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

SEP 29 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carbaryl: Review of a Special Study (DNA Binding) submitted by the Registrant.

P.C. Code: 056801
Submission: S469034
MRID No: 432822-01
DP Barcode: D204990

FROM: Timothy F. McMahon, Ph.D., Pharmacologist 7/24/94
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

TO: Linda Propst / PM 73
Special Review and Reregistration Division (7508W)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia Van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (7509C)

Registrant: Rhone Poulenc Ag Company

Action Requested: Review of a special study (DNA binding) submitted by t registrant

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Summary:

A study entitled, "Investigation of the Potential for Protein- and DNA-Binding of Carbaryl" was submitted for review (MRID # 432822-01) in response to the carbaryl Data Call-in.

In this study, [1-^{14}C]-naphthyl-N-methylcarbamate (14-C carbaryl) was tested for the ability to bind to liver DNA in male CD1 mice treated with a single radiolabelled dose of carbaryl (75 mg/kg) or in mice pretreated with 8000 ppm (approximately 1143 mg/kg/day) unlabelled carbaryl in the diet for two weeks followed by a single 75 mg/kg radiolabelled dose. Binding of radiolabel to chromatin protein isolated from the livers of mice treated wit a single dose or in pretreated mice was similar (specific activities ranging from 340.3-537.0 dpm/mg). No radioactivity was detectable in DNA samples isolated from mice treated with radiolabelled carbaryl (Covalent Binding Index < 0.1). According to the report, this maximum binding ability of carbaryl is more than 5 orders of magnitude below the Covalent Binding Index of aflatoxin B_1, and more than 4000 times lower than the Covalent Binding Index for 2-acetylaminofluorene. This study demonstrated the interaction of carbaryl with chromatin protein, but no significant interaction with DNA in the liver of male CD1 mice. The possible toxicologic significance of chromatin protein binding of carbaryl in this study was not discussed.

Classification: acceptable

This study was not conducted to satisfy a specific guideline requirement, bu fulfills the purpose for which it was conducted.

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Reviewed by: Timothy F. McMahon, Ph.D. 9/24/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 9/26/94
Head, Section I, Toxicology Branch II (7509C)

Data Evaluation Record

Study type: Special Study- DNA Binding

MRID number: 432822-01 P.C. Code: 056801

Test material: 1-naphthyl-N-methylcarbamate; Carbaryl

Study number: CB93/52

Testing Facility: CIBA-GEIGY Limited
Toxicology Services/Cell Biology, CH-4002 Basel

Sponsor: Rhone-Poulenc Ag Company

Title of report: Investigation of the Potential for Protein- and DNA-Binding of Carbaryl

Author(s): P. Sagelsdorff

Study completed: 4/28/94

Executive Summary: In a special study (MRID # 432822-01), [1-^{14}C]-naphthyl-N-methylcarbamate (14-C carbaryl) was tested for the ability to bind to liver DNA in male CD1 mice treated with a single radiolabelled dose of carbaryl (75 mg/kg) or in mice pretreated wi 8000 ppm (approximately 1143 mg/kg/day) unlabelled carbaryl in the diet for two weeks followed by a single 75 mg/kg radiolabelled dose. Binding of radiolabel to chromatin protein isolated from the livers of mice treated with a single dose or in pretreated mice was similar (specific activities ranging from 340.3-537.0 dpm/mg). No radioactivity was detectable in DN samples isolated from mice treated with radiolabelled carbaryl (Covalent Binding Index < 0.1). According to the report, this maximum binding ability of carbaryl is more than 5 orders of magnitude below the Covalent Binding Index of aflatoxin B_1, and more than 4000 times lower than the Covalent Binding Index for 2-acetylaminofluorene. This study demonstrated the interaction of carbaryl with chromatin protein, but no significant interaction with DNA in the liver of male CD1 mice treated with either a single 75 mg/kg dose or in mice pretreated with 8000 ppm (1143 mg/kg/day) carbaryl in the diet followed by a single 75 mg/kg radiolabelled dose.

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Core Classification: acceptable

This study was not conducted to satisfy a specific guideline requirement, but fulfills the purpose for which it was conducted.

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I. MATERIALS AND METHODS

A. Test Materials

[1]: [1-^{14}C]-naphthyl-N-methylcarbamate
specific activity: 22.04 mCi/mMol (110 µCi/mg)
lot number: CSL-92-360-5-31
storage: -20 °C , protected from light
purity: > 98% by TLC

[2]: unlabelled 1-naphthyl-N-methylcarbamate
lot number: Sevin®, lot 198
purity: 99.6%

B. Control Materials: none stated

C. Test System

Animals (male CD-1 mice) were obtained from Charles River Wiga, Sulzfeld, FRG and were quarantined at least one week prior to use in the study. Mice were housed in macrolor cages during acclimation and pretreatment. All glass metabolism cages were used after test article administration. Mice received food (Nafag Gossau, CH) and tap water *ad libitum*.

E. Experimental Design and Dosing

Table 1
Assignment of Experimental Groups and Animal Numbers

Group No.	Animal No.	Pretreatment with Unlabelled Carbaryl	Treatment with Radiolabelled Carbaryl
1	1-4	none	75 mg (8 mCi)/kg
2	5-8	1143 mg/kg/day dietary	75 mg (8 mCi)/kg
3	9-12	none	none
4	13-17, 23	1143 mg/kg/day dietary	none
5	18-22	none	none

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Animals in group 1 were used for determination of DNA and protein binding of carbaryl, while those animals in group 2 were used to analyze for any possible enzyme induction effect and/or inhibition at high dose levels effect on DNA binding. Animals in group 3 were untreated and were used to determine DNA binding *in vitro* during the DNA isolation process to demonstrate that sample preparation was not contaminated with radioactivity. Group 4 animals were treated and the livers stored for eventual biochemical analyses. Animals in group 5 received control diet and the livers stored for eventual analyses.

Radiolabelled compound was dissolved in 0.5% aqueous carboxymethylcellulose and administered as a single dose at a dose volume of 10 ml/kg. For those mice receiving repeated carbaryl exposure, five kilograms of pelleted diet containing 8000 ppm carbaryl was prepared by Ciba-Geigy. It was stated that about 100g of the prepared diet was stored at -80 ^{o}C for possible later analysis of concentration and homogeneity. No data were provided on dosing solution concentration.

After administration of radiolabelled compound, animal nos 1 and 5 as well as untreated animal no. 9 were placed in 3 all glass metabolism cages for collection of urine and exhaled breath. Aliquots of urine and absorbed exhaled breath were counted for radioactivity by liquid scintillation counting.

Twenty-four hours following administration of radiolabel, mice were killed by open heart puncture under carbon dioxide anesthesia. Livers, kidneys, and bladders of group 1-3 mice were excised and frozen in liquid nitrogen. Frozen tissues were stored at -80 ^{o}C until processing for determination of DNA and protein binding of carbaryl.

F. Isolation of DNA

The organs of two identically treated mice were pooled, homogenized, and the DNA and chromatin protein isolated according to the method of Sagelsdorff et al. (1983). Essentially, chromatin was precipitated with the non-ionic detergent Nonident P40. The chromatin pelle was blended in a denaturing lysing medium, deproteinated with chloroform/isoamyl alcohol/phenol (CIP), extracted with diethyl ether, and the DNA was then further purified by adsorption on a hydroxyapatite column, dialysis and precipitation with ethanol. The highly purified DNA (protein contamination less than 1%) was dissolved and an aliquot was used for UV-determination of the DNA content. Another aliquot was mixed with scintillation cockt for the determination of radioactivity.

Chromatin protein was precipitated with acetone from the CIP extract and dissolved in 1% sodium dodecylsulfate (4 to 5 times). The final precipitate was dissolved and counted for radioactivity. The amount of chromatin protein was measured according to Smith et al. (1985).

It is noted that control experiments were performed on mice nos. 11 and 12 which received no radioactivity, and *in vitro* incubations were performed on the chromatin pellet isolated from the livers of mice nos. 9 and 10 (untreated control) in which the pellet was incubated with the labeled supernatant of the first chromatin precipitation step of the DNA isolation procedure from 2 treated mice. The DNA isolated after this *in vitro* incubation was used to show that the purification steps were sufficient to remove non-covalently bound radiolabelled carbaryl and its radiolabelled metabolites from DNA.

G. Calculation of the Covalent Binding Index

Specific radioactivity of DNA was converted to the units of the Covalent Binding Index (CBI) according to the following equation (Lutz, 1979):

$$CBI = \frac{\mu mol\ chemical\ bound\ /\ mol\ DNA\ nucleotide}{mmol\ chemical\ applied\ /\ kg\ body\ weight} = \frac{dpm/mg\ DNA}{dpm/kg\ body\ weight} \times 309 \times 10^6$$

H. Compliance

A signed statement of **no data confidentiality** was provided.

A signed statement of **compliance** with 40CFR Part 160 was provided

A signed statement of **quality assurance** was provided.

II. RESULTS

a) Disposition

A comparison of the disposition in mice dosed singly with 75 mg/kg radiolabelled carbaryl with those given a pretreatment of 8000 ppm carbaryl in the diet for 2 weeks followed by a single dose of 75 mg/kg labelled carbaryl was presented in table 2, page 21 of the report. These data showed no significant effect of pretreatment on the percentage of a dose of carbaryl excreted in urine (30.9% in pretreated vs 33.2% non-pretreated) or as exhaled breath (0.003% in pretreated vs 0.005% in non-pretreated) in mice dosed singly vs those pr treated.

b) Food Consumption

Food consumption data showed a marked decrease in those mice receiving test chemical in the diet vs those not receiving test chemical in the diet (Figure 1, page 24 of the report). After 4 days, food consumption returned to normal in group 4 mice, and increased briefly in group 2 mice before returning to control levels by one week after the start of the dietary treatment.

c) Body Weight

Group mean body weight over the course of the study was presented graphically for all groups on page 25 of the report. These data show that for those groups receiving carbaryl in the diet (groups 2 and 4), body weight was decreased over the 2 week period during which test article was administered. Over the 2 week period, group mean body weight appeared decreased anywhere from 5-10 grams vs untreated mice. However, there did not appear to be a corresponding effect on mean liver weight (Figure 4, page 27), despite the decrease in food consumption and body weight for these groups of mice. Relative liver weight as a percentage of body weight was, however, slightly increased (Figure 5, page 28).

d) DNA and Protein Binding of Carbaryl

Results of DNA binding experiments are reproduced below (Table 3 of the report). These data show that interaction of radiolabelled carbaryl with chromatin protein resulted in specif activities from 340-537 dpm/mg or 7-11 pmol/mg protein. Pretreatment of mice with 1143 mg/kg/day carbaryl in the diet showed similar results as for the single dose study. There wa no significant level of binding of carbaryl to DNA in any treated group as shown by the low values for Covalent Binding Index. According to the report (page 18), the data, when converted to the CBI of 0.1, shows that the possible DNA binding capacity of carbaryl is mo than 5 orders of magnitude lower than the value for aflatoxin B_1 and more than 4000 times lower than the CBI for the hepatocarcinogen 2-acetylaminofluorene. However, it is evident that some binding of carbaryl to chromatin protein occurs. The possible deleterious effect of such binding was not discussed in this report.

Table 3
Radioactivity in liver chromatin protein and DNA isolated 24 hours after oral administration o
14-C carbaryl to male mice.

Group No.	1	1	2	2	3	3
Animal No.[a]	1-2	3-4	5-6	7-8	9-10	11-12
Pretreatment[b] [ppm]	no	no	8000	8000	0	0
Chemical dose [mg/kg b.w.]	73.7	73.6	74.0	74.9	0	0
Radioactivity dose [dpm/kg b.w. x 10^{10}]	1.80	1.79	1.80	1.83	0	0
Binding to chromatin protein [dpm/mg]	376.6	537.0	340.3	364.3	32.5	0
[pmol/mg]	7.7	11.0	7.0	7.5	–	–
DNA Amount in vial [mg]	0.85	0.95	0.73	0.57	0.59	0.64
Gross activity [cpm]	11.7	10.7	10.2	9.5	10.0	10.1
Specific activity [dpm/mg][c]	<3.98	<3.57	<4.67	<5.99	<5.78	-
Binding to DNA [CBI units][d]	<0.07	<0.06	<0.08	<0.10	-	-

Footnotes to Table 3

a - The livers of 2 identically treated mice were pooled for DNA isolation.

b - Animals were pretreated for 2 weeks with a diet containing 8000 ppm carbaryl.

c - On the basis of 53 background values compiled over the last 5 years, a respective value 11.8 cpm with a standard deviation of 0.97 cpm was calculated. Assuming that a vial containing DNA from a treated animal with low amount of radioactivity shows the same statistical variation as the background, a limit of detection for radioactivity in a vial can be calculated on a level of 2 S.D. to be 2.7 cpm over the background rate.

d - Covalent Binding Index.

III. CONCLUSIONS

Binding of radiolabel to chromatin protein isolated from the livers of mice treated with a single dose or in pretreated mice was similar (specific activities ranging from 340.3-537.0 dpm/mg). No radioactivity was detectable in DNA samples isolated from mice treated with radiolabelled carbaryl (Covalent Binding Index < 0.1). According to the report, this maximum binding ability of carbaryl is more than 5 orders of magnitude below the Covalent Binding Index of aflatoxin B_1, and more than 4000 times lower than the Covalent Binding Index for 2-acetylaminofluorene. This study demonstrated the interaction of carbaryl with chromatin protein, but no significant interaction with DNA in the liver of male CD1 mice treated with either a single 75 mg/kg dose or in mice pretreated with 8000 ppm (1143 mg/kg/day) carbaryl in the diet followed by a single 75 mg/kg radiolabelled dose. The possible toxicologic significance of the interaction of carbaryl with chromatin protein in this study was not discussed.

IV. CORE CLASSIFICATION: acceptable

This study was not conducted to satisfy a specific guideline requirement, but satisfies the purpose for which it was conducted.