9-30-91

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

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SEP 30 91

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: PP#9F03775/FAP#9H05583 - Quinclorac - Use of FACET® Herbicide on Rice - Submission of Addendum/ Responses Regarding a Rabbit Developmental Toxicity Study (#90/5091), Two-Generation Reproduction Study (#88/0321), and Several Mutagenicity Studies (#88/5520, 86/0214, 86/0371, 86/0018, and 90/0008)

Shaughnessy No.: 128974
TOX Chem No.: 325A
Project No.: 1-1323
Submission No.: S396352, S396354, S396356

FROM: William B. Greear, M.P.H. William B Greear 9/3/91
Review Section IV, Toxicology Branch I
Health Effects Division (H7509C)

TO: Vickie Walters/Robert J. Taylor, PM Team 25
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Marion P. Copley, D.V.M., Section Head Marion Copley 9/19/91
Review Section IV, Toxicology Branch I
Health Effects Division (H7509C)

I. CONCLUSIONS

BASF's comments on several toxicological studies have been considered and two new studies have been reviewed by Toxicology Branch I (TB-I). The studies have been classified as follows:

	Study	Study No.; Date	Classification
83-3	Developmental Toxicity (Rabbit)	88/0099; 4/5/88	Minimum Data[1]

	Study	Study No.; Date	Classification
84-2	Mutagenic (CHO/HGPRT)	90/0008; 10/26/89	Acceptable[1]

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84-2	Mutagenic (Micronucleus)	86/0018; 2/3/86	Unacceptable
84-2	Mutagenic (Struct. Chrom. Aberr.)	86/0371; 11/25/86	Acceptable[1]
84-2	Mutagenic (Ames)	88/0358; 8/18/88	Acceptable[1]
84-2	Mutagenic (CHO/HGPRT)	86/0214; 7/18/86	Unacceptable
84-2	Mutagenic (Struct.) Chrom. Aberr.)	87/0005; 1/9/87	Unacceptable

[1] - fulfills current regulatory requirements

[DERs of the two new studies are attached. The conclusions made based on additional information/comments provided by BASF on previously reviewed toxicological studies have been responded to by TB-I and are presented herein in lieu of supplemental DERs.]

II. ACTION REQUESTED

Under a cover letter dated May 15, 1991, Bob Rohde of the BASF Corporation has submitted a response to the deficiencies TB-I noted in the two-generation reproduction study (BASF #88/0321, 7/21/88) and food consumption data (in German - not translated). Mr. Rohde indicated that the food consumption data will be translated and submitted by June 15, 1991. In addition, BASF's submitted responses to the deficiencies noted in the rabbit developmental toxicity study (BASF #88/0099) and four mutagenicity studies (BASF #86/0013, 2/3/86; 86/0371, 11/25/86; 88/0358, 8/18/88; 86/0214, 7/18/86) and submitted two new mutagenicity studies (BASF #90/0008, 10/26/89; 87/0555, 1/9/87).

III. DISCUSSION

The following new mutagenicity studies were submitted for evaluation. The DERs are attached.

1. Report on the Study of a Point Mutation Test Carried Out on CHO Cells (HGPRT Locus) of Reg. No. 150 732 (BAS 514). Dr. R. Jackh. October 26, 1989. BASF Reg. Doc. No. 90/0008. pp. 30.

   The study is acceptable.

2. Comparative in vitro Cytogenetics Investigations in Human Lymphocytes with Reg. No. 150 732, batch CH 384 121 and Reg. No. 150 732. Dr. G. Englehardt. Jan. 9, 1987,. BASF Reg. Doc. No. 87/0555. pp. 22.

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The study is unacceptable.

The sponsor has submitted responses to deficiencies noted in TB-I's review of one developmental toxicity study and several mutagenicity studies. The responses will be discussed below study by study.

3. Report on the Study of the Prenatal Toxicity of Reg. No. 150 732 in Rabbits after Oral Administration (gavage) (BASF #88/0099, 4/5/88). MRID No. 416805-01-Addendum; BASF #90/5091; 9/26/90. This takes the place of a DER supplement. See HED DOC# 81 61 for original DER

Dynamac Comment No. 1: Data on the stability and homogeneity of dosing levels should have been submitted.

BASF Response: Data on the stability of dosing solutions have been submitted in this submission.

TB-I Response: The stability data are acceptable. The data show that after approximately 2 months the concentrations of the 70,200 and 600 mg/kg/day dosing solutions ranged from 68, 187-191, and 571-574 mg/kg/day, respectively. TB-I notes that homogeneity data were not submitted; however, this is not essential.

Dynamac Comment No. 2: Individual data for food consumption, number of corpora lutea, and fetal sex were not submitted.

BASF Response: The data mentioned above were only kept as raw data. The new data has been used to generate the appropriate tables and are enclosed.

TB-I Response: The data are acceptable and adequately reflect the results that were summarized in the initial submission.

Dynamac Comment No. 3: Too few animals were evaluated in the high-dose group.

BASF Response: None.

TB-I Response: This is a minor deficiency. Occasionally, toxicity is observed in the high- dose group; however, sufficient data were available at lower doses to determine a NOEL. The highest dose level was a LEL for developmental effects.

Dynamac Comment No. 4: The study was conducted according to OECD GLPs and not EPA's GLPs.

BASF Response: None.

TB-I Response: The study appears to have been well conducted and is acceptable.

The study is classified as Minimum Data and the following NOELs and LELs are established:

Maternal NOEL = 70 mg/kg/day

Maternal LEL = 200 mg/kg/day (based on decreased body weight gain and food consumption)

(Additional signs observed in the maternal rabbits at 600 mg/kg/day were increased water consumption, increased mortality, and discoloration of the kidney.)

Developmental NOEL = 200 mg/kg/day

Developmental LEL = 600 mg/kg/day (based on an increase in resorptions and post-implantation loss, a decrease in the number of live fetuses and decreased body weights.)

4. Report on the Reproduction Study with Registration No. 150 732 in Rats; Continuous Dietary Administration Over Two Generations (Two Litters in the First and One Litter in the Second Generation (BASF #88/0321, 7/21/88)). MRID No. 418742-01 and 02-Addendum. The deficiencies that the sponsor has responded to in the submission will be discussed under Project No. 1-1530 which contains the remainder of the data/information submitted on the 2-generation production study.

5. BASF's responses to the deficiencies in previously reviewed mutagenicity studies are provided in an attachment from I. Mauer dated 8/31/91.

IV. DATA REQUIREMENTS (40 CFR 158.340) TERRESTRIAL FOOD-USE

Quinclorac No. 325A
Updated - September, 1991

Facet[R] Herbicide	Required	Satisfied
Formulation		
81-1 Acute Oral Toxicity	Y	Y
81-2 Acute Dermal Toxicity	Y	Y
81-3 Acute Inhalation	Y	Y
81-4 Primary Eye Irritation	Y	Y
81-5 Primary Dermal Irritation	Y	Y
81-6 Dermal Sensitization	Y	Y
Technical		
81-1 Acute Oral Toxicity	Y	Y
81-2 Acute Dermal Toxicity	Y	Y
81-3 Acute Inhalation Toxicity	Y	Y
81-4 Primary Eye Irritation	Y	Y
81-5 Primary Dermal Irritation	Y	Y
81-6 Dermal Sensitization	Y	Y
81-7 Acute Delayed Neurotoxicity (Hen)	N	-
81-8 Acute Neurotoxicity (Rat)	R	-
82-1 Subchronic Oral (Rodent)	Y	Y
82-1 Subchronic Oral (Nonrodent)	Y	Y
82-2 21-Day Dermal	Y	W
82-3 90-Day Dermal	N	-
82-4 90-Day Inhalation	N	-
82-6 28-Day Neurotoxicity (Hen)	N	-
82-7 90-Day Neurotoxicity (Rat)	R	
83-1 Chronic Toxicity (Rodent)	Y	Y
83-1 Chronic Toxicity (Nonrodent)	Y	Y
83-2 Carcinogenicity (Rat)	Y	Y
83-2 Carcinogenicity (Mouse)	Y	Y
83-3 Developmental Toxicity (Rat)	Y	Y
83-3 Developmental Toxicity (Rabbit)	Y	Y
83-4 Reproduction	Y	Y
83-6 Postnatal Developmental Toxicity	R	-
84-2 Mutagenicity - Gene Mutation	Y	Y
84-2 Mutagenicity - Structural Chromosomal Aberration	Y	Y
84-4 Mutagenicity - Other Genotoxic Effects	Y	Y

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Technical	Required	Satisfied
86-1 General Metabolism	Y	Y
86-2 Domestic Animal Safety	N	-
86-3 Dermal Penetration	N	-
86-4 Visual System Studies	N	-

Y = Yes; N = No; W=Waived; R= Reserved;
[1] = The 1-year chronic study in dogs is acceptable in lieu of the subchronic study.

[All toxicological data requirements have been satisified at this time.]

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8613

Reviewed By: Irving Mauer, Ph.D., Geneticist 07/18/91
Toxicology Branch I, IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief 7/22/91
Toxicology Branch I, IRS (H7509C)

DATA EVALUATION RECORD 008613

I. SUMMARY

MRID No.: 41680503
ID No.: 9F03755
RD Record No.: S-396354
Caswell No.: 325A
Project No.: 1-1323-M

Study Type: (84-2) Mutagenicity - Gene mutation in mammalian cells in vitro (CHO/HGPRT)

Chemical: Quinclorac

Synonyms: FACET Herbicide

Sponsor: BASF, RTP (NC)

Testing Facility: BASF AG, Ludwigshafen-am-Rhein (FRG)

Title of Report: Report on the Study of a Point Mutation Test Carried Out on CHO Cells (HGPRT Locus) of EPA Registration No. 150732 (BAS 514).

Author: R. Jackh

Study Number: 90-0008

Date of Issue: October 26, 1989

TB Conclusions:

Negative for inducing forward mutation at the HGPRT locus of CHO cells exposed to concentrations producing severe toxicity (2000 ug/mL), with or without metabolic activation.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - Reg. No. 150732 (BAS 514 . . . H) (quinclorac technical)

Description: Crystalline white powder
Batch (Lot): N57 Tox Charge III$_{-2}$
Purity (%): 97.38
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism: Established mammalian cell strain

Species: Chinese hamster ovary (CHO)
Strain: K1 (HGPRT$^{+/-}$)
Source: Flow Labs, Meckenheim (FRG)

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of quinclorac at the hypoxanthine-guanine phosphoribosyl transferase locus (HGPRT) when administered in vitro to cultures of Chinese hamster ovary (CHO) cells, according to established (referenced) published procedures, as well as OECD Guideline No. 476 for this type of assay.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice was provided.

D. Procedures/Methods of Analysis - Employing doses determined in an earlier CHO/HGPRT study, replicate monolayer cultures of heterozygous (HGRPT) CHO cells (cleansed of spontaneous HGPRT $^{-/-}$ mutants by HAT treatment) were exposed for 4 hours to test substance, both in the absence and presence of a mammalian metabolizing enzyme system consisting of the microsomal (S9) fraction of livers from Aroclor 1254 pretreated male SD rats, supplemented with NADP(H)-generating cofactors (S9-mix). In addition to solvent (DMSO) controls, additional sets of cultures were treated with the mutagens bromdeoxyuridine (BrdU, 50 ug/mL) and 3-methylcholanthrene (MCA, 10 ug/mL), to serve as positive controls for, respectively, the nonactivated (-S9) and activated (+S9) test series.

After 8 days subculturing (to allow expression of induced mutants), all cell cultures were exposed to 6-thioguanine (TG) for a further 7 days in order to select for TG-resistant colonies presumed to be HGPRT $^{-/-}$ mutants (and kill all other HGPRT cell types). The cultures were then

Stained (methanol/Giesma) cells were scored for cloning efficiency (CE, a measure of cytotoxicity) as well as TG-resistant mutant colonies.

Two separate trials were run (EXPERIMENTS 1 and 2). Criteria for both assay acceptance (parameters of expressed values), as well as evaluation of response (positive/negative/equivocal), were presented.

E. Results - In neither experiment did the test substance induce any increases over solvent controls in numbers of presumed HGPRT concurrent mutants, or exceed the upper value for the background mutation rate for this cell strain (15×10^{-6}), even at severely toxic concentrations (see Report Tables attached to this DER). In contrast, both (positive) mutagens were clearly positive, inducing mutation rates averaging 10 to 30 times negative/solvent controls.

The investigator concluded that quinclorac technical was not mutagenic in these assays.

F. TB Evaluation - ACCEPTABLE under assay conditions considered adequate to generate valid results.

Attachments (Report Data Tables)

Attachment

(Data Tables)

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Pages 12 through 15 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.

✓ FIFRA registration data.

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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.

Reviewed By: Irving Mauer, Ph.D., Geneticist 7/19/91
Toxicology Branch I, IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief 7/22/91
Toxicology Branch I, IRS (H7509C)

DATA EVALUATION RECORD 008613

I. SUMMARY

MRID No.: 41680504
ID No.: 9F03755
RD Record No.: S-396354
Caswell No.: 325A
Project No.: 1-1323-M

Study Type: (84-2) Mutagenicity - Chromosome damage in vitro (human lymphocytes)

Chemical: Quinclorac

Synonyms: FACET Herbicide

Sponsor: BASF, RTP (NC)

Testing Facility: BASF AG, Ludwigshafen-am-Rhein (FRG)

Title of Report: Comparative in vitro Cytogenetic Investigations in Human Lymphocytes with Registration No. 153732, batch CH384 121, and Registration No. 150732, batch N32.

Author: G. Engelhardt

Study Number: 87-0005

Date of Issue: January 9, 1987

TB Conclusions:

Positive for chromosome aberrations at high (cytoxic) concentrations (1000 ug/mL) in human lymphocyte cultures, but only without activation.

Classification (Core-Grade):

UNACCEPTABLE, due to discrepancies in reporting; as well as lacking preliminary cytotoxicity testing, assays with metabolic activation, and many procedural details; also, only one dose level tested.

II. DETAILED REVIEW

A. Test Material - Registration No. 150732

Description: White crystals
Batches: (1) CH 384 21; (2) N_{32}
Purity (%): (1) Not given; (2) 96.5
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Blood cells (lymphocytes), drawn fresh from a single donor.

Species: Volunteer blood donor
Age: (Unstated)
Source: (Unstated)

C. Study Design (Protocol) - This study was designed to assess the clastogenic (structural chromosome-damaging) potential of quinclorac when administered in vitro to cultures of human lymphocytes, according to procedures (referenced publication, and OECD Guidelines) for this type of assay.

A Statement of Quality Assurance measures (inspections/audits) was provided. A Statement of Good Laboratory Practice was also provided.

D. Procedures/Methods of Analysis - Based on the results of a previous study with Batch N32 of the test article (increased chromosome damage at 1000 ug/mL without activation) in the same test system, duplicate cultures of human lymphocytes were exposed to a single dose of quinclorac [for an unstated length of time], and harvested 24 later. In addition to untreated and solvent (DMSO) controls, mitomycin-C (MC, 0.05 ug/mL) served as positive control.

Two to three hours prior to harvest, Colcemid (1.33 ug/mL) was added to arrest mitosis at metaphase, following which the cells were prepared for microscopy on glass slides by conventional cytological methods for cytogenetic analysis. Dried slide preparations were stained (Giesma/Titrisol) and sealed under synthetic mounting medium. One hundred metaphases per culture on coded slides were scored according to the conventional cytogenic array of chromosome aberrations for gaps, as well as for both structural (single and complex) as well as numerical (aneuploidy/polyploidy) changes. Group aberration data were analyzed for significance by Fisher-Yates. Mitotic indices (percent metaphases among 1500

cells/culture) were calculated for all groups, to provide a measure of cytotoxicity.

E. Results - Data were summarized (Report Table 1), but as well results from individual cultures of all test groups were presented (Report Tables 2 to 6).

Compared to background control values of 3.5 to 4.0 percent chromosomally aberrant cells including gaps but only 0.5 to 1.5 percent aberrant metaphase excluding gaps, 1000 ug/mL of quinclorac resulted in significant ($p < 0.01$, Fisher-Yates) increases in chromosome aberrations whether calculated with (23.5% and 18.0%) or without (14.5% and 9.0%) staining gaps (Table 1, attached). Slightly greater damage was recorded in MC-exposed (positive control) cultures.

Mitotic indices in test culture did not appear to have been affected, as indicated by the following tabulation presented in the text (Section 4.2 of the Final Report):

Test Groups	1st Culture	2nd Culture	Mean
Untreated control	10.13	11.47	10.80
Solvent control 0.1 mL DMSO/mL	7.80	8.67	8.27
1000 ug/mL Batch CH 384 121	9.33	10.80	10.07
1000 ug/mL Batch N_{32}	6.67	7.13	6.93
Mitomycin C	1.40	1.60	1.53

The author concluded that quinclorac was positive for chromosome aberrations, as confirmed by comparable values in assays with two different batches of the test material (ruling out the presence of mutagenic impurities). However, as discussed in the report from the previous study, he reiterates his concern that the cause for the increased clastogenicity may represent effects secondary to "special culture conditions, i.e., cytotoxicity and low solubility of the test substance."

F. TB Evaluation - Although confirming a presumptively

positive result in a previous assay with another batch of the test article in the same test system, the inadequate and incomplete reporting of this study renders it UNACCEPTABLE, because of major procedural deficiencies:

1. It is not clearly apparent in this Final Report how many assays with which batch of test material are reported herein.

2. No preliminary cytotoxicity (or solubility) tests were performed to support the author's disclaimer.

3. Only one dose was programmed.

4. No assays with metabolic activation were performed, nor any results with such activation alluded to.

Attachment (Report Summary Data Table)

ATTACHMENT

(Summary Table)

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Pages _____ through _____ are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

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Irving Mauer, Ph.D.
(H7509C)

Quinclorac

(Company responses to previous DERs)

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(1) Mouse Micronucleus (Study No. 86/0018) MRID 410635-29
Response 10/31/90

The study author reported no increased formation of micronuclei in bone marrow cells from male and female mice treated acutely by oral gavage with test material at doses up to 2000 mg/kg (animal toxicity encountered), at sampling times up to 48 hr post-dose (HDT only).

Dynamac judged this study unacceptable for failing to include a HDT-72 hr post-dose harvest (to detect any micronuclei following severe mitotic delay).

The Company responded by citing publications by both the EEC Directive (79/831, Annex V, Part B-12, November 1989) and the Gene-Tox Program (MUT. RES. 239:29-80, 1990), the first of which indicated that sampling later than 48 hrs is not necessary, while the second revised an earlier (1983) Gene-Tox Micronucleus Report, and recommended the last sample be no later than 60 hr after final treatment. Further, no mitotic delay was apparent in this study, as evidenced by PCE/NCE ratios within the normal (background) range.

EPA (IM) Appraisal of Company Response - While the peak formation of chemically induced micronuclei in bone marrow PCEs usually occurs between 24 and 48 hrs after treatment, in certain cases it may be as late as 72 hrs (or later) post-dose, for example, with 7,12-dimethybenzanthracene (Salmone et al., MUT. RES. 74:347-356, 1980), for which maximum responses were repeatedly reported not sooner than 55 to 60 hrs after treatment (see summary of DMBA studies in Ashley and Mirkova, ENVIRON, MOLEC. MUTAG. 10:297-305, 1987). The reason suggested for such peak activity later than 48 hrs post-dose was the slow absorption of the chemical and transport to the target, as well as a requirement for metabolic activation to mutagenic derivatives of such indirect mutagens. Therefore, whereas the sampling recommendations of some expert panels (above citations) brought forward by BASF are less rigorous, others (notably, for example, the ASTM Task Group on the Micronucleus Test, chaired by J.T. MacGregor of the USDA, 1987 et seq.) continue to advise sampling up to 72 hrs, the maximum also authorized by the EPA Toxic Substances Control Act Health Effects Test Guidelines (40 CFR Part 798.5395).

The request for upgrading this study is denied, not only for the reason (failure to sample up to 72 hrs post-treatment) given by the contract reviewer (as cited above for the Dynamac evaluation), but additionally because:

1. The assay did not even provide analysis of the final sampling time recommended by one of the company's cited sources (the revised 1990 Gene-Tox Report), namely, 60 hr.

2. No evidence was provided that the orally administered test substance even at clinically toxic doses, or its metabolic products, were absorbed from the gut and transported in sufficient quantities to the target, bone marrow cells, to produce any effect (cytotoxic, or mutagenic).

Hence, this study remains UNACCEPTABLE.

(2) Chromosome Aberrations in Human Lymphocytes (Study 86/0371) MRID 410761-03 Rayens 10/31/90

The study author reported that the test article (analytical-grade quinclorac, purity > 98%) induced significant increases in structural chromosome aberrations, whether calculated with or without (staining) gaps, and both in the presence and absence of metabolic (rat liver S9) activation, (but) only at the highest scorable concentrations (1000 ug/mL/-S9; 2000 ug/mL/+S9), which were also cytotoxic (depression of mitotic index).

The Dynamac reviewer judged the study unacceptable due to "the lack of definitive result" and recommended a repeat assay, preferably "performed with new donor cells" in which appropriate pH measurements should be included, and any variations during the experiments be corrected. [pH changes alone have been demonstrated to cause aberrations, and this chemical has been reported to cause acidic changes in a previous UDS assay at doses of 253 ug/mL and above.]

In the Company response, BASF noted that the culture medium used in its assay (designated "1a," from GIBCO) contains two indicators one of which would turn the medium yellow below pH 6.5, and the other red above 7.8. The investigators recorded no color changes during the conduct of the aberration assay, ". . . Therefore, in contrast to the UDS test, it can be observed that there was no significant change in pH values . . . ." Further, the Company's respondent stated that the positive results have since been confirmed in a repeat assay with technical grade quinclorac (batch N_{32}) [submitted as Project No. 30M03 83/8368, report dated January 17, 1987].

EPA (IM) Appraisal of Company Response:

The additional information submitted by BASF is sufficient to upgrade the study to ACCEPTABLE, in demonstrating that the purified (analytical) grade of the test article is clastogenic but only at toxic doses.

(3) Bacterial Mutation (Ames/E.coli) (Study 88/0358) MRID 401635-28 (BASF Addendum #88155JC, 8/18/88; MRID #416605-02)

The study report recorded negative results for the induction of reversions in the standard battery of Ames strains of Salmonella typhimurium, or in E.coli WP2-uvrA cultures exposed under nonactivated or S9-activated conditions, to soluble concentrations of test article up to the limit dose (5000 ug/plate), which also proved to be nontoxic.

The Dynamac reviewer, however, judged the study unacceptable because the activated series was conducted with "an excessive concentration of S9 liver homogenates in the S9 Mix (30%)," and recommended a repeat with the conventional screening concentration of S9 (4% of the Mix), as recommended by Maron and Ames (1983). As well, the report lacked results for chemical analysis on test material solutions.

The BASF response insisted that the concentration of S9 was in line with Tox. Method 005 of the Ecological and Toxicological Association of Dyestuff industry (as indicated in the ETAD protocol attached to this rebuttal). Further, since quinclorac is "not readily metabolized in several animal species" (as documented by several metabolism studies submitted to the Agency), a higher S9 concentration was felt to be needed in order to enhance the production of any active metabolites.

Additionally, accompanying its response the company submitted analytical data regarding stability of the test article in both DMSO and water, which indicates that the test article is stable (retains 99 to 101% of initial concentration) over the period of the assay (48 hr).

The Agency's (IM) appraisal (and rereview) tends to agree with the company's rebuttal, insofar as the positive controls responded appropriately to the more potent S9 with values (10 to 70 times solvent control), comparable to those achieved with the conventional S9 concentrations (which may go as high as 10 percent, as recommended by the Maron/Ames publication cited by the Dynamac reviewer). Additionally, we no longer require concentration control analyses of all working solutions for such in vitro short-term testing; the stability data submitted with the current company response suffices.

Hence, this study can be upgraded to ACCEPTABLE, and a repeat assay is not needed.

(4) CHO/HGPRT Forward Mutation (Study 86/0214) MRID 41[illegible]51-0[illegible]

The study reported that repeat experiments (three) of 4-hr exposures of nonactivated CHO cells to six concentration of test article in (aqueous) culture medium ranging from 46.4 to 2150 ug/mL in each trial resulted in repeated lethality at the HDT (i.e. no mutant colonies recovered from cultures exposed at higher doses, 464 and 1000 ug/mL), but nondose-related mutant frequencies ranging from approximately 5 to 30 X 10^{-6} (corrected for cytotoxicity) at lower doses (46.4 to 215 ug/mL). No mutants were induced in the single series of activated CHO cultures exposed to the same schedule of test doses. From these results the study author concluded that the test material was not mutagenic in this test system.

The Dynamac reviewer judged the study unacceptable, mainly due to the inability to interpret the scatter of presumptively positive results in the nonactivated assays at lower doses, and attributes this to the poor solubility of quinclorac in the solvent selected, namely, an aqueous culture medium. Additionally, the study author failed to correct the limited data generated for cytotoxicity. Finally, the single activated assay (reportedly with zero mutants) falls because an "unacceptably excessive" strength of S9 liver homogenate in the S9 Mix (30%) was employed. Finally, neither a GLP nor QAS statement was provided in the Final Report.

Company's Respondent acknowledged the low solubility of the test article in an aqueous medium (resulting in poor control of accurate concentration levels), but lamented ". . . there is no other way to proceed in an in vitro assay" [sic!]. The respondant also admits to fore[illegible]g QA/GLP adherence, but promises to correct this in a repeat to be submitted to the Agency. Finally, the use of 30 percent [illegible] was justified on the basis of other published literature (notably Li, 1984[1]), plus the fat that the positive control (3-MCA) responded appropriately.

The Agency (IM) Appraisal: That the study author can confess an inability to employ a suitable solvent for this test article is amazing, considering that other in vitro assays evaluating quinclorac's potential for mutagenicity were conducted appropriately (in DMSO). Hence, we agree with the Dynamac review

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[1] A.P. Li: "Use of Arochlor 1254-indued rat liver homogenate of promutgens in Chinese Hamster ovary cells." Environ. Mutagnesis 6, 539-544 (1984).

with reference to both her judgment of the nonactivated portions of the study ("difficult to interpret;" "inappropriate solvent"), but would add that the S9 series also failed to include a repeat.

This study remains unacceptable.