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

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JUN 13 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTAN

MEMORANDUM

SUBJECT: Section 18 Emergency Exemption for the use of DCNA on peanuts in Oklahoma- re-consideration of earlier request (Tox ref. 8-0657, Record no. 220100)

TO: Jim Tompkins
Emergency Response Section
Registration Support and Emergency Response Branch
Registration Division (TS-767)

FROM: Margaret L. Jones M.L. Jones 6/9/88
Review Section III
Toxicology Branch (TS-769)

THROUGH: Marcia van Gemert, Ph.D., Head
Review Section III
Toxicology Branch (TS-679) M. van Gemert 6/9/88

and Theodore M. Farber, Ph.D., Chief
Toxicology Branch (TS-769)

Chemical: DCNA technical; Botran™; Dicloran — Tox. Chem. 311
Record No. 222449 — Tox. Project no.
CAS: 99-30-9 — Registrant NOR-AM
ID: 88-OK-01

Action Requested: Re-consider the Section 18 for the use of Botran™ on peanuts in Oklahoma. This request was denied on May 11, 1988 (Memorandum: Jones to Tompkins) based on continued data gaps. A similar request was denied by Toxicology Branch in 1987 for the same reason.

Background: Portions of data missing when the 1988 request from Oklahoma was considered have been located in Registration Division. These data have been forwarded with a request to determine if they support a Section 18 for peanuts in Oklahoma.

Data Considered Previously:

2-year dog feeding study, NOEL = 2.5 mg/kg/day

Data Reviewed for this request:

1. Ames Salmonella test for induction of reverse mutations:

DCNA technical induced reverse mutations when tested at 5000 and 1500 ug/plate in strains TA 1538 and TA 98 (± S9) and in TA 100 (- S9). DCNA technical did not induce reverse mutations in TA 1535, TA 1537 (± S9) or in TA 100 (+ S9). A repeat assay confirmed the results of the first study.

Study: Acceptable.

2. Chromosomal aberrations in human lymphocytes in culture:

DCNA technical was found to be positive in cells tested without metabolic activation at the mid and high doses (10 and 20 ug/ml) at which chromosomal aberrations were slightly increased over the low dose. In cells tested with metabolic activation the study was negative at all doses.

DCNA technical induced a slight increase in aberrations in human lymphocytes in culture.

Study: Acceptable.

Other Data forwarded with this request:

The 21-day dermal toxicity study is not a requirement for the Section 18. In the interests of a timely response, this study will be reviewed and forwarded as a follow-up to this request.

Data Currently Lacking:

Mouse oncogenicity study
DNA Repair (other genotoxic effects) study
Acute inhalation for technical DCNA

The 21-day dermal study, previously noted as missing, has been received and is under review. This study is not a requirement for an emergency exemption (Sec. 18) and will be forwarded when reviewed. The DNA Repair study has not been received by Toxicology Branch to date. Verbal communication indicates the mouse study is expected in August, 1988.

A search of the files indicates there is no acceptable acute inhalation toxicity study for technical DCNA. There are 21 day inhalation studies in the dog and rat, and a subchronic inhalation study in the rabbit, however these are core grade supplementary. Toxicology Branch would like to know whether this information agrees with other Agency records. If the study is not located within the Agency, the data requirements should be revised to include acute inhalation toxicity or a reason why this study should not be required.

Efforts to Obtain Missing Data: It is apparent from the above that the PM and Registrant are making efforts to obtain the data for Botran. Toxicology Branch will appreciate efforts to locate the acute inhalation toxicity study, recently discovered to be missing from the file.

Effect of tolerance on the Acceptable Daily Intake: The Residue Chemistry Branch will analyze the effect of the requested tolerance on the ADI.

Acceptable Daily Intake: The no observed effect level for the 2-year dog study was 2.5 mg/kg/day. With a safety factor of 100, the ADI is 0.03 mg/kg/day.

Discussion and Conclusions: According to information in a letter from the registrant (NOR-AM to Ms. Rossi, 8 February 1988), a number of uses have been dropped from the label. Emergency Response Section is aware of these changes and will request a revised TAS analysis to determine the percent of the Acceptable Daily Intake (ADI) used by the existing and proposed use. If the TMRC including the proposed use on peanuts (which adds approximately 4.26%) exceeds 100% of the ADI, then it would not be toxicologically supported.

Guideline Series 84: Mutagenicity

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Reviewed by: Margaret L. Jones M. L. Jones 7 June 1988
Section III, Toxicology Branch (TS-769)
Secondary reviewer: Kerry Dearfield, Ph.D. [illegible] 6.8.88
Scientific Mission Support Staff, Toxicology Branch (TS-769)
Date: [illegible] 6/9/88

DATA EVALUATION REPORT

CHEMICAL: 2,6-Dichloro-4-nitroaniline

STUDY TYPE: Salmonella/mammalian activation gene mutation assay

ACCESSION NUMBER: 405088-01

SYNONYMS/CAS NO.: DCNA; Botran™; Technical Dicloran; 99-30-9

SPONSOR: NOR-AM

TESTING FACILITY: Schering Agrochemicals Limited, formerly, FBC Limited, Saffron Walden, Essex, UK

TITLE OF REPORT: T103 TECHNICAL DICLORAN: Ames bacterial mutagenicity assay

AUTHOR: E. Jones, L.A. Fenner

STUDY NUMBER: TOX/87/199-85; TOX 87222; SMS 44/87647

REPORT ISSUED: July, 1987

CONCLUSIONS: Executive Summary: DCNA technical was tested for induction of reverse mutations in S. typhimurium tester strains at 5000, 1500, 500, 150, and 50 ug/plate with and without metabolic activation. DCNA technical induced reverse mutations when tested at 5000 and 1500 ug/plate in strains TA 1538 and TA 98 (± S9) and in TA 100 (- S9). DCNA technical did not induce reverse mutations in TA 1535, TA 1537 (± S9) or in TA 100 (+ S9). Positive controls confirmed the sensitivity of the tester strains. A repeat assay confirmed the results of the first study.

Study: Acceptable.

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SALMONELLA

A. MATERIALS

1. Test Material: Name: 2,6-Dichloro-4-nitroaniline, DCNA (MCPB Acid); Description: yellow powder; Lot No.: CR 20642/3; Purity: 97.5% ± 1.0% w/w (analysis no. T00202); Contaminants, if any, are unknown; Solvents used: dimethyl sulfoxide (DMSO), no lot number;

2. Control Materials:
Negative Control: untreated group with bacteria and buffer but without solvent
Solvent Control: DMSO, amount not specified
Positive Control: known positive controls were used to test each strain with and without S-9 activation, as follows:
2-Aminoanthracene (2 ug/plate) for TA 1535, TA 1537 with S9
2-Aminoanthracene (0.5 ug/plate) for TA 1538, TA 98, TA 100 with S9
2-Nitrofluorene (2 ug/plate) for TA 1538, without S9
2-Nitrofluorene (1 ug/plate) for TA 98, without S9
9-Aminoacridine (80 ug/plate) for TA 1537, without S9
N-ethyl-N'-nitro-N-nitrosoguanidine (5 ug/plate) for TA 1535, without S9
N-ethyl-N'-nitro-N-nitrosoguanidine (3 ug/plate) for TA 100, without S9

3. Activation: S9 derived from Aroclor 1254 induced male rats. As described in the protocol, S9 mix contained 10% (v/v) S9, $MgCl_2$ (8 mM), KCl (33 mM), sodium phosphate buffer pH 7.4 (100 mM), glucose-6-phosphate (5 mM), NADP (4 mM). Cofactors were to be filter-sterilized before use.

4. Test organisms:
TA 98, TA 100, TA 1535, TA 1537, TA 1538 strains of S. typhimurium, laboratory of origin not reported. Tester strains were tested "routinely" for cell membrane permeability and for ampicillin resistance. Subcultures were grown in 'Oxoid' nutrient broth.

5. Test concentrations used:
Preliminary cytotoxicity test:
With and without metabolic activation: 5000, 500, 50, 5 ug/plate
Full mutagenicity assay:
With and without metabolic activation: 5000, 1500, 500, 150, and 50 ug/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay: standard plate assay

a. Protocol: See appended pages 1-4 (pages 16 and 19 of report TOX 87222)

b. Evaluation criteria: The following method of assessment

is found in appended page 3. "The mean number of revertant colonies for all treatment groups is compared with those obtained for negative and positive control groups. The effect of metabolic activation is assessed by comparing the results obtained both in the presence and absence of the liver microsomal fraction for each treatment group.

A compound is deemed to provide evidence of mutagenic potential if (1) a statistically significant dose-related increase in the number of revertant colonies is obtained in two separate experiments, and (2) the increase in the number of revertant colonies is at least twice the concurrent solvent control value."

2. Preliminary cytotoxicity assay Test article was evaluated at concentrations of 5000, 500, 50, and 5 ug/plate in the presence and absence of S9 to determine growth of background lawn and number of revertant colonies. Strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 were tested in this method.

Results: Precipitation was observed at 5000 ug/plate. The test substance was not found to be toxic to the tester strains in the preliminary assay. For this reason, 5000 ug/plate was chosen as the high dose for the full assay.

3. Mutagenicity assay - Test article was evaluated at concentrations of 5000, 1500, 500, 150, and 50 ug/plate with and without metabolic activation. Replicate assays using three plates per dose per strain were used. Negative controls and solvent controls were used for each strain with and without metabolic activation.

Results: Statistically significant results are shown in Table I. Dicloran technical was positive in S. Typhimurium strains TA 1538 and TA 98 with and without metabolic activation and positive in TA 100 without metabolic activation. Positive controls demonstrated the sensitivity of the tester strains and negative (solvent-EtOH) controls showed results similar to concurrent and historical controls. A repeat assay confirmed the results of the first test.

In the original and repeat assays, Dicloran technical was negative for induction of revertant colonies in S. typhimurium strains TA 1535 and TA 1537 with and without S9 and TA 100 with S9.

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4. Toxicology Branch discussion/conclusions:

According to the reported results, DCNA (Dicloran technical) was mutagenic in Salmonella typhimurium tester strains TA 1538 and TA 98 with and without metabolic activation, and TA 100 without metabolic activation. DCNA was not mutagenic in S. typhimurium tester strains TA 1535 and TA 1537 with and without metabolic activation, or in TA 100 with metabolic activation. Positive controls demonstrated the sensitivity of the tester strains. Solvent and negative controls showed no evidence of mutagenicity in these strains.

5. The assay was performed under GLP Regulations and quality assurance and GLP compliance statements were included in the report.

6. CBI appendix not attached.

Table I

Dicloran Technical (DCNA)
Mean Revertant Colony Counts in Salmonella Strains Testing Positive for Induction of Revertants (Ames)

Strain	Dose (ug/plate)	+/- S9	Test I (SD)	Test II (SD)
TA 1538	5000	-	33 (3.5)	51 (7.2)
	1500	-	21 (4.0)	29 (10.7)
	500	-	16 (1.2)	29 (5.5)
	150	-	15 (2.5)	24 (2.5)
	50	-	10 (2.6)	11 (3.5)
	0	-	9 (2.0)	8 (2.1)
	solvent	-	11 (4.4)	10 (1.5)
	5000	+	37 (13.0)	43 (8.2)
	1500	+	19 (1.7)	43 (6.7)
	500	+	8 (1.0)	22 (0.6)
	150	+	11 (1.7)	10 (4.9)
	50	+	7 (3.8)	10 (1.5)
	0	+	8 (2.0)	9 (3.5)
	solvent	+	10 (3.2)	10 (2.3)
TA 98	5000	-	48 -	84 (9.0)
	1500	-	71 (7.2)	76 (21.0)
	500	-	50 (7.0)	60 (1.5)
	150	-	47 (2.6)	38 (10.5)
	50	-	29 (6.1)	25 (2.6)
	0	-	29 (7.5)	33 (4.7)
	solvent	-	29 (7.8)	34 (4.9)

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Strain	Dose (ug/plate)	+/- S9	Test I (SD)	Test II (SD)
TA 98	5000	+	47 (6.5)	89 (8.1)
	1500	+	65 (12.0)	73 (11.7)
	500	+	35 (6.2)	40 (8.5)
	150	+	22 (4.4)	27 (1.2)
	50	+	21 (4.9)	19 (4.0)
	0	+	16 (2.6)	21 (2.1)
	solvent	+	21 (2.0)	24 (6.6)
TA 100	5000	-	185 (19.0)	199 (26.6)
	1500	-	166 (28.5)	154 (10.5)
	500	-	109 (20.1)	95 (4.6)
	150	-	87 (9.1)	86 (12.4)
	50	-	80 (7.0)	93 (5.1)
	0	-	93 (10.5)	74 (14.6)
	solvent	-	74 (2.5)	84 (13.0)

DCNA science review

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Pages 9 through 12 are not included in this copy.

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___ Description of the product manufacturing process

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Guideline Series 84: MUTAGENICITY

Reviewed by: Margaret L. Jones M.L. Jones 8 June 1988
Section III, Toxicology Branch (TS-769C)
Secondary reviewer: Kerry Dearfield, Ph.D.
Scientific Mission Support Staff, Toxicology Branch (TS-769C) 6/8/88
Date:

DATA EVALUATION REPORT

CHEMICAL: 2,6-Dichloro-4-nitroaniline

STUDY TYPE: Mammalian cells in culture chromosomal aberration

ACCESSION NO.: 405088-02

SYNONYMS/CAS NO.: DCNA; Botran™; Technical Dicloran; 99-30-9

SPONSOR: NOR-AM

TESTING FACILITY: Huntingdon Research Centre
Huntingdon, Cambridgeshire, England

TITLE OF REPORT: T105 TECHNICAL DICLORAN: Metaphase chromosome analysis of human lymphocytes cultured in vitro

AUTHORS: Allen, J. et. al.

STUDY NUMBERS: TOX/87/199-188; SMS 43/871138; TOX 87225

REPORT ISSUED: January 20, 1988, Study completed.

CONCLUSIONS: Executive Summary: DCNA was tested in human lymphocytes in culture at 20, 10, and 2 ug/ml, with and without metabolic activation. Two tubes were used at each dose level and four for controls. Positive controls demonstrated the sensitivity of the test system.

In cells tested without metabolic activation the study was positive at the mid and high doses (10 and 20 ug/ml). The results were slightly increased over the low dose.

In cells tested with metabolic activation the study was negative at all doses.

DCNA technical induced a slight increase in aberrations in human lymphocytes in culture.

Study: Acceptable.

MAMMALIAN CELLS IN CULTURE: ABERRATIONS

A. MATERIALS

1. Test Material: Name: 2,6-Dichloro-4-nitroaniline acid (DCNA) technical grade; yellow powder, stability unreported, purity 97.5% (w/w), impurities not reported; Batch no.: CR 20642/3 Contaminants: not reported; Solvent: dimethylsulfoxide (DMSO),

2. Control Materials:
Negative: untreated cultures with and without metabolic activation (S9)
Solvent: Dimethylsulphoxide (DMSO), 10 ul/ml, with and without metabolic activation (S9); sterile distilled water
Positive: without activation: ethylmethanesulfonate (EMS), 500 ug/ml
Positive: with activation: cyclophosphamide, 20 ug/ml

3. Activation: S9 derived from: Aroclor 1254 induced rat liver homogenate from CD rats of Sprague-Dawley origin; S9 mix was 0.1 ml S-9 fraction, 0.02 ml $MgCl_2$ (0.4 M), 0.5 ml Na_2HPO_4 (0.2 M), 0.005 ml glucose-6-phosphate (1.0 M), 0.04 ml NADP (0.1 M), 0.335 ml Distilled water; the ingredients were mixed and then filter-sterilized.

4. Test cells: mammalian cells in culture: Human lymphocytes were separated from human blood (collected aseptically and diluted with tissue culture medium) by centrifugation 'Histopaque-1077). After three washes and sedimentation at 200 x 'g' for 10 minutes cells were suspended at 1.15×10^6 cells per ml for the preliminary toxicity test and 1.07×10^6 cells per ml for the main metaphase analysis study. Cells were suspended in RPMI 1640 + 20% fetal calf serum (Gibco) + 2% phytohaemagglutinin (Wellcome). Cells were incubated at 37°C in humid atmosphere with 5% CO_2 for 48 hours.

For the preliminary cytotoxicity assay:
After incubation, 250 ul of S9 were added to one set of cultures with 12.5 ul of various dilutions of test substance. Final concentrations of test compound were 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, and 20 ug/ml. After two hours cells with S9 were centrifuged, pellet discarded, resuspended in fresh medium and incubated for an additional 22 hours.

For the main metaphase analysis assay:
After incubation, 1.25 ml S9 were added to one set of cultures with 50 ul aliquots of test substance for final concentrations of 2, 10, and 20 ug/ml. The remaining procedure was similar to that described above and next.

After 70 hours incubation, colchicine was added to each culture to arrest mitotic activity. After 2 hours incubation the cell suspensions were made hypotonic, incubated 10 minutes and centrifuged for 10 minutes at 110 x 'g'. Cell pellets were 'fixed' with 3 parts methanol: 1 part glacial acetic acid. Prior to slide preparation, pellets were aspirated through a hypodermic needle, centrifuged at 200 x 'g' for 10 minutes and resuspended in a small volume of fixative. Spreads of cells were made by

dropping 2-3 drops of cell suspensions onto precleaned slides, then allowed to air-dry. Slides were stained using Giemsa solution and mounted in DPX.

5. Examination of cells: Cells were examined for mitotic index using light microscope at 160X magnification. Metaphase figures were calculated from number of metaphase cells and total number of nuclei. The criteria for selection of dose levels for the metaphase analysis were as follows:

a. High dose was the concentratior of test compound expected to cause a 50% reduction in mitotic index compared with concurrent solvent control value, or
b. Where no appreciable reduction in mitotic index was observed, the maximum soluble concentration was used.
c. Mid and low doses were 50% and 10% respectively of the high dose.

6. Test compound concentrations used:

Preliminary cytotoxicity assay:
With and without S9: 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.50, 5, 10, and 20 ug/ml

Mutagenicity assay:
With and without S9: 2, 10, and 20 ug/ml

B. TEST PERFORMANCE

1. Cell treatment:

Cell treatment appears in section A.4. Test cells. The procedure for examination of cells and counting appears in this section.

2. Protocol: see Appended pages 1-3 (pages 13-15 from report No. TOX 87223).

3. Preliminary cytotoxicity assay: The highest concentration tested in the preliminary assay did not cause reduction in mitotic index compared with concurrent controls, therefore, 20 ug/ml was chosen as the high dose for the main assay, and 10 and 2 ug/ml were selected to be the mid and low doses for the assay.

4. Mutagenicity assay and study author's conclusions: Results appear in Appended pages 4-5 (pp 19-20 from report No.: TOX 87223). Positive controls confirmed the sensitivity of the target cells.

MAMMALIAN CELLS IN CULTURE: ABERRATIONS

Study authors found positive controls statistically significantly increased the incidence of metaphase anomalous figures when compared to solvent controls. In the assay without metabolic activation,

slightly higher incidences of chromosomal damage were observed at 20 and 10 ug/ml. These increases were statistically significant according to the Fischer's exact test. (10 ug/ml, $p<0.05$, 20 ug/ml, $p<0.01$). The numbers were small and the authors concluded they were not increased over historical controls (0-5.25%) and therefore did not indicate clastogenic activity.

Study authors concluded technical dicloran was not mutagenic at any dose with metabolic activation.

5. Reviewer's discussion/ conclusions: In cells without metabolic activation there was a statistically significant increase in mean percent of aberrant cells at the mid and high doses. The increases at 10 and 20 were 1.5 ($p<0.05$) and 2.5 ($p<0.01$) respectively in relation to concurrent controls. The authors concluded the results were not significant based on historical controls. However, the historical data were not included in the study report and lacking such information, the study must be judged as reported. The study is slightly positive at 10 and 20 ug/ml.

6. This assay was performed under GLPs with Quality Assurance statements.

7. CBI appendix not attached.

DCNA science review

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Pages 17 through 21 are not included in this copy.

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