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

003583

MEMORANDUM

CASWELL 165A

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Henry Jacoby, PM#21
Registration Division (TS-767)

FROM: Irving Mauer, Ph.D.
Geneticist, Section V
Toxicology Branch/HED (TS-769)

THRU: William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: VITAVAX (carboxin). Review of studies in response to Registration Standard data gaps. Action: 661
CASWELL#165A

Accession #'s: 251655
251656
251657
251658

Registration No.: 400-81

Registrant: Uniroyal Chemical
Bethany, CT

Action Requested: Review the following mutagenicity studies submitted in response to data gaps identified in the Carboxin RS:

(1) Mutagenicity Evaluation of Technical Grade VITAVAX Lot #965 98+% A.I. in the Ames Salmonella/Microsome Plate Test (Acc. #251655), performed by Litton Bionetics, Kensington, MD, Report #LBI-20895, September, 1982.

(2) Evaluation of Vitavax Techical Grade in the Primary Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Assay (Acc. #251656), performed by Litton Bionetics, Kensington, MD. Report #LBI-20991, October, 1982.

(3) Mutagenicity Evaluation of Techical Grade Vitavax Lot #956, 98+% A.I. in an in vitro Cytogenetic Assay Measuring Chromosome Aberration Frequencies in Chinese Hamster (CHO) Cells (Acc. #251657), performed by Litton Bionetics, Kensington, MD, Report #LBI-20990, September 14, 1982.

(4) in vivo Bone Marrow Chromosome Study in Rats (Acc. #251658), performed by Hazleton Laboratories America, Report #HLA298-199, July 29, 1983.

TB Evaluation: A summary of reported results and TB evaluation (DATA REVIEWS Attached) of these studies follows:

Study	Acc. #	Result	Classified
(1) Ames	251655	Negative	Acceptable
(2) UDS	251656	Positive	Acceptable
(3) in vitro cytogenetics	251657	Positive	Acceptable
(4) in vivo cytogenetics	251658	Negative	Not Acceptable

TB Recommendations: In view of the valid positive results for cytogenetic and DNA damage (studies 2 and 3), but negative in bacteria, Toxicology Branch strongly recommends a gene mutation assay in mammalian cells (e.g., L5178Y, or CHO, or V79, inter alia).

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Caswell No.: 165A
Shaughnessy No.: 090201-5
Registration No.: 400-81

Chemical: VITAVAX (carboxin)

Study Type: Mutagenicity: Reverse Mutation in Bacteria

Citation: Mutagenicity Evaluation of Technical Grade Vitavax Lot #965, 98+% A.I. in the Ames Salmonella/Microsome Plate Test.

Accession No./MRID No.: 251655/NA

Sponsor/Contracting Lab.: UniroyalChemical/Litton Bionetics, Inc.

Report No./Date: LBI #20895/September, 1982

Test Material: Technical grade Vitavax, Lot #956, 98+% a.i., a pale-yellow powder, dissolved in DMSO for assay.

Procedures: Cultures of Salmonella typhimurium strain TA-100 were tested at two-fold increments of test compound from 1.22 to 10,000 ug/plate for dose selection. From toxicity and solubility results of this preliminary test, mutagenicity assays were performed at 7 doses ranging from 1 through 5000 ug/plate (3 plates per dose) in repeat tests on strains TA-1535, TA-1537 and TA-100 (with single tests on TA-1538 and TA-98), both in the absence (i.e., directly) and presence of metabolic activation (MA) provided by the S-9 fraction of Aroclor-stimulated hepatic microsomal enzymes from a SD adult male rat, plus regenerating co-factors (D-G-6-P, $MgCl_2$, KCl, $NaPO_4$). Concurrent controls consisted of solvent (negative); and bacterial mutagens specific to each strain (NaAz, 2-NF, 9-AAc) for non-MA tests, and 2-AAnth for all strains in activated tests (positive controls). No statistical analyses were performed.

Results: The preliminary toxicity test revealed the test substance to be toxic at 2500 ug/plate and higher, accompanied by precipitation. No increase over control in revertents were observed for any Vitavax-treated strain at any dose level, either in non-MA or activated tests. The responses in positive control plates ranged from 10-85x those in DMSO controls.

The authors concluded that: "Technical grade Vitavax ..... did not exhibit genetic activity ..... and was considered not mutagenic under these test conditions according to (our) evaluation criteria" [Test material producing a positive response equal to 3 times the normal background solvent values for strains TA-1535, TA-1537 and TA-1538; twice control for TA-98 and TA-100, accompanied by the demonstration of dose-related increases in revertents.]

TB Evaluation: ACCEPTABLE DATA (Negative for reverse gene mutation in the Ames Salmonella/Microsome Assay.)

TOXICOLOGY BRANCH: DATA REVIEW

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Caswell No.: 165A
Shaughnessy No.: 090201-5
Registration No.: 400-81

Chemical: VITAVAX (carboxin)

Study Type: Mutagenicity: UDS in Rat Hepatocytes

Citation: Evaluation of Vitavax Technical Grade in the Primary Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Assay.

Accession No./MRID No.: 251656/NA

Sponsor/Contracting Lab.: Uniroyal Chemical/Litton Bionetics, Inc.

Report No./Date: LBI #20991/October, 1982

Test Material: VITAVAX Technical (Lot #956, 98+% a.i.), dissolved in DMSO for the assay.

Procedures: Hepatocytes from an adult male F344 rat (80% viability by Trypan Blue exclusion) were cultured for 5 hr in WME, then exposed for 18 hrs to 9 concentrations of test substance ranging from 0.513 ug/ml to 256 ug/ml in approximately two-fold steps, plus a constant 1 uCi/ml tritiated thymidine; counted to estimate cell survival (relative to negative control), and then processed for measuring UDS by conventional procedures. Stained, coded, coverslip preparations were scored for "net nuclear grain counts" (= nuclear grain count less the average number of grains in 3 nuclear-sized background areas adjacent to each nucleus) for 50 randomly selected cells per preparation (150 total nuclei for 3 coverslips). The averages for each treatment were compared to those from the concurrent solvent control (DMSO). The mutagen, 2-AAF, known to be active in this assay, was run concurrently as a positive control.

Results: Test material was toxic at the HDT (3.2% cell survival), less so in a dose-responsive manner at 103 ug/ml (19.4% survival), 51.3 ug/ml (37.3% survival) and 25.6 ug/ml (67.6% survival), but not toxic at levels of 10.3 ug/ml and below (90%+, and normal cell morphology).

The minimal criteria for determining a positive result in this assay (mean net nuclear grain count exceeding 6.26; and/or at least 10% of nuclei containing 6 or more net grains; and/or at least 2% of the nuclei containing 20 or more net grains) were achieved or exceeded in a dose-dependent response for 5 treatment levels of Vitavax, in the range 5.13 to 103 ug/ml, an increase clearly evident in the absence of moderate to severe toxicity. The positive control (2-AAF) performed as expected, thus indicating this population of hepatocytes was considered to have normal responsiveness.

The authors concluded that: "..... Technical Grade Vitavax induced significant increases in the nuclear labeling of primary rat hepatocytes for an applied concentration range of 5.13 ug/ml to 103 ug/ml. Treatment with 256 ug/ml was excessively toxic. The increases in nuclear labeling were dose-related, and all three minimum criteria used to indicate UDS were exceeded for the moderately toxic treatment with 51.3 ug/ml. The test material was therefore evaluated as active in the Primary Rat Hepatocytes UDS Assay."

TB Evaluation: Acceptable Data (Positive for UDS in rat hepatocytes).

TOXICOLOGY BRANCH: DATA REVIEW 003583

Caswell No.: 165A
Shaughnessy No.: 090201-5
Registration No.: 400-81

Chemical: VITAVAX (carboxin)

Study Type: Mutagenicity: Chromosome Aberrations in vitro (CHO Cells)

Citation: Mutagenicity Evaluation of Technical Grade Vitavax, Lot #956, 98% A.I. in an in vitro Cytogenetic Assay Measuring Chromosome Aberration Frequencies In Chinese Hamster Ovary (CHO) Cells.

Accession No./MRID No.: 251657/NA

Sponsor/Contracting Lab.: UniroyalChemical/Litton Bionetics, Inc. Kensington, MD 20895

Report No./Date: LBI #20990/September 14, 1982

Test Material: VITAVAX Technical (Lot #956, 98% a.i.), diluted in DMSO for the assay.

Procedures: Cultures of CHO (strain WBI) were exposed to test material at half-log concentrations ranging from 17 to 1670 ug/ml (limit of solubility in aqueous medium = 3.3 mg/ml), both in the absence and presence of a metabolic activation system (MA) provided by Aroclor-stimulated rat liver microsomal enzymes (S9 fraction) plus co-factors (NADP + isocitrate). Controls were run concurrently: negative (no additions); solvent (1% DMSO); mitomycin-C (MC) for non-activated cultures, and cyclophosphamide (CP) for activated cultures, as positive controls. After incubation (10 hr. without MA; 2 hr with MA), colcemid was added to all cultures to arrest cells in metaphase, followed by hypotonic treatment (0.075 M KCl), fixation (3 parts methanol: 1 part acetic acid), and coded microscope slides made. (5% Giemsa strain). Separate (replicate) experiments were run with and without MA. Standard "blind" scoring procedures were used (100 cells per dose), and both structural and numerical aberrations recorded and analyzed statistically (against solvent control) by Student's t-test.

Results: Suppression of cell growth (as evidenced by dose-dependent reduced confluence) occurred at all dose levels in non-MA cultures, and doses above 500 ug/ml were toxic. No significant increase in aberration induction, however, was found at any dose up to toxic levels, in contrast to the significant amount and severity of chromosomal damage induced by the positive control (MC). In a second experiment without MA, a range of toxic doses (400-1200 ug/ml)

also resulted in strong suppression of mitotic activity (data not provided), such that no cytogenetic scores could be obtained at doses above 500 ug/ml (confirming the negative result of the first trial).

In the presence of MA, cytotoxicity (reduced confluence, and mitotic inhibition) also limited the scoring of metaphase cells to doses of 500 ug/ml and more in the first trial. Again, in contrast to a positive result effected by the indirect mutagen, CP, there was no significant induction of aberrations by the test compound up to the toxic level. In a repeat experiment employing a series of toxic dose levels (400-1400 ug/ml), readable metaphases could be scored at all doses except the HDT, and a slight but not significant increase in aberrations recorded at both 600 and 1200 ug/ml, but not at 800 ug/ml.

A third trial with MA in the narrow range, 500 to 900 ug/ml (at 50 ug/ml intervals), revealed a dose-related reduction in cell growth (almost complete lethality at 850 and 900 ug/ml), as well as highly significant increases ($p < 0.01$) in aberrations at all doses above 650 ug/ml, as well as the appearance of a chromosome-type break level (and some complex re-arrangements) not heretofore observed in either historical lab controls or in these Vitavax-treated cultures, a large proportion of which confined to the long arm of the X-chromosome. Even in the absence of the X-chromosome damage, positive results ($p < 0.01$ for aberrations per cell) were still recorded at all doses except at 700 ug/ml and the highly toxic 900 ug/ml.

The authors concluded that: "Vitavax is positive in the test for chromosome aberrations with metabolic activation. No chromosome damage was detected in the test without activation under the conditions of this assay."

TB Evaluation: Acceptable Data (Positive with MA).

TOXICOLOGY BRANCH: DATA REVIEW

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Caswell No.: 165A
Shaughnessy No.: 090201-5
Registration No.: 400-81

Chemical: VITAVAX (carboxin)

Study Type: Mutagenicity: Chromosome aberrations in rats

Citation: Final Report
In Vivo Bone Marrow Chromosome Study in Rats with VITAVAX

Accession No./MRID No.: 251658/NA

Sponsor/Contracting Lab.: Uniroyal Chemical Company/Hazelton Laboratories America, Inc.
Vienna, Virginia

Report No./Date: HLA 298-199/7-29-83

Test Material: Off-white solid designated VITAVAX, 98%, suspended in 0.5% CMC for oral gavage administration.

Procedures: Groups of 20 male (average weight = 300 g) and 20 female (200 g) SD-CD rats (age not stated) were dosed orally once with test substance at 200, 600 and 2,000 mg/kg (HDT = 50% of the LD_{50}, reported by the sponsor in a separate study involving "albino rats" as 3820 mg/kg p.o., but no data presented here). Sub-groups of 5 male:5 female were sacrificed 6, 12, 24 and 48 hours after acute administration. A comparable group was administered vehicle and sacrificed at the same time intervals, while 5 male:5 female were given 40 mg/kg cyclophosphamide (CP) orally and killed 24 hours later (positive control). Femoral bone marrow was collected from sacrificed animals 2 hours following administration of the mitotic-arresting agent, colchicine (single dose i.p., 2 mg/kg), cells processed into microscope slide preparations according to standard techniques (2 slides per animal), coded blind, and 50 metaphases per animal scored for both structural and numerical aberrations. Mean mitotic indices and modal numbers, percent aberrant cells and mean number of aberrations per cell for each group were statistically analyzed by Kruskal-Wallis non-parametric ANOVA and all pair-wise group comparisons.

Results: Only very mild clinical effects ("depressed", rough coat, urine stains) but no deaths were observed in a few animals of the mid- and high-dose groups, and slight to moderate dose-dependent body weights losses (none apparently statistically significant, according to the report) observed at these levels in groups sacrificed 24- and 48 hours post-treatment.
No statistically significant increases in the frequency of

chromosomal aberrations were found, however, at any of the four sampling times in animals treated with test substance at any dose, when compared to vehicle control values, in contrast to expected increases in CP-treated animals (P = 0.002 for percent aberrant cells; P = 0.002 for average number of aberrations per cell). Neither were any statistically significant differences recorded in modal number of chromosomes per cell (2 n = 42 in the rat) in any Vitavax-treated group, nor between mean mitotic indices of any test group and vehicle control.

Hence, the authors concluded that: "..... under the conditions of this study, VITAVAX is considered not to be clastogenic at any of the dose levels tested."

TB Evaluation: UNACCEPTABLE DATA. Although the procedures and reporting are adequate, this study does not qualify as a comprehensive assay of the potential of the test substance to induce chromosome aberrations, mainly due to: (i) insufficient dosage to induce clinical toxicity in the animals, or cytotoxicity in the target cells; and (ii) lack of a multiple dosage schedule to assay cumulative effects.