.33	0.95 ±0.19	0.55 ±0.09	0.20 ±0.05	0.08 ±0.03	23.06 ±1.44
Females	26.28 ±2.24	12.74 ±1.71	5.25 ±0.55	3.14 ±0.25	1.13 ±0.25	0.47 ±0.09	0.22 ±0.03	49.23 ±2.44
				Feces				
Males	27.21 ±3.27	30.12 ±1.09	10.14 ±1.07	3.16 ±0.51	1.35 ±0.16	0.47 ±0.17	0.29 ±0.11	72.94 ±1.81
Females	19.83 ±1.99	14.52 ±1.23	6.33 ±0.78	2.53 ±0.44	1.07 ±0.27	0.47 ±0.13	0.20 ±0.04	44.94 ±2.13

[a] Rats received 14 daily doses of unlabeled NC-302 followed by one dose of [^{14}C-phenyl]NC-302.

[b] Mean values ± S.E. of five rats.

Source: CBI pp. 13 and 14.

B. Blood [^{14}C] concentrations of NC-302 equivalents in both sexes increased following dosing and reached maximum levels (3.6 ppm) at 9 hours postadministration (Figure 1, CBI p. 23). [^{14}C] levels then decreased steadily to less than 0.015 ppm by 168 hours postadministration with estimated biological half-lives of 18.9 and 19.8 hours for males and females, respectively.

[^{14}C] residue levels in the tissues at 24 and 168 hours after [^{14}C]NC-302 dosing are presented in Appendix B (CBI Tables 5 and 6, pp. 16 and 17). Tissues containing the highest levels of [^{14}C]NC-302 equivalents at 24 hours postadministration were plasma (3.11 and 2.95 ppm in males and females, respectively), blood (1.85 and 1.72 ppm), kidneys (1.28 and 1.17 ppm), and liver (1.08 and 0.96 ppm). Residue levels in all other tissues were less than 0.67 ppm. At 168 hours postadminstration, residue levels in all tissues were less than 0.03 ppm.

C. The metabolite profiles in the 0- to 48-hour urine and feces of the male and female rats are shown in Table 2. Approximately 77 and 98 percent of radioactivity in the urine from the males and females, respectively, was extractable with ethyl acetate (non-polar metabolites). The major nonpolar metabolites detected were NC-302 acid (accounting for 22 and 73 percent of urinary radio-activity in males and females, respectively) and PPA (accounting for 25 and 10 percent). β-glucuronidase treatment of the water-soluble metabolites from male rats converted about 42 percent of the aqueous [^{14}C] to ethyl-acetate-soluble material. The major metabolite detected in this fraction was PPA together with a small amount of NC-302 acid. The parent compound NC-302 was not detected in the urine of either sex.

Approximately 84 percent of the fecal radioactivity from male and female rats was ethyl acetate extractable (Table 2). The major fecal metabolites were NC-302 acid (males, 43 percent and females, 47 percent of fecal radioactivity) and PPA (males and females, 18 percent). About 5 to 6 percent of the administered test material was excreted unchanged.

More than 92 percent of the radioactivity in the plasma, liver, and kidneys taken 24 hours postadministration was extractable with ethyl acetate (Table 3). The major metabolite detected was NC-302 acid, which accounted for 84 to 96 percent of the total radioactivity in each tissue.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The effect of repeated administration of NC-302 on its metabolic fate in rats was investigated. Test animals were administered [^{14}C-phenyl]NC-302 (1.5 mg/kg) following repeated oral administration of unlabeled NC-302 daily for 2 weeks at 1.5 mg/kg/day.

001 100

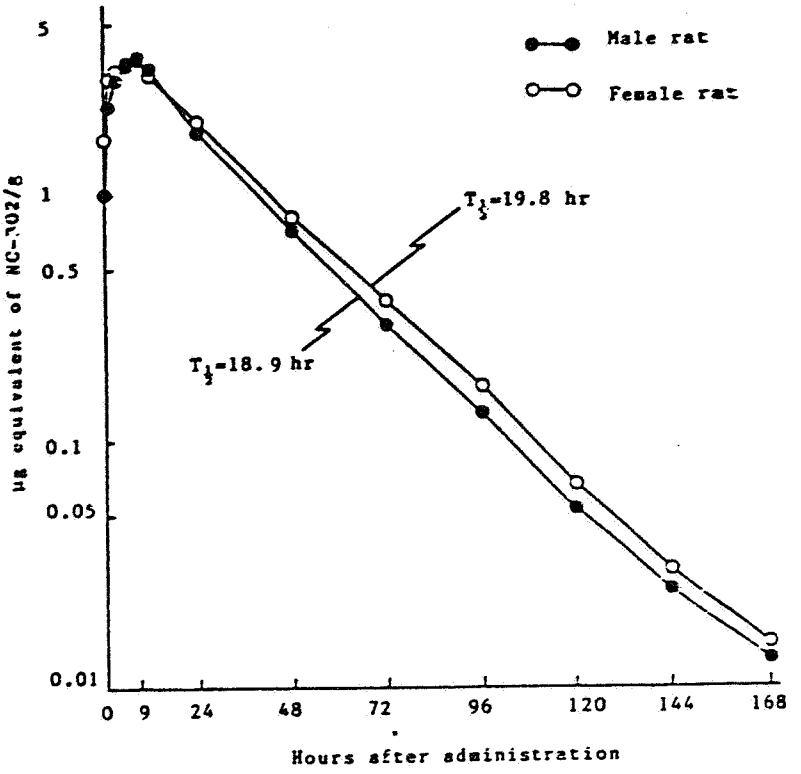

Fig. 1 Blood concentrations of radioactivity after administration of ^{14}C-NC-302 at 1.5 mg/kg to pretreated rats.

Each point represents the mean value of 5 rats.

SOURCE: CBI page 23.

TABLE 2. Excretion of NC-302 and Its Metabolites in the Urine and Feces of Male and Female Rats after Oral Administration of [^{14}C-phenyl]NC-302[a,b]

Compound[c]	Males				Females			
	Urine		Feces		Urine		Feces	
Organic soluble	76.8	(13.8)	84 3	(48.3)	97.9	(38.2)	84.0	(28.8)
NC-302	<0.1	(<0.1)	4.9	(2.8)	<0.1	(<0.1)	5.8	(2.0)
NC-302 phenol	0.2	(<0.1)	0.3	(0.2)	0.3	(0.1)	0.9	(0.3)
NC-302 acid	22.3	(4.0)	42.7	(24.4)	73.2	(28.6)	47.0	(16.1)
AM-1 (unknown)	11.7	(2.1)	0.7	(0.4)	4.9	(1.9)	0.9	(0.3)
AM-2[d]	9.3	(1.7)	11.0	(6.3)	3.2	(1.2)	3.2	(1.1)
PPA	25.2	(4.5)	17.8	(10.2)	9.6	(3.8)	18.1	(6.2)
Origin	4.4	(0.8)	2.4	(1.4)	3.6	(1.4)	2.0	(0.7)
Others	3.7	(0.7)	4.5	(2.6)	3.1	(1.2)	6.1	(2.1)
Aqueous soluble	23.2	(4.2)	3.8	(2.2)	2.1	(0.8)	3.2	(1.1)
Residues		--	11.9	(6.8)		--	12.8	(4.4)
Total	100.0	(18.0)	100.0	(57.3)	100.0	(39.0)	100.0	(34.3)

[a] Rats received 14 daily doses of unlabeled NC-302 and then one dose of [^{14}C-phenyl]NC-302.

[b] Values are percent of [^{14}C] in the sample; those in parentheses are percent of administered [^{14}C] dose in excreta from 0-48 hours.

[c] Metabolites were separated by TLC using the following solvent system--benzene: ethanol:acetic acid (20:1:1, v/v/v).

[d] Presumed to be hydroxylated NC-302 acid.

Source: CBI pp. 18 and 19.

TABLE 3. NC-302 and Its Metabolites in Plasma, Kidney, and Liver of Male and Female Rats 24 Hours After Oral Administration of [^{14}C-phenyl]NC-302[a,b]

	Males			Females		
Compound[c]	Plasma	Liver	Kidney	Plasma	Liver	Kidney
Organic soluble	100.0	92.5	97.4	99.7	94.5	98.1
NC-302	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
NC-302 phenol	0.2	1.0	1.2	0.2	0.7	1.0
NC-302 acid	95.5	83.6	88.8	93.9	86.5	88.3
AM-1 (unknown)	0.6	0.5	0.5	0.4	0.5	0.5
AM-2[d]	2.2	0.7	1.2	3.6	2.0	1.7
PPA	0.2	1.9	1.2	0.1	1.5	1.7
Origin	0.2	2.0	2.8	0.2	0.7	2.9
Others	1.0	2.8	1.7	1.3	2.6	2.0
Aqueous soluble	< 0.1	2.7	0.1	0.3	0.1	< 0.1
Residues	--	4.8	2.5	--	5.4	1.9

[a] Rats received 14 daily doses of unlabeled NC-302 and then one dose of [^{14}C-phenyl]NC-302.

[b] Values are percent of [^{14}C] in the sample.

[c] Metabolites were separated by the TLC solvent system--benzene:ethanol:acetic acid (20:1:1, v/v/v).

[d] Presumed to be hydroxylated NC-302 acid.

Source: CBI p. 21.

Peak [^{14}C] concentrations in the blood (3.6 µg equivalents/g) of both sexes occurred at 9 hours postadministration. The biological half-lives of radiolabel in blood were about 20 hours. Within 4 days the majority of the radioactivity administered was excreted into the urine and feces, and no radiolabel carbon was detected in the expired air. Seven days postadministration, the radioactive residues remaining in the rat bodies were only 1 percent of the dose. Distribution levels of [^{14}C] in the tissues were measured at 24 and 168 hours after [^{14}C-phenyl]NC-302 administration, and no significant [^{14}C] residues were observed.

The primary metabolites identified in rat excreta were NC-302 acid and PPA. In plasma, liver, and kidneys the major metabolite at 24-hour postadministration was NC-302 acid. Aqueous soluble metabolites in urine of male rats were increased over rats that were not repeatedly dosed. One of the aqueous soluble metabolites was identified as PPA conjugates.

From these results, it was concluded that there was no significant difference in the metabolic fate of NC-302 between repeated and nonpretreated rats.

B. A quality assurance statement was not provided.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study provides information on the metabolism of NC-302 in rats following pretreatment with unlabeled NC-302 for 14 days. An adequate number of animals was used, and the methods used are standard for similar studies. The results indicate that female rats excreted more radiolabel into the urine and less into the feces than did males. However, it is not clear why sex differences were not apparent in the tissue distribution of [^{14}C]NC-302 metabolites and biological half-lives of [^{14}C] disappearance from whole blood.

Several metabolites were identified in the rat excreta, with NC-302 acid and PPA accounting for most of the urinary and fecal radioactivity. Females excreted more NC-302 acid in the urine than the males. A small percentage of unchanged NC-302 was detected in the feces of both sexes. Radiolabel was widely distributed throughout the tissues following dose administration with the blood, liver, and kidneys having the highest concentrations. The major metabolite found in the plasma, liver, and kidneys was NC-302 acid.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 3-6, and Appendix B, CBI Tables 5 and 6, pp. 16 and 17.

APPENDIX A

Materials and Methods

ASSURE 005546

Page_____ is not included in this copy.

Pages 106 through 109 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

CBI Tables 5 and 6

Table 5 Mean concentrations of radioactivity in tissues after administration of ^{14}C-NC-302 at 1.5 mg/kg to pretreated male rats.

Tissues	24hr	168hr
W.blood**	1.85 ± 0.14 (2.55)*	0.01 ± 0.01 (0.02)
Plasma**	3.11 ± 0.26 (4.30)	0.02 ± 0.01 (0.04)
Liver	1.08 ± 0.04 (1.30)	0.01 ± 0.00 (0.02)
Kidney	1.28 ± 0.07 (1.79)	0.01 ± 0.00 (0.02)
Heart	0.48 ± 0.05 (0.59)	<0.01 (<0.01)
Lung	0.60 ± 0.05 (0.74)	<0.01 (<0.01)
Brain	0.04 ± 0.00 (0.05)	<0.01 (<0.01)
Pancreas	0.35 ± 0.03 (0.46)	<0.01 (<0.01)
Spleen	0.22 ± 0.02 (0.27)	<0.01 (<0.01)
Eye	0.13 ± 0.01 (0.15)	<0.01 (<0.01)
Testis	0.30 ± 0.03 (0.48)	<0.01 (<0.01)
Epididymis	0.47 ± 0.03 (0.75)	0.01 ± 0.01 (0.01)
Fat	0.18 ± 0.02 (0.42)	0.02 ± 0.01 (0.05)

Values are expressed as ppm (μg equivalent of NC-302/g) and mean ± S.E of 5 rats.

* ; Values in parentheses denote mean concentrations in tissues of non-pretreated rats.

** ; μg/ml.

Table 6 Mean concentrations of radioactivity in tissues after administration of ^{14}C-NC-302 at 1.5 mg/kg to pretreated female rats.

Tissues	24hr	168hr
W.blood	1.72 ± 0.18 (1.60)*	0.02 ± 0.00 (0.02)
Plasma**	2.95 ± 0.27 (2.58)	0.03 ± 0.00 (0.03)
Liver	0.96 ± 0.08 (0.68)	0.01 ± 0.00 (<0.01)
Kidney	1.17 ± 0.12 (1.22)	0.02 ± 0.00 (0.03)
Heart	0.47 ± 0.05 (0.41)	<0.01 (<0.01)
Lung	0.60 ± 0.09 (0.47)	<0.01 (<0.01)
Brain	0.03 ± 0.00 (0.03)	<0.01 (<0.01)
Pancreas	0.33 ± 0.03 (0.32)	<0.01 (<0.01)
Spleen	0.21 ± 0.02 (0.18)	<0.01 (<0.01)
Eye	0.10 ± 0.01 (0.13)	<0.01 (<0.01)
Ovary	0.48 ± 0.04 (0.46)	<0.01 (0.01)
Uterus	0.67 ± 0.08 (0.66)	<0.01 (<0.01)
Fat	0.24 ± 0.04 (0.58)	0.01 ± 0.00 (0.03)

Values are expressed as ppm (μg equivalent of NC-302/g) and mean ± S.E of 5 rats.
* ; Values in parentheses denote mean concentrations in tissues of non-pretreated rats.
** ; μg/ml.

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EPA: 68-02-4225
DYNAMAC No. 1-11A-6
March 25, 1986

DATA EVALUATION RECORD

NC-302

Metabolic Study in Rats

STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., and Takano, S. The accumulation in tissues and metabolites in plasma and liver after repeated oral administration of NC-302 in rats (1.5 mg/kg/day). (Unpublished study [No. unavailable] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E.I. Du Pont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.) Accession No. 073546.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-25-86

005546

1. CHEMICAL: NC-302; ethyl-2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]-propionate.

2. TEST MATERIAL: [^{14}C-quinoxaline]NC-302 had a specific radioactivity of 7.8 mCi/mmol and a radiochemical purity of greater than 98 percent.

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: Hirata, H., Onitsuka, H., and Takano, S. The accumulation in tissues and metabolites in plasma and liver after repeated oral administration of NC-302 in rats (1.5 mg/kg/day). (Unpublished study [No. unavailable] prepared and submitted by Biological and Chemical Research Laboratory, Nissan Chemical Industries, Ltd., Tokyo, Japan, for E.I. Du Pont de Nemours and Co., Inc., Wilmington, DE; dated February 1985.) Accession No. 073546.

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7. CONCLUSIONS:

A. The accumulation of radiolabel in the tissues and the distribution of metabolites in the plasma and liver of Sprague-Dawley rats were investigated following repeated oral administration of [^{14}C-quinoxaline]NC-302 at 1.5 mg/kg for 28 days.

Concentrations of [^{14}C] in the blood of male and female rats were monitored at 24-hour intervals during the 28-day dosing period and for 7 days postadministration. Radioactivity levels in the blood of male rats plateaued after 5 days of administration, whereas in females the blood levels reached a plateau after 3 days of dosing. The plateau levels were about 3 to 4 µg NC-302 equivalents/g and about twofold higher than the concentrations found in the blood 24 hours after the first dose. The levels of radioactivity found in the blood of male rats were slightly higher than those found in the females throughout the 28-day dosing period. After dosing was discontinued, the female rats cleared the radiolabel from the blood more slowly than did the males.

Levels of radioactivity in selected tissues were compared between male rats receiving a single oral dose of [^{14}C]NC-302 and male rats dosed daily for 28 days. Twenty-four hours after the final dose, the radiolabel concentrations in the tissues (except for the adipose tissue) of the 28-day dosed rats were 1.2- to 2-fold higher than the singly dosed rats. Elimination rates of radioactivity from the tissues (except the spleen) were similar for male rats that were dosed for 1 or 28 days. Adipose tissue [^{14}C] residues in rats administered [^{14}C-quinoxaline]NC-302 for 28 days were 2.4-fold higher (at 24 hours postadministration) and were eliminated at a much slower rate than the residues in fat from rats given a single oral dose.

The major metabolite identified in the plasma and liver of [^{14}C-quinoxaline]NC-302-dosed male rats after 1 day and 28 days of dose administration was 2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]-propionic acid (NC-302 acid). The data also indicate increased covalent binding of NC-302 and/or its metabolites to the liver following repeated administration of NC-302.

B. The study authors' conclusions concerning NC-302 residue accumulation in tissues and binding to liver are not substantiated by the data provided.

Items 8-10--see footnote 1.

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[1]Only items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test animals were 5-week-old male and female CD Sprague-Dawley rats. [^{14}C-quinoxaline]NC-302 was diluted with unlabeled NC-302 in 1 percent aqueous Tween 80. The rats received a single daily oral dose of [^{14}C-quinoxaline]NC-302 at 1.5 mg/kg/day (2 µCi/mL/135 g rat) via stomach tube for 28 days.

2. To monitor [^{14}C] levels in blood, blood was taken from the tail veins of three male and three female rats at 24-hour intervals during administration of [^{14}C-quinoxaline]NC-302 and at the following time periods after 28 days of dose administration: 24, 48, 72, 96, 120, 144, and 168 hours. Blood samples were weighed and radioassayed by liquid scintillation counting (LSC) using standard techniques.

   [^{14}C] tissue distribution was determined in four or five male rats after sacrifice at 24, 72, 120, and 168 hours following a single oral dose or 28 daily doses of [^{14}C-quinoxaline]NC-302. Whole blood, plasma, liver, fat, kidneys, lungs, heart, and spleen were taken and radioassayed. Whole blood and tissues were radioassayed by LSC following combustion using standard techniques.

   Whole-body autoradiography was conducted on male rats (number not specified) sacrificed 24, 72, and 120 hours after 28 daily oral doses. Following sacrifice, the rats were frozen in a dry ice:acetone bath for 30 minutes. The frozen carcass was placed in 5 percent (w/v) aqueous carboxymethyl cellulose at -70°C and mounted onto a microtome stage (maintained in a cryostat at -20°C). Thin sections (50 µm) were freeze dried and exposed to X-ray film.

3. Plasma and liver samples were obtained 24 hours after either a single oral administration or 28 days of dosing with [^{14}C-quinoxaline]NC-302. Plasma was diluted with water, the solution was adjusted to pH 2 with HCl, and the radioactivity was extracted twice with ethyl acetate. The liver was homogenized and radioactivity in the precipitate was then extracted with methanol. The acetone and methanol extracts were combined, diluted with water, and adjusted to pH 5 with 0.1 M acetate buffer. The radioactivity was extracted with chloroform. The solvent extracts were concentrated and analyzed for metabolites by thin-layer chromatography (TLC) using three solvent systems. NC-302 and the metabolites were identified by cochromatography with the following authentic compounds: NC-302, 2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]-propionic acid (NC-302 acid), 4-(6-chloro-2-quinoxalinyloxy)-phenol (NC-302 phenol), and 6-chloroquinoxaline-2-one (CQO).

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. The [^{14}C] concentrations found in the whole blood in male and female Sprague-Dawley rats during the 28-day [^{14}C-quinoxaline]-NC-302 dosing period are presented in Appendix B (CBI Table 1, p. 12). The patterns of [^{14}C] concentrations in the whole blood were similar for both male and female rats. Following the first day of dose administration, the blood [^{14}C] levels were 2.037 and 1.492 ppm in males and females, respectively. The [^{14}C] concentrations reached a plateau level of about 3 ppm after 3 days of dosing in females (see Appendix B, CBI Figure 1, p. 18). In the male rats, blood [^{14}C] levels plateaued after 5 days of dosing at approximately 4 ppm. Following the final dose, radioactivity levels in the blood steadily decreased to about 0.199 and 0.365 ppm by 168 hours in male and female rats, respectively.

[^{14}C] residue levels found in tissues of male rats at various intervals after a single oral dose or 28 daily oral doses of [^{14}C-quinoxaline]NC-302 are presented in Appendix B (CBI Tables 2 and 3, pp. 13 and 14). The concentrations in whole blood, plasma, and tissues 24 hours after 28 days of dosing (CBI Table 3) were 1.2- to 2-fold higher than the corresponding levels in tissues from rats given a single dose, except for fat, which was 2.4-fold higher (CBI Table 2). In addition, similar rates of radiolabel elimination were noted for the tissues of both groups of animals at 72, 120, and 168 hours after dosing, except for the adipose tissue from the rats dosed for 28 days (Appendix B, CBI Figures 2-5, pp. 19-22).

Whole-body autoradiograms of male rats given daily oral doses of [^{14}C-quinoxaline]NC-302 for 28 days supported the tissue radioanalysis. At 24 hours after the last dose, high concentrations of radioactivity were observed in the large intestine, blood, liver, kidneys, lungs, and teeth. By 72 hours, radioactivity was still present in the intestines, but had decreased in all other tissues, and by 120 hours only trace amounts of radioactivity were observed.

B. Metabolic profiles in the plasma of rats after 1 and 28 days of [^{14}C-quinoxaline]NC-302 dosing were almost identical (Table 1). Approximately 98 percent of the total plasma radioactivity was ethyl acetate extractable, and the primary metabolite, NC-302 acid, accounted for 93 percent of the sample radioactivity. After a single dose, 94 percent of the radioactivity in liver was extracted in ethyl acetate and NC-302 acid accounted for 85 percent of the radioactivity extracted. However, after repeated dosing only 72 percent of the radioactivity was extractable and the proportion of NC-302 acid dropped to 72 percent and that of polar metabolites and bound residues increased (Table 1).

TABLE 1. Relative Concentrations of NC-302 and Its Metabolites in the Plasma and Liver of Male Rats after a Single Oral Dose and Repeated Dosing for 28 Days with [^{14}C-Quinoxaline]NC-302[a]

Compounds	Plasma[b]		Liver[b]	
	1	28	1	28
Organic soluble	98.2	97.8	93.7	87.1
NC-302	<0.1	<0.1	<0.1	<0.1
NC-302 phenol	<0.1	<0.1	0.8	0.6
NC-302 acid	93.2	92.6	84.7	72.2
CQO	<0.1	0.4	<0.1	<0.1
Origin	<0.1	<0.1	4.6	9.0
Others	5.0	4.8	3.6	5.3
Aqueous soluble	1.8	2.2	3.1	2.8
Residues	--	--	3.2	10.1

[a]Values are percent of radioactivity in the sample.

[b]Metabolites were separated by TLC.

Source: CBI pp. 15 and 17.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The metabolism of NC-302 in rats was investigated with respect to residue accumulation in tissues and metabolite formation in the plasma and liver after repeated oral administration of [^{14}C-quinoxaline]NC-302 at 1.5 mg/kg for 28 days.

Blood [^{14}C] concentrations plateaued after 3 to 5 days of dosing in male and female rats. The plateau levels were about 3 to 4 µg NC-302 equivalents/g and were approximately twofold higher than the blood concentrations found after 1 day of dosing.

The concentrations of radioactivity in whole blood, plasma, liver, kidneys, lungs, heart, and spleen at 24 hours after the final (28-day) dose were less than two times the concentrations found in the rat tissues at 24 hours after a single [^{14}C-quinoxaline]-NC-302 dose. The [^{14}C] residues in the fat tissue from the 28-day dosed rats were 2.4-fold higher than the fat tissue from single-dosed animals. The radioactivity elimination rates from tissues of the 28-day and single dose male rats were similar, except for adipose tissue, which was eliminated at a much slower rate in the 28-day dosed rats.

In whole-body autoradiograms taken at 120 hours after 28 days of dosing, radioactivity was hardly observed in any of the tissues. It was concluded that significant accumulation in the tissues would not occur by repeated administration of NC-302.

About 90 percent of the radioactivity in the livers of 28-day dosed rats was extractable with acetone and methanol. There was only a small percentage of covalent binding by NC-302 and/or its metabolites to the liver. NC-302 acid was the major metabolite in liver and plasma after both a single oral dose and 28 days of oral administration.

B. A quality assurance statement was not provided.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study provides some useful information on tissue accumulation of residues and metabolite formation in rats after repeated oral administration of NC-302. The results indicate that radiolabel originating from [^{14}C-quinoxaline]NC-302 reached plateau levels in the blood of male and female rats after 3 to 5 days of repeated dosing. Concentrations of radioactivity in the blood and tissues of rats receiving repeated administration of [^{14}C]NC-302 were about twofold higher than the levels detected in the rats that received a single dose. However, it appears that the rate of [^{14}C] elimination from the fat of animals receiving repeated dosing was very slow, although the total concentration of residues were quite small. Moreover, the metabolite distribution in the livers of rats given 28 daily oral doses of [^{14}C-quinoxaline]NC-302 indicates increased binding of [^{14}C] residues to liver tissues.

The study authors concluded that a significant accumulation of residues in the tissues would not occur by repeated administration of NC-302. However, by 7 days after the final dose, there was no significant decrease in residue levels detected in the adipose tissue of the rats that received repeated administration. In addition, radiolabel was also released more slowly from the spleen, blood, and lungs of the 28-day dosed rats when compared to the singly dosed rats. Following metabolite analysis of liver samples from rats given 1 or 28 oral doses of [^{14}C-quinoxaline]NC-302, the study authors concluded that only a small percentage of covalent binding by NC-302 and/or its metabolites occurred; yet there was a threefold increase (3 to 10 percent of sample radioactivity) in the amount of radiolabel that remained bound to the liver following repeated dosing. These results do not support the study authors' conclusions because they show that low levels of the test material reached the target organs but were retained for a long time. Hence, accumulation could occur with repeated exposure.

In addition to the residue accumulation, there is the possibility of metabolites being formed that are not [^{14}C] labeled and that could also bind to the liver. Hence, further investigation of NC-302 metabolism and binding to the liver should be conducted using NC-302 with the [^{14}C] label at another position.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 3-7, and Appendix B, CBI Tables 1-3, pp. 12-14, and CBI Figures 1-5, pp. 18-22.

APPENDIX A

Materials and Methods

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____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

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APPENDIX B

CBI Tables 1-3 and
CBI Figures 1-5

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073546

EPA: 68-02-4225
DYNAMAC No. 1-011A-7
March 25, 1986

DATA EVALUATION RECORD

NC-302

Metabolic Study in Rats

STUDY IDENTIFICATION: Hawkins, D. R., Elsom, L. F., Davidson, C., and Roberts, D. The biokinetics and metabolism of [^{14}C]NC-302 in the rat. (Unpublished study No. NSA 12/8361 prepared and submitted by Huntingdon Research Centre, Huntingdon, Cambridgeshire, England, for E. I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 14, 1983.) Accession No. 073546.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-25-86

1. CHEMICAL: NC-302; etnyl-2-[4-(6-chloro-2-quinoxalyloxy)phenoxy]propionate.

2. TEST MATERIAL: [^{14}C-quinoxaline]NC-302, ethyl-2-(4-(6-chloro-2-quinoxalyloxy-U-^{14}C)phenoxy)propionate, had a specific activity of 7.79 mCi/mmol and radiochemical purity of greater than 98 percent.

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: Hawkins, D. R., Elsom, L. F., Davidson, C., and Roberts, D. The biokinetics and metabolism of [^{14}C]NC-302 in the rat. (Unpublished study No. NSA 12/8361 prepared and submitted by Huntingdon Research Centre, Huntingdon, Cambridgeshire, England, for E. I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated February 14, 1983.) Accession No. 073546.

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Date: 4/7/86

Clint Skinner, Ph.D.
EPA Section Head

Signature: [illegible]
Date: 4-15-86

7. CONCLUSIONS:

A. The absorption, distribution, and elimination of radiolabel were investigated following oral administration of [^{14}C-quinoxaline]-NC-302 at 1.5 mg/kg to male and female Sprague-Dawley rats. Most of the radioactive material was eliminated by way of the urine and feces within 4 days following dosing. The major route of elimination in male rats was by fecal excretion, whereas female rats excreted equal amounts of radiolabel (about 48 percent of the dose) into the urine and feces. No [^{14}C] was released with expired air. Approximately 50 percent of the administered dose was excreted in the bile of cannulated rats regardless of sex, suggesting that the apparent difference in urinary excretion between the males and females was not a difference in absorption. However, only one male and one female were used to measure biliary excretion.

Absorption and distribution of dosed radioactivity were similar for male and female rats. Maximum plasma concentrations of NC-302 equivalents occurred at 6 hours postadministration. However, a difference in the elimination rates of radioactivity from the plasma was evident, with males and females having average biological half-lives of 35.2 and 26.7 hours, respectively. Radiolabel was distributed throughout the tissues of male and female rats by 6 hours following [^{14}C]NC-302 administration, and the plasma, blood, gastrointestinal tract, liver, kidneys, thyroid, and ovaries had the highest concentrations of radioactivity.

A major radioactive metabolite (more polar than the paremt compound) was detected in the urine, feces, and bile of male amd female rats. A sex-related difference for this metabolite was observed in the urine; the females excreted more of the metabolite into the urine than the male rats. However, the proportions of this component in the feces and bile were similar for both sexes. A small amount of the administered radioactivity was excreted as unchanged NC-302 in the feces of both sexes; however, it was not detected in the urine or bile of either sex.

B. The study is acceptable.

Items 8-10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. [^{14}C-quinoxaline]NC-302 was dissolved in aqueous 1 percent Tween 80 and administered to male and female Sprague-Dawley rats in single oral doses at 1.5 mg/kg (approximately 31 µCi/kg).

---

[1]Only items appropriate to this DER have been included.

2. For the excretion studies, three males and three females were housed individually in metabolism cages and their urine, feces, and expired air samples were collected separately. Urine was collected at the following times after dose administration: 8, 24, 48, 72, 96, and 120 hours. Feces were collected at 24-hour intervals until 120 hours postadministration, and [^{14}C] in expired air was trapped during 0-24 hours and 24-48 hours following dose administration. The animals were sacrificed 5 days postadministration, and the liver and gastrointestinal tract were removed for examination. Urine, feces, expired air, and cage washings were radioassayed by liquid scintillation counting (LSC) using standard techniques.

   To assess biliary excretion, a cannula was inserted into the bile ducts of one male and one female rat. Bile was collected at 1-hour intervals for 48 hours. Urine and feces were collected at 24 and 48 hours postadministration. The rats were sacrificed 48 hours after dosing, and the livers and gastrointestinal tracts were collected and stored at -20°C prior to analysis.

3. [^{14}C] levels in plasma were monitored by collecting blood from the tail veins of three male and three female rats before dosing and at 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, and 168 hours after dose administration.

   [^{14}C] residues were determined in the tissues of groups of two rats (one male and one female) sacrificed at 6, 24, 48, 96, and 168 hours following administration. The following tissues were taken at sacrifice and radioassayed: liver, lungs, gonads, eyes, kidneys, adrenals, thyroid, heart, brain, spleen, pancreas, thymus, gastrointestinal tract, muscle (longissimus dorsi), fat (perirenal), and blood.

4. For the analysis of metabolites, samples of urine and bile were applied directly to thin-layer chromatography (TLC) plates. The plates (Kiesegel F-254) were then developed in two different solvent systems. Fecal samples were extracted with methanol, and the concentrated methanol extracts were analyzed by TLC as above. Analysis of urine and bile for conjugated metabolites was performed by adjusting aliquots of each to pH 5 with acetic acid and then incubating with B-glucuronidase/sulfatase for 18 hours at 37°C; the hydrolyzed samples were then spotted on TLC plates and developed as described above.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Five days following oral administration of [^{14}C-quinoxaline]-NC-302 at 1.5 mg/kg, male rats excreted about 20 percent of the

dose in the urine and 74.5 percent in the feces whereas females excreted about 48 percent of the dose in the urine and 49 percent in the feces (Table 1). Elimination of [^{14}C] in the urine and feces reached maximum levels at 24 and 48 hours after dosing, then the rate of elimination gradually decreased. During the initial 48 hours of dosing, no radioactivity was detected in the expired air. The total recovered radioactivities from the urine, feces, gastrointestinal tract, livers, and carcasses of the male and female rats averaged 104 and 105 percent, respectively (Table 2).

Forty-eight hours after a single oral dose of [^{14}C]NC-302 was administered to rats with cannulated bile ducts, 52 and 49 percent of the dosed radioactivity was excreted in the bile of a male and a female, respectively (Table 3). Biliary excretion of radioactivity occurred at a fairly steady rate during the 0- to 48-hour collection period, particularly from 12 to 40 hours with 1 to 2 percent of the dose being excreted per hour (see Appendix B, CBI Table 4, p. 19). Additionally, the radioactivity excreted in the urine and bile plus that remaining in the liver and carcass indicated that approximately 67 and 89 percent of the oral dose was absorbed by the male and female rats, respectively.

B. Peak [^{14}C] concentrations in plasma of male and female rats occurred at 6 hours postadministration and accounted for 9.19 and 10.50 µg NC-302 equivalents/mL (ppm), respectively (Table 4). Plasma [^{14}C] concentrations decreased steadily to less than 0.40 ppm by 168 hours postadministration. The biological half-lives of radiolabel in the plasma from 6 to 168 hours averaged 35.2 and 26.7 hours for male and female rats, respectively. The mean area under the plasma concentration-time curve for up to 168 hours corresponded to 512.7 and 453.6 µg/hr/mL for male and female rats, respectively.

[^{14}C] residues measured in the tissues of male and female rats at various intervals following dosing are presented in Appendix B (CBI Table 8, p. 23). Peak concentrations of radioactivity occurred in the tissues at 6 hours postadministration. In males, the tissues containing the highest concentrations of radiolabel were plasma (9.93 ppm), blood (6.07 ppm), gastrointestinal tract (4.12 ppm), thyroid (3.36 ppm), liver (3.32 ppm), and kidneys (3.11 ppm). In females, the tissues containing the highest concentrations of radioactivity were plasma (11.11 ppm), blood (6.73 ppm), gastrointestinal tract (6.37 ppm), liver (3.69 ppm), kidneys (3.16 ppm), ovaries (3.13 ppm), and thyroid (2.82 ppm). The remaining tissues contained less than 2.30 ppm. [^{14}C] residues levels in the tissues declined steadily to less than 0.14 ppm by 168 hours postadministration.

C. The proportions of radioactive components detected in the 8- to 48-hour urine samples from male and female rats dosed with [^{14}C-quinoxaline]NC-302 are shown in Table 5. The TLC system resolved the raw urine into six major radioactive components;

TABLE 1. Recovery of Radioactivity in Urine and Feces Following Oral Administration of [^{14}C-Quinoxaline]MC-302 to Rats

Sex	Percent Recovery of Administered Radioactivity[a] at Postadministration Day						
	8	24	48	72	96	120	Total
				Urine			
Males	0.53	6.12	6.50	3.93	1.96	1.20	20.23
Females	1.34	17.77	15.44	7.49	4.26	2.06	48.35
				Feces			
Males	--[b]	19.64	26.62	15.16	8.79	4.31	74.52
Females	--[b]	1.33	26.42	12.04	6.14	3.22	49.14

[a]Mean values of three rats.

[b]Fecal samples were collected over 24-hour intervals.

Source: CBI p. 17.

TABLE 2. Cumulative Recovery of Radioactivity Following Oral Administration of [^{14}C-Quinoxaline]NC-302 to Rats[a]

	Percent Recovery of Administered Radioactivity	
	Males[b]	Females[b]
Urine[c]	21.00	49.84
Feces	74.52	49.14
Expired Air	ND[d]	ND
Gastrointestinal Tract	2.23	1.51
Liver	0.61	0.48
Carcass	5.63	3.79
Total	103.99	104.75

[a]Values expressed as percent of administered [^{14}C] dose.

[b]Mean values of three rats.

[c]Includes cage wash.

[d]None detected.

Source: CBI p. 16.

TABLE 3. Cumulative Excretion and Retention of Radioactivity Following Oral Administration of [^{14}C-Quinoxaline]NC-302 to Rats with Cannulated Bile Ducts

	Percent Recovery of Administered Radioactivity	
	Male	Female
Excreted in		
Urine[a]	5.66	13.51
Feces	18.77	8.62
Bile	52.03	49.06
Retained in		
Gastrointestinal tract	2.33	5.46
Liver	1.80	3.51
Carcass	7.93	23.22
Total	88.52	103.38

[a]Includes cage wash.

Source: CBI p. 18.

TABLE 4. [^{14}C] Concentrations in Plasma of Rats after Oral Administration of [^{14}C-Quinoxaline]NC-302

Time (hours)	[^{14}C] in plasma (µg equivalents/mL)	
	Males[a]	Females[a]
0.5	3.95	3.45
1	6.04	5.62
2	8.40	8.64
4	9.12	10.46
6	9.19	10.50
12	8.22	8.96
24	6.86	6.48
48	4.13	3.40
72	2.47	1.79
96	1.49	1.05
120	1.04	0.58
168	0.39	0.16

[a]Mean values of three rats.

Source: Mean values calculated by our reviewers. Individual data used from CBI p. 20.

TABLE 5. Radioactive Components in 8- to 48-Hour Urine Samples of Male and Female Rats after Oral Administration of [^{14}C-Quinoxaline]NC-302

Component[b]	R_f[c]	[^{14}C] in untreated urine[a]		[^{14}C] in β-glucuronidase-treated urine[a]	
		Males	Females	Males	Females
1	0.61	0.1 (1)	ND[d]	0.2 (2)	ND
2	0.50	2.3(18)	21.0(63)	3.1(24)	22.1(67)
3	0.45	1.7(14)	1.7 (5)	2.0(16)	2.0 (6)
4	0.37	1.6(13)	2.0 (6)	2.0(16)	2.8 (8)
5	0.27	0.4 (3)	0.7 (2)	1.0 (8)	1.6 (5)
6	0.20	0.9 (7)	1.0 (3)	1.0 (8)	1.1 (3)
Origin	0.00	5.2(42)	6.2(19)	3.0(24)	2.9 (9)
Others	--	0.3 (2)	0.7 (2)	0.4 (3)	0.7 (2)

[a] Values expressed as percent of administered [^{14}C] dose; values in parentheses are percent of sample radioactivity.

[b] Components were separated by the TLC solvent system benzene:ethyl acetate:acetic acid (4:2:1, v/v).

[c] R_f of authentic NC-302 was 0.72.

[d] None detected.

Source: Values calculated by our reviewers from data presented on CBI pp. 26 and 28.

one of which was identified. In male rats, components with R_f values of 0.50, 0.45, and 0.37 accounted for 18, 14, and 13 percent of the total 8- to 48-hour urinary radioactivity, respectively, with 42 percent remaining at the origin. After treatment with β-glucuronidase/sulphatase, the material remaining at the origin decreased to 24 percent of the sample radioactivity and there were corresponding increases to 24, 16, and 16 percent of the radioactivity in the components with R_f values of 0.50, 0.45, and 0.37, respectively.

In the 8- to 48-hour sample of urine from the female rats, the component with R_f values of 0.50 was present in a significantly larger proportion than in the urine of the males. This component accounted for 63 percent of the total urinary radioactivity, and material remaining at the origin accounted for 19 percent of the sample radioactivity. Following incubation of the urine from female rats with β-glucuronidase/sulphatase there was a decrease in radioactive material at the origin to 9 percent of the urinary radioactivity. Unchanged parent compound was not detected in the urine from either sex.

Bile from male and female rats was resolved by TLC as one major component (R_f 0.50), which accounted for 45 and 51 percent of the total biliary radioactivity, respectively (Table 6). Material remaining at the origin accounted for 48 and 41 percent of the biliary radioactivity in male and female rats, respectively. Following incubation of the bile with β-glucuronidase/sulphatase, there was an increase to 54 and 64 percent in male and female rats, respectively, for the component with an R_f value of 0.50; there were corresponding decreases to 40 and 22 percent, respectively, in the material remaining at the origin. The parent compound, [^{14}C-quinoxaline]NC-302, was not detected in the bile of either sex.

Methanol extracts from 24- and 48-hour fecal samples were analyzed by TLC. Several radioactive components were resolved; the component with an R_f value of 0.50 accounted for 62 to 70 percent of the total sample radioactivity (Table 7). The parent compound was also present at low concentrations, approximately 2 to 6 percent of the sample radioactivity.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The results obtained after oral dosing of [^{14}C]NC-302 at 1.5 mg/kg to male and female rats showed a sex-related difference in metabolism. Approximately 21.0 and 49.8 percent of the administered radioactivity was recovered in the urine of male and female rats, respectively. This variance could not be attributed to a difference in the extent of absorption; monitoring the radiolabel in the bile ducts of cannulated rats showed that almost

TABLE 6. Proportions of Radioactive Components in the 0- to 48-Hour Bile from Male and Female Rats after Oral Administration of [^{14}C-Quinoxaline]NC-302

Metabolite[b]	R_f[c]	[^{14}C] in untreated bile[a]		[^{14}C] in β-glucuronidase-treated bile[a]	
		Males	Females	Males	Females
1	0.50	23.6(45)	24.8(51)	28.4(54)	32.2(66)
2	0.37	ND[d]	1.0 (2)	ND	1.9 (4)
Origin	0.00	25.5(48)	20.0(41)	21.4(40)	10.7(22)
Others	--	3.8 (7)	2.6 (5)	3.2 (6)	3.7 (8)

[a] Values expressed as percent of administered [^{14}C] dose; values in parentheses are percent of sample radioactivity.

[b] Components were separated by the TLC solvent system benzene:ethyl acetate:acetic acid (4:2:1, v/v).

[c] R_f of authentic NC-302 was 0.72.

[d] None detected.

Source: Values calculated by our reviewers from data presented on CBI pp. 31 and 32.

TABLE 7. Proportions of Radioactive Components in the Methanol Fecal Extracts (0-24 and 24-48 hours) of Male and Female Rats after a Single Oral Dose of [^{14}C-Quinoxaline]NC-302

Component[a]	R_f	Percent of fecal radioactivity in males		Percent of fecal radioactivity in females	
		0-24 hr	24-48 hr	0-24 hr	24-48 hr
1[b]	0.72	5	2	6	4
2	0.61	2		3	1
3	0.50	70	66	62	67
4	0.45	5	6	7	7
5	0.37-0.32	9	14	8	11
Origin	0.00	6	8	6	7
Others	--	3	4	8	3

[a]Components were separated by the TLC solvent system benzene:ethyl acetate:acetic acid (4:2:1, v/v).

[b]Corresponds to authentic NC-302.

Source: CBI p. 33.

equal amounts of the administered dose (49 to 52 percent) were excreted by both sexes as were the terminal biliary elimination half-lives. The higher excretion of radiolabel in the urine of intact females compared to that of bile-duct-cannulated female rats indicated that extensive reabsorption of excreted biliary radioactivity may occur. The lower urinary excretion in male rats would indicate that enterohepatic circulation of NC-302 and/or its metabolites is less extensive than in female rats.

A major radioactive component more polar than NC-302 was detected in the urine and feces and also in the bile where it was partly present as a conjugate. This metabolite was probably formed to about the same extent by both male and female rats, but a greater proportion was eliminated in the urine by females (22 percent of dose) when compared to male rats (3 percent of dose). Several other components were also detected in the urine of male and female rats, each representing about 1 to 2 percent of the administered dose.

B. This study was conducted in compliance with Good Laboratory Practice regulations as set forth in 'Title 21 of the U.S. Code of Federal Regulations, Part 58.' The study report was audited by HRC Quality Assurance Unit and considered to be an accurate presentation of the data produced during the course of the study.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Although this study was adequately conducted, one major deficiency is that the metabolites were not identified; consequently, it is difficult to correlate the TLC data for the excreta, bile, and tissues. Other studies with this compound suggest that the major component with an R_f value of 0.5 is the NC-302 acid.

The results indicate that NC-302 is largely absorbed from the gastrointestinal tract following oral administration at 1.5 mg/kg. Radiolabeled material originating from [^{14}C-quinoxaline]NC-302 was eliminated in the urine and feces; females excreted more radioactivity in the urine and less in the feces than male rats. A major radioactive component was detected in the excreta, and female rats excreted more of this component into the urine than the males. Only small amounts of unchanged NC-302 were detected in the feces of both sexes. Radiolabel was widely distributed throughout the tissues after dose administration, with plasma, blood, gastrointestinal tract, liver, kidneys, and thyroid having the highest concentrations. Male and female tissues contained similar levels of [^{14}C] residues.

Other deficiencies were noted. The studies investigating the biliary excretion and tissue distribution of NC-302 employed only one rat per sex. A more accurate quantitative representation of radiolabel concentrations could have been obtained by utilizing more test animals. This is especially true for biliary excretion, since [^{14}C] residue

levels in bile were found to be similar for males and females whereas [^{14}C] levels in urine were vastly different. Also, in CBI Appendix 3 (page 49) the authors indicated that two rats/sex were dosed for the study on biliary excretion, but data were only provided for one rat/sex. No explanation was given for this discrepancy.

However, these deficiencies are minor, and we assess that they do not detract from the importance or validity of the data or from conclusions based on the data reported.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 2-5, and Appendix B, CBI Table 4, p. 19, and CBI Table 8, p. 23.

APPENDIX A

Materials and Methods

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APPENDIX B

CBI Tables 4 and 8

TABLE 4

Rates of excretion of radioactivity in bile of rats after an oral dose of ^{14}C-NC 302
Results are expressed as % dose/stated time interval

Time (h)	23♂	24♀	Mean	SD
0 - 1	.04	.05	.05	.01
1 - 2	.11	.18	.15	.05
2 - 3	.19	.43	.31	.17
3 - 4	.32	.56	.44	.17
4 - 5	.42	.55	.49	.09
5 - 6	.56	.55	.56	.01
6 - 7	.76	.55	.66	.15
7 - 8	.85	.66	.76	.13
8 - 9	.98	.71	.85	.19
9 - 10	1.03	.75	.89	.20
10 - 11	1.20	.41	.81	.56
11 - 12	1.40	.80	1.10	.42
12 - 13	1.64	1.05	1.35	.42
13 - 14	2.01	1.17	1.59	.59
14 - 15	2.21	1.45	1.83	.54
15 - 16	2.33	1.14	1.74	.84
16 - 17	2.57	1.29	1.93	.91
17 - 18	2.31	.28	1.30	1.44
18 - 19	2.13	1.57	1.85	.40
19 - 20	1.98	1.35	1.67	.45
20 - 21	1.83	1.59	1.71	.17
21 - 22	1.46	1.40	1.43	.04
22 - 23	1.46	1.66	1.56	.14
23 - 24	1.40	1.58	1.49	.13
24 - 25	1.21	1.46	1.34	.18
25 - 26	1.05	1.58	1.32	.37
26 - 27	1.01	1.53	1.27	.37
27 - 28	1.15	1.38	1.27	.16
28 - 29	1.05	1.29	1.17	.17
29 - 30	1.06	1.45	1.26	.28
30 - 31	1.29	1.25	1.27	.03
31 - 32	1.11	1.32	1.22	.15
32 - 33	1.14	1.31	1.23	.12
33 - 34	1.13	1.03	1.08	.07
34 - 35	.87	1.30	1.09	.30
35 - 36	.95	1.26	1.11	.22
36 - 37	.92	1.31	1.12	.28
37 - 38	.93	1.35	1.14	.30
38 - 39	.91	1.22	1.07	.22
39 - 40	1.01	1.15	1.08	.10
40 - 41	.94	1.01	.98	.05
41 - 42	.54	.88	.71	.24
42 - 43	.55	.84	.70	.21
43 - 44	.30	.84	.57	.38
44 - 45	.60	.74	.67	.10
45 - 46	.45	.67	.56	.16
46 - 47	.44	.67	.56	.16
47 - 48	.23	.49	.36	.18

SD Standard deviation
h Hours

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TABLE 8

Concentrations of radioactivity in the tissues of rats after a single oral dose of ^{14}C-NC 302 (1.5 mg/kg)
Results are expressed as µg equivalents ^{14}C-NC 302/g tissue

Animal No.	13♂	14♀	15♂	16♀	17♂	18♀	19♂	20♀	21♂	22♀
Time of sacrifice (hrs)	6.00	6.00	24.00	24.00	48.00	48.00	96.00	96.00	168.00	168.00
Adrenal glands	1.90	1.66	.944	.974	.591	.360	.256	.199	.000	.000
Thyroid	3.36	2.82	1.03	1.53	1.21	.000	.280	.435	.000	.000
Eyes	.668	.577	.326	.351	.177	.123	.051	.062	.000	.000
Brain	.188	.225	.096	.072	.047	.034	.015	.022	.003	.002
Thymus	.554	.690	.334	.356	.197	.118	.050	.070	.014	.009
Testes/Ovaries	1.24	3.13	.683	1.55	.335	.544	.103	.315	.016	.028
Spleen	.652	.772	.410	.445	.228	.176	.070	.088	.009	.013
Muscle	.682	.651	.318	.293	.200	.112	.060	.062	.009	.009
Heart	1.50	1.86	.949	.868	.488	.285	.165	.137	.022	.014
Pancreas	1.32	.885	.906	.569	.464	.324	.127	.189	.039	.029
Lungs	1.87	2.28	1.31	.913	.564	.361	.183	.211	.028	.028
Fat	.596	.776	.535	.518	.762	.239	.302	.247	.131	.081
G.I. Tract	4.12	6.37	4.15	2.94	1.58	1.16	.448	.458	.054	.110
Liver	3.32	3.69	1.99	2.11	1.52	.726	.331	.352	.033	.058
Kidneys	3.11	3.16	1.97	2.48	1.91	1.16	.440	.604	.060	.060
Whole-blood*	6.07	6.73	3.53	2.48	1.84	1.31	.533	.660	.073	.071
Plasma*	9.93	11.11	5.99	5.35	2.98	1.93	.906	1.13	.106	.090

* Concentrations are expressed as µg equivalents/ml

005546

EPA: 68-02-4225
DYNAMAC No. 1-011A-8
March 25, 1986

DATA EVALUATION RECORD

NC-302

Metabolic Study in Rats

STUDY IDENTIFICATION: Hawkins, D. R., Down, W. H., Moore, D. H., Ballard, S. A., and Whitby, B. R. The accumulation of NC-302 and its metabolites in the tissues of rats during repeated oral administration. (Unpublished study No. HRC/NSA 13/83182 prepared and submitted by the Department of Chemical Metabolism and Radiosynthesis, Huntingdon Research Centre, Huntingdon, Cambridgeshire, England, for E.I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated April 15, 1983.) Accession No. 073546.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-25-86

1. CHEMICAL: NC-302; ethyl-2-[4-(6-chloro-2-quinoxalyloxy)-phenoxy]-propionate.

2. TEST MATERIAL: [^{14}C-quinoxaline]NC-302 had a specific activity of 7.79 mCi/mmol and a radiochemical purity of at least 98 percent.

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: Hawkins, D. R., Down, W. H., Moore, D. H., Ballard, S. A., and Whitby, B. R. The accumulation of NC-302 and its metabolites in the tissues of rats during repeated oral administration. (Unpublished study No. HRC/NSA 13/83182 prepared and submitted by the Department of Chemical Metabolism and Radiosynthesis, Huntingdon Research Centre, Huntingdon, Cambridgeshire, England, for E.I. DuPont de Nemours and Co., Inc., Wilmington, DE; dated April 15, 1983.) Accession No. 073546.

5. REVIEWED BY:

Lynne L. W. Binari, M.S.
Principal Reviewer
Dynamac Corporation

Signature: [signature]
Date: 3-26-86

Charles E. Rothwell, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: [signature]
Date: 5-26-86

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Metabolism
Technical Quality Control
Dynamac Corporation

Signature: [signature]
Date: March 25, 1986

Whang Phang, Ph.D.
EPA Reviewer

Signature: [signature]
Date: 6/18/86

Clint Skinner, Ph.D.
EPA Section Head

Signature: [signature]
Date: 4.18.86

7. CONCLUSIONS:

A. The accumulation and distribution of radiolabel in the tissues of male Sprague-Dawley rats were investigated during and after repeated oral administration of [^{14}C-quinoxaline]NC-302 at 1.5 mg/kg for 28 days.

Tissue radioanalysis and whole-body autoradiograms showed that maximal concentrations of radiolabel were attained 24 hours following the seventh daily dose of [^{14}C]NC-302. Of the tissues that were radioassayed, the blood, liver, and kidneys contained the highest concentrations of radioactivity. Autoradiograms showed high levels of radioactivity in the gastrointestinal tract, liver, kidneys, blood, lungs, skin, fur, and teeth. From the 7th to the final dose (at 28 days), radioactivity levels declined in most tissues, except in the fat, suggesting either a decrease in the absorption of orally administered NC-302 or an increased rate of elimination. Following the final dose, tissue [^{14}C] levels decreased steadily over an 8-day postadministration period, except in the fat. Radiolabel concentrations in the fat were fairly constant throughout the dosing and postadministration periods at about 1.0 to 1.5 ppm.

B. This study provides supplementary data.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Male CD Sprague-Dawley rats that were 7 weeks of age were used in the study.

[^{14}C-quinoxaline]NC-302 was mixed with unlabeled NC-302 to a specific activity of 6.773 µCi/mg (2.52 mCi/mmol) in 1 percent aqueous Tween 80.

[^{14}C-quinoxaline]NC-302 was administered daily to each rat for periods of up to 28 days by oral intubation at a dose of 1.5 mg/kg.

---

[1] Only items appropriate to this DER have been included.

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2. [^{14}C] concentrations in various tissues were determined in groups of three rats sacrificed 24 hours following the 3rd, 7th, 14th, 21st, and 28th dose and also during a withdrawal period at 2, 4, 6, and 8 days after the 28th dose. At sacrifice, blood, liver, kidneys, muscle (gastrocnemius), and fat (subcutaneous or supra-renal) were taken and radioassayed by liquid scintillation counting (LSC) following combustion using standard techniques.

3. For whole-body autoradiograms, a single male rat was sacrificed 24 hours following the 3rd, 7th, 14th, 21st, and 28th daily dose of [^{14}C]NC-302, and the body was frozen in a dry ice:petroleum ether bath. Sagittal sections (20 µm) of the carcass were freeze dried and exposed to X-ray film. The relative concentrations of radioactivity indicated on the autoradiographs for the various tissues were estimated by visual inspection.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. [^{14}C] concentrations determined in tissues of male rats during and after the administration of 28 daily oral doses of [^{14}C-quinoxaline]NC-302 (1.5 mg/kg) are presented in Table 1 and Figure 1 (CBI, p. 13). Maximum radioactivity levels were noted after 7 days of [^{14}C]NC-302 administration. [^{14}C] concentrations declined to approximately half of each tissue maxima by the 21st day of [^{14}C]NC-302 dose administration, except in the fat where they were only slightly depressed. The levels of radioactivity in the tissues following the 28th dose increased and were similar to those observed after the 14th dose. During the withdrawal period, levels declined steadily to less than 0.20 ppm, except fat where [^{14}C] concentrations remained constant at about 1 ppm throughout the 8-day withdrawal period.

B. Whole-body autoradiograms of male rats during the 28-day dosing period supported the above distribution patterns of radiolabel, with no extensive accumulation of radioactivity in any tissue being apparent. Maximal concentrations of radioactivity were observed after the 7th dose and levels diminished gradually thereafter, with the lowest amounts of radioactivity being observed after the 28th dose. The tissues containing the highest concentrations of radioactivity at each time interval were the gastrointestinal tract, liver, kidneys, blood, lungs, muscle, fat, skin, and fur.

APPENDIX A

Materials and Methods

TABLE I. Mean Concentrations of Radioactivity in the Tissues of Male Rats During and After the Administration of 28 Consecutive Daily Oral Doses of [^{14}C]NC-302 (1.5 mg/kg/day)

Dosing Period (days)[c]	Mean Concentration of Radioactivity (ppm[a])[b] Tissue				
	Whole blood	Liver	Kidney	Muscle	Fat
3	5.00±0.58	3.19±0.53	3.33±0.70	0.61±0.14	1.35±0.18
7	5.97±0.68	4.58±0.40	4.20±0.62	0.68±0.09	1.49±0.16
14	3.80±1.83	3.39±1.34	3.63±1.34	0.48±0.28	1.30±0.36
21	2.73±0.40	2.63±0.42	2.36±0.30	0.30±0.05	1.18±0.07
28	3.33±0.98	2.55±0.31	3.46±0.54	0.38±0.12	1.40±0.36
Withdrawal period					
28 + 2	1.33±0.18	1.20±0.17	1.52±0.41	0.15±0.00	0.97±0.21
28 + 4	0.56±0.32	0.47±0.25	0.59±0.37	0.07±0.04	1.26±0.27
28 + 6	0.54±0.36	0.34±0.21[d]	0.43±0.35	0.08±0.04[d]	1.07±0.20
28 + 8	0.20±0.10	0.13±0.06[d]	0.12±0.07	0.03±0.02	0.96±0.25[d]

[a] ppm = µg equivalents NC-302/g tissue.

[b] Mean values ± S.D. of three rats.

[c] Rats were sacrificed at 24 hours after the indicated number of consecutive daily oral doses of [^{14}C]NC-302.

[d] These values (taken from CBI Table 1, p. 10) differ slightly from those reported in CBI Table 2, pp. 11-12.

FIGURE 1

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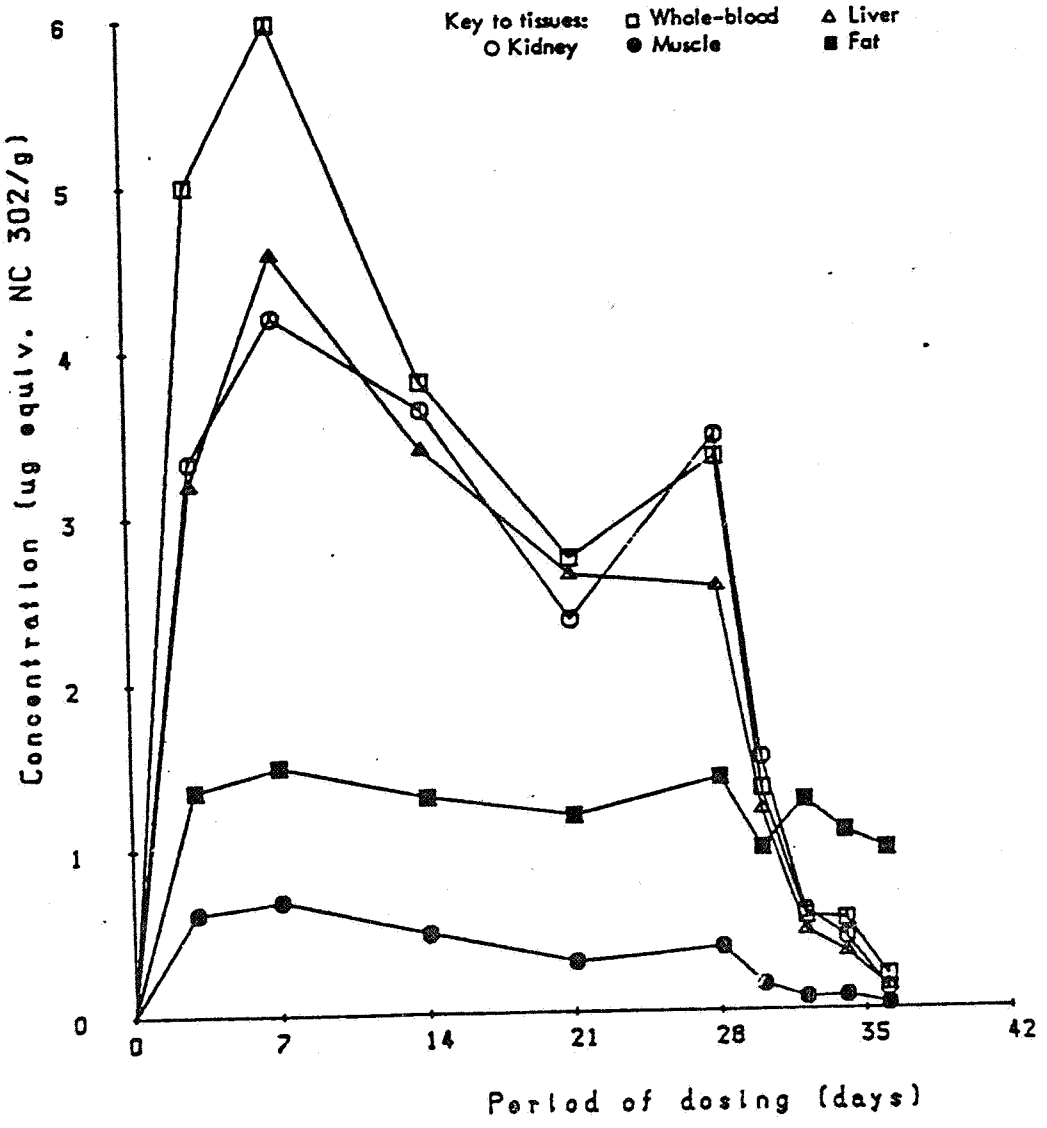
Mean concentrations of radioactivity in the tissues of male rats during and after 28 daily oral doses of ^{14}C-NC-302 (1.5 mg/kg/day)



SOURCE: CBI page 13.

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13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Tissue concentrations and distribution of radioactivity were determined in rats during and after 28 days of consecutive oral administration of [^{14}C]NC-302 at a nominal dosage of 1.5 mg/kg/day. Rats were sacrificed at 24-hour intervals after the respective number of daily doses.

Mean concentrations of radioactivity were maximal after 7 days of administration of [^{14}C]NC-302 (5.97, 4.58, 4.20, 1.49, and 0.68 µg equivalents NC-302/g tissue in the blood, liver, kidneys, fat, and muscle, respectively). Between 7 and 28 days of dosing, mean concentrations of radioactivity in each tissue were slightly decreased compared with those found after 7 days. After cessation of [^{14}C]NC-302 dosing, mean tissue concentrations of radioactivity declined relatively quickly during 8 days to 0.20, 0.13, 0.12, and 0.03 µg/g in blood, liver, kidneys, and muscle, respectively. Mean concentrations in fat, however, remained essentially the same between the time of the final (28th) dose of [^{14}C]NC-302 and 8 days of withdrawal (about 1.0 µg/g).

Whole-body autoradiography indicated similar patterns of distribution of radioactivity throughout the dosing period, and there was no gross accumulation of radioactivity in any tissue. Maximal concentrations of radioactivity were apparent after the 7th dose and levels diminished gradually thereafter, the lowest concentrations occurring after 28 daily doses. The tissues containing the greatest concentrations of radioactivity at each time were the gastrointestinal and urinary tracts, liver, blood, skin, fur, muscle, fat, some endocrine and secretory glands, connective tissue, and, to a lesser extent, some parts of the reproductive system.

The available data (quantitative and qualitative) indicated that maximal levels of radioactivity in most tissues occurred after 7 days of [^{14}C]NC-302 dosing and declined slightly thereafter during 28 days of dosing. This may have been either due to a decline in the extent of absorption of orally administered [^{14}C]NC-302 or to an increase in the rate of elimination of NC-302 and/or its metabolites, which could be due to enzyme induction. Concentrations of radioactivity in fat, however, remained essentially constant between 3 and 28 days of dosing as well as during the 8-day recovery period. No extensive accumulation of radiolabel occurred in fat during the dosing period, with maximum levels of only about 1.5 µg/g.

B. The report was audited by the HRC Quality Assurance Unit and was considered to be an accurate presentation of the data produced during the course of the study. Authorization was by Kenneth W. G. Shillam, B.Sc., Ph.D., F. I. Biol., director, Quality Assurance.

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14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: The purpose of this study was to provide information on the accumulation and distribution of radiolabel in certain tissue of male rats after repeated administration of [^{14}C-quinoxaline]NC-302. Using tissues from three animals at each specified interval, the triplicate radioassay of each tissue resulted in a more accurate determination of tissue radioactivity concentrations. However, the results of this study and the conclusions of the authors could have been further supported by additional data on [^{14}C] levels in urine and feces as well as other tissues that were not radioassayed. Although the whole-body autoradiograms helped to support the radioassay results and also indicated other areas of radiolabel accumulation, this information is only qualitative in nature. Another deficiency in this study was the large variability in the actual doses administered (Table 2). On 11 of the 28 days in which the rats were dosed, the administered dose level deviated from the nominal dose level by more than 10 percent. The largest deviation occurred on day 17 when the rats received only 53 percent of the nominal dose. This deviation may have caused the noticeable drop in tissue residues detected in the rats sacrificed after 21 days of dosing (see Figure 1). Consequently, the results of this study are limited.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-5.

TABLE 2. Daily Doses of [^{14}C]NC-302 Administered Orally to Rats

Day of Dosing	Administered Dose (mg NC-302/kg)	% Deviation from Nominal Dose (1.5 mg/kg)[a]
1	1.43	- 4.7
2	1.74	+16.0
3	1.46	- 2.7
4	1.21	-19.3
5	1.45	- 3.3
6	1.58	+ 5.3
7	1.61	+ 7.3
8	1.68	+12.0
9	1.50	0.0
10	1.90	+26.7
11	1.22	-18.7
12	1.42	- 5.3
13	1.70	+13.3
14	1.59	+ 6.0
15	1.50	0.0
16[b]	1.15	-23.3
17[b]	0.80	-46.7
18	1.66	+10.7
19	1.50	0.0
20	1.47	- 2.0
21	1.45	- 3.3
22	1.48	- 1.3
23	1.59	+ 6.0
24	1.19	-20.7
25	1.33	-11.3
26	1.52	+ 1.3
27	1.43	- 4.7
28	1.42	- 5.3

[a] Calculated by our reviewers.

[b] Study stated dose preparations on days 16 and 17 underwent frothing resulting in reduced volumes administered to animals.

Source: CBI p. 23.

ASSURE 005546

Page_____ is not included in this copy.

Pages 169 through 173 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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____ Description of the product manufacturing process.

____ Description of quality control procedures.

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____ Sales or other commercial/financial information.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

November 22, 1985

SUBJECT: Assure™, Review of a Dermal Absorption Study in Rats

TO: Caroline A. Gregorio
Toxicologist
Review Sec III

FROM: Robert P. Zendzian PhD
Pharmacologist
Toxicology Branch

Action Requested

Review the following dermal absorption study.

Rat dermal absorption of 14C-labeled Assure™ herbacide, S.G. Hundley, A.M. Sarrif & R.L. Fisher, Haskell Laboratory for Toxicology and Industral Medicine, du Pont. May 1, 1985

Conclusions

Core Classification Acceptable (limited)

Dermal absorption of Assure™ for ten hours of exposure at doses of .19, 1.9 and 19 mg/rat was 8.38, 3.27 and 2.98 % respectively. The latter value may be erroniously high. No absorption values for intermediate exposure durations can be determined from this study. Carcasses of the male animals sacrificed at one hour should be analyzed in order to determine the total absorption at that time. This study design was experimental and should not be considered acceptable in the future.

Discussion

The experimental design of this dermal absorption study is a variation of the basic design in that groups of animals are not killed for determination of body burden at each time interval (0.5, 1, 2, 4 and 10 hours). Rather a single group for each dose is carried for the maximum time with blood samples taken at the usual time intervals. At the request of this reviewer additional groups of males, for each dose, were carried for one hour and blood samples taken the

carcasses were preserved frozen but not analyzed. Considering the data generated it is not possible to determine absorption rates for the intermediate periods for each dose dispite the fact that blood concentration appears to increase in a linear fashion after the second hour. This is due to our lack of information on the kinetics of distribution of the compound into the tissue with time.

Additional insight into this question may be gained from the males that were not cannulated and only carried for one hour. In order to determine the relative tissue distribution it is necessary to determine the quantity of material (percent of dose) in the carcasses of these animals.

This modified design was accepted by this reviewer from the standpoint of determining if it could produce usable data. The data produced is usable for ten hours exposure but not for intermediate exposure durations with the proviso that the data for carcass at 19 mg may well be wrong. It is not possible to generate usable information for intermediate exposure durations from this data. Also the experimental design does not allow identification and possible compensation for outlieing or apparently erronious data. The protocol cannot be considered acceptable for future use.

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Date Evaluation Report

Compound Assure™, DPX-Y6202, ethyl, 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]-,propanoate

Citation

Rat dermal absorption of 14C-labeled Assure™ herbacide, S.G. Hundley, A.M. Sarrif & R.L. Fisher, Haskell Laboratory for toxicology and Industral Medicine, du Pont. May 1, 1985

Reviewed by Robert P. Zendzian PhD
Pharmacologist 11/22/85

Core Classification Acceptable (limited)

Conclusions

Dermal absorption of Assure™ for ten hours of exposure at doses of .19, 1.9 and 19 mg/rat was 8.38, 3.27 and 2.98 % respectively. The latter value may be erroniously high. No absorption values for intermediate exposure durations can be determined from this study. Carcasses of the male animals sacrificed at one hour should be analyzed in order to determine the total absorption at that time. This study design was experimental and should not be considered acceptable in the future.

Materials

Radiolabeled [quinoxalin-phenyl-^{14}C(U)] DPX-Y6206
Daiichi Chemical Co, 58.0 uCi/mg; 99% radio chemical purity
New England Nuclear (13.9 uCi/mg; 97% radio chemical purity

Nonlabeled DPX-Y6202 99% pure

Assure™, (Quinoxaline -phenyl(U) -^{14}C)
9.5% Active ingredient
90.5% Inert ingredients

Charles River Rats

Experimental Design

Eight male rats were assigned to each dose group which received doses of 0.19, 1.9 or 19.0 mg/rat. Eight female rats received a dose of 0.19 mg/rat. The material used for the high dose was radiolabeled Assure™ concentrate and the lower dose materials represented 10 and 100 fold dilutions, in distilled water, of the concentrate.

"Jugular-vein cannulas were surgically inserted into four of the eight rats at each exposure level three days prior to dermal exposure according to published procedures". "All rats (cannulated and non-cannulated) were shaved the day before dermal exposure over a 3" by 3" area on the back extending from the shoulder blades."

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On the day of exposure the shaved area was cleaned with acetone and the dose applied to a 2" by 2" marked area. Applicators were analysed for residual material. Non-occlusive gauze was used to cover the application site. Cannulated rats were placed in "Roth"-type class metabolism units and the non-cannulated rats were placed in restrainers above funnels equipted to collect feces and urine.

"The cannulated rats were maintained for 10 hours and the non-cannulated rats for one hour following application. Urine and feces were collected at 1, 2, 4 and 10 hours from the cannulated rats and at the 1-hour time point for restrained rats."

"Blood samples (0.5 ml) were drawn from each cannulated rat at 0.5, 1, 2, and 4 hours. After the 1- and 4-hour collections, 0.5 ml blood from a "donor" rat was given to each canulated rat to prevent adverse health effects from blood loss."

At termination the animals were anesthetized and a terminal blood sample obtained by cardiac puncture. Residual urine was collected from the urinary bladder and added to the terminal urine collection. The gauze and the skin at the application site were collected. The following organs and tissues were collected from all cannulated rats;

heart	brain
lungs	G.I. tract
liver	muscle
spleen	fat
kidneys	abdominal skin
testes	bone marrow

The samples and the residual carcass were stored frozen. The carcasses for the non-cannulated rats were stored frozen.

All samples were analyzed for radioactivity which was expressed as compound. However, the carcasses of the noncannulated rats were not analyzed.

Results

The blood concentration of compound is presented in Table 1 below.

Table 1. Mean blood concentration of compound, as ug equivalents per ml of whole blood. Data from table 2 of the report.

Sample time (hour)	Cannulated Rats 0.19 mg Female	0.19 mg Male	1.9 mg Male	19 mg Male
0.5	0.003 ±0.001	0.007 ±0.005	0.041 ±0.007	0.33 ±0.07
1.0	0.010 ±0.007	0.022 ±0.015	0.125 ±0.015	0.76 ±0.16
2.0	0.033 ±0.023	0.039 ±0.027	0.242 ±0.024	1.36 ±0.30
4.0	0.089 ±0.056	0.074 ±0.033	0.391 ±0.051	2.16 ±0.28
10.0	0.226 ±0.111	0.187 ±0.070	0.930 ±0.136	4.98 ±0.81
	Non-Cannulated Rats			
Sample Time	Female	Male	Male	Male
1 hour	0.014 ±0.012	0.006 ±0.004	0.143 ±0.095	1.04 ±0.37

Data on total dose absorption is presented below for the ten hour cannulated male rats, the only animals for which the total dose distribution can be determined.

Table 2. Mean percent of dose absorbed and dose distribution for male cannulated rats. Data derived from tables 10, 11 and 12 of the report.

	dose mg/rat 0.19		1.90		19.0	
Sample	Mean % of of dose absorbed	% distribution of absorbed dose	Mean % of of dose absorbed	% distribution of absorbed dose	Mean % of of dose absorbed	% distribution of absorbed dose
Blood	0.85	10.14	0.49	14.98	0.23	7.55
Organs & Tissues	2.63	31.38	1.05	32.11	0.56	18.62
Urine & Feces	0.91	10.86	0.10	3.06	0.04	1.26
Carcass	4.00	47.73	1.63	49.85	2.07	68.46
Total	8.38	100.00	3.27	100.00	2.98	100.00

The mean blood concentration data of the cannulated male rats, from Table 1, is plotted in figures 1, 2 and 3. On inspection the data for the 0.19 mg rats appear linear from 1 to 10 hours and the data from the 1.9 and 19 mg rats appear linear from 2 to 10 hours. The nonlinear portion of the curves probably represents an early lag in absorption. Dispite appearances in visually comparing the figures, the linear portions of the respective curves are not parallel. This can be shown by calculating the slope for the concurrent linear portions of each curve (2 to 10 hours) as follows.

$$\text{Slope} = \frac{\text{Concentration at 10 hours - Concentration at 2 hours}}{8 \text{ hours}}$$

Dose (mg/rat)	Slope (ug eq/ml/hr)
0.19	0.0185
1.90	0.0860
19.00	0.4525

Discussion

In general the data produced in this study follows the most common pattern seen in dermal absorption studies, the quantity absorbed, per unit time, increases with dose but the percent of dose absorbed decreases with dose. This is particularly illustrated by the data in Table 2 for absorption over 10 hours. As the dose increases the percent of the dose that is absorbed decreases. However, there appears to be a lack of pattern in the percent distribution of the absorbed dose for each of the doses. Considering the individual samples, for blood, organs and tissues, and urine and feces the mean percent of dose abosrbed decreases with inreasing dose. For the carcass the mean percent absorbed decreases from the .19 mg dose to the 1.9 mg dose but for the 19 mg dose the percent in the carcass is higher than for the 1.9 mg dose.

It appears most likely that the quantity found in the carcass is too high (erroniously?) making the mean percent of dose absorbed for the carcass too high and the mean total percent of dose absorbed therefore too high. However one cannot eliminate the possibility that the kinetics of distribution have changed with the high dose. As seen in Table 1 the blood concentration in the 19 mg animals at 10 hours is 5 times the blood concentration in the 1.9 mg animals

at 10 hours. Yet at the same time the blood concentration in the 1.9 mg animals is four times the blood concentration in the .19 mg animals with only a two percent increase in the % distribution of the absorbed dose in the carcass of the 1.9 mg animals and a less than one percent increase in the organs and tissues. Because of the lack of intermediate time sacrifices it is not possible to determine if a significant change in distribution kinetics has occured.

The experimental design of this dermal absorption study is a variation of the basic design in that groups of animals are not killed for determination of body burden at each time interval (0.5, 1, 2, 4 and 10 hours). Rather a single group for each dose is carried for the maximum time with blood samples taken at the usual time intervals. Considering the data generated it is not possible to determine absorption rates for the intermediate periods for each dose dispite the fact that blood concentration appears to increase in a linear fashion after the second hour. This is due to our lack of information on the kinetics of distribution of the compound into the tissue with time.

Additional insight into this question may be gained from the males that were not cannulated and only carried for one hour. In order to determine the relative tissue distribution it is necessary to determine the quantity of material (percent of dose) in the carcasses of these animals.

This modified design was accepted by this reviewer from the standpoint of determining if it could produce usable data. The data produced is usable for ten hours exposure but not for intermediate exposure durations with the proviso that the data for carcass at 19 mg may well be wrong. Also it does not allow identification and possible compensation for outlieing or apparently erronious data. The protocol cannot be considered acceptable for future use.

Figure 1. Mean blood concentration of Assure™ with time following a single dermal dose of 0.19 mg to 4 cannulated male rats.

Figure 2. Mean blood concentration of Assure® with time following a single dermal dose of 1.90 mg to 4 cannulated male rats.

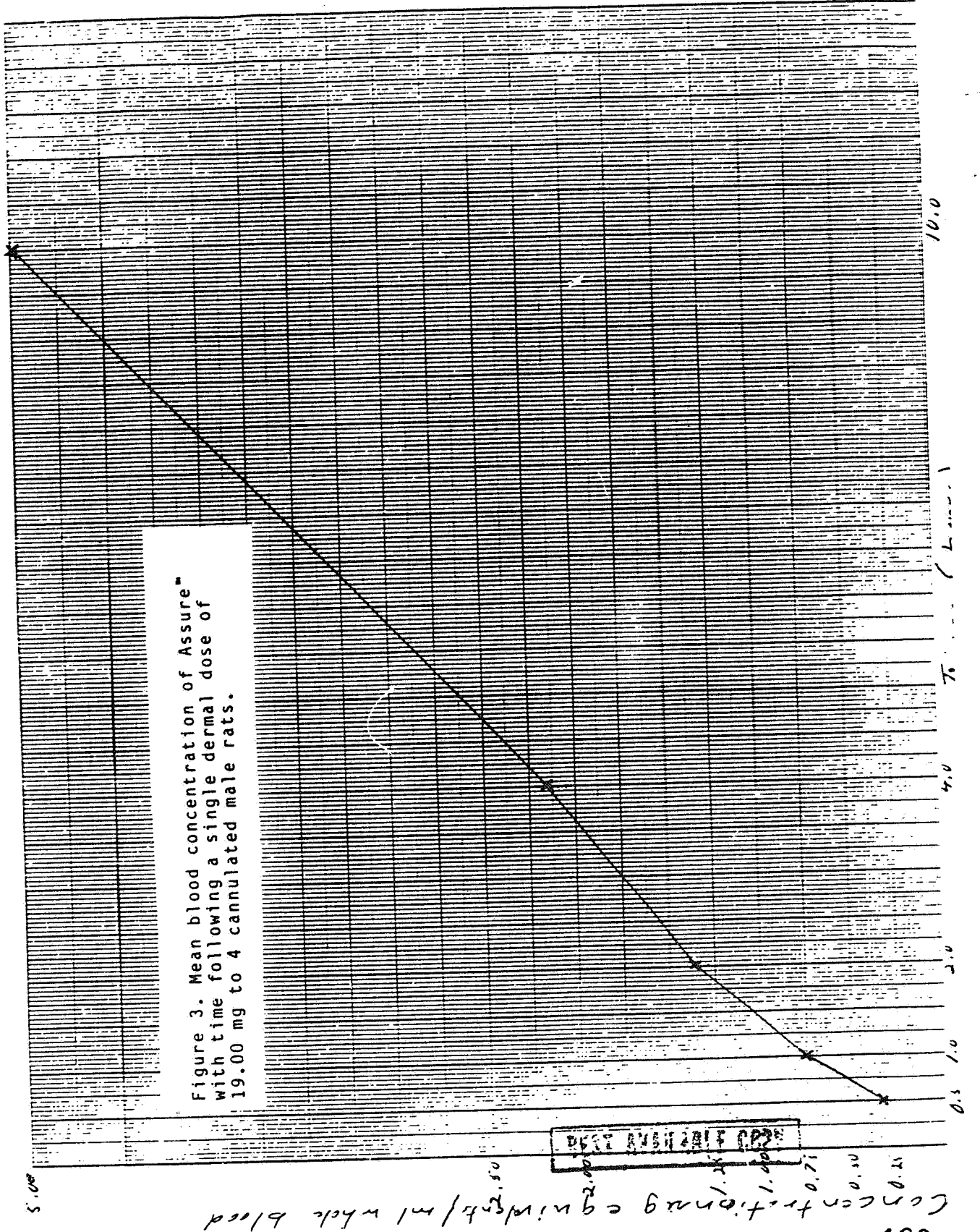

Figure 3. Mean blood concentration of Assure™ with time following a single dermal dose of 19.00 mg to 4 cannulated male rats.