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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: November 29, 2001

MEMORANDUM

SUBJECT: **Acifluorfen:** Response to BASF's **Phase 3 Comments** on the Preliminary Risk Assessment Document for Sodium Acifluorfen.

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INTRODUCTION:

The registrant, BASF, submitted a Phase 3 response to the Preliminary Risk Assessment Document for Sodium Acifluorfen. The registrant's comments have been reviewed. Each comments relative to issues of health risk assessment is addressed in detail below.

DISCUSSION:

Human Health Risk Assessment

Health Effects Division Chapter for the Reregistration Eligibility Decision Document

1. Submission of 5 new studies and information needed to upgrade a mouse lymphoma assay:

BASF Comments:

In the memorandum that precedes the table of contents for this chapter, it is noted that a subchronic mouse study and hepatic cell proliferation study have been submitted. It is also be noted that three Ames assays and information needed to upgrade a mouse lymphoma assay have also been submitted. The MRID numbers assigned to the Ames assays are MRID 45393902, 45323501 and 45393901. The information needed to upgrade the mouse lymphoma assay has not been assigned an MRID number.

HED Response:

A subchronic mouse study (4-week feeding study; MRID 45323503) has been reviewed [See Toxicology Review (TXR) No. 014642 dated July 24, 2001] and the executive summary is presented in the Appendix.

A hepatic cell proliferation study in Primary Mouse Hepatocytes (MRID 45323502) has been reviewed (See TXR No. 014638 dated July 24, 2001) and the executive summary is presented in the Appendix.

The Ames assay (MRID 45323501) has been reviewed (See TXR No. 014640 dated July 24, 2001) and the executive summary is presented in the Appendix.

The Ames assay (MRID 45393901) has been reviewed (See TXR No. 014641 dated July 24, 2001) and the executive summary is presented in the Appendix.

The Ames assay (MRID 45393902) has been reviewed (See TXR No. 014641 dated July 24, 2001) and the executive summary is presented in the Appendix.

The Mouse Lymphoma Study (MRID No. 01227390) was previously reviewed by HED however, the study was classified unacceptable (TXR No. 003556, dated 1/26/1984) because mutation frequencies and positive controls were not available. The supporting data (dated February 20, 2001) to Mouse Lymphoma Study was previously submitted also (February 28,

1984). The data was already reviewed by HED (TXR No. 003963 dated 9/17/1984) and HED found that the supporting data was satisfactory. The study was **reclassified as Acceptable and satisfies the guideline data requirement for a mutagenicity study (84-2).** It was concluded that acifluorfen did not induce forward mutation either with or without metabolic activation.

2. Page 3. Rat Developmental Toxicity Study:

BASF Comments:

The Agency states that a developmental neurotoxicity study in the rat is required based on increased incidence of dilated lateral ventricles of the brain in the rat developmental toxicity study (MRID 00122743). The reviewer states that this effect indicates that administration of the compound results in neurotoxicity.

First, it should be noted that dilated ventricles were described in the EPA reviews and summaries of this study as only "slightly dilated." Second, the slightly dilated ventricles are not likely to be adverse effects, but are more likely secondary to decreased fetal body weight... Decreases in fetal body weight are often associated with delayed development...This indicates that it is the decreased fetal body weight, not the compound exposure, which results in slightly dilated ventricles of the brain.

HED Response:

It is not clear whether there is a relationship between decreased fetal weight and slightly dilated ventricles of the brain. However, neurotoxicity in the form of clinical signs (such as decreased motor activity) and slightly dilated lateral ventricles of the brain was observed in the developmental toxicity study in rats. Therefore, **the Hazard Identification Assessment Review Committee (HIARC) recommended a developmental neurotoxicity study in order to further define the neurotoxic potential in the developing fetus**.

3. Q1* Calculation:

BASF Comments:

A. Non-genotoxic mechanism of action has been demonstrated for the mouse liver tumors observed with acifluorfen. Therefore, a threshold (MOE) approach should be used for risk assessment.

NOTE: BASF presented a position paper on this issue to EPA (from K. Blundell, BASF to Ms. Christina Scheltema, Chemical Review Manager, SRRD, MRID No. 45323500 dated Feb. 2, 2001). A summary of that petition is presented as Appendix 1 in the phase 3 comments.

B. The Agency has calculated a Q1* of 5.33×10^{-2} $(mg/kg/day)^{-1}$. This was based upon male mouse liver tumors (adenomas and carcinoma combined). The dose levels used

were 0, 29, 62 and 157 mg/kg/day with liver tumor incidence values of 9/58, 21/60, 16/56 and 40/59 from control to high dose, respectively.

BASF has calculated the Q1* value and determined a value of 1.4×10^{-2}. The discrepancy between the methods used by BASF and the Agency is in the dose levels. The actual reported test material intake for males were 119, 259 and 655 mg/kg/day. The Agency has erroneously reduced the reported dose levels by 24% to correct for the test material being a 24% aqueous solution of acifluorfen, resulting in doses for the Q1* assessment of 29, 62, and 157 mg/kg/day.

Further support for the dose levels being sodium acifluorfen is given in the analytical verification method in Appendix E of the report. The 240 g/L (240 mg/mL) aqueous solution was used as the stock solution. From that, 2 dilutions were made, 1:100 to produce a 2.4 mg/mL solution and 1:1000 to produce 0.24 mg/mL solution. The 2 dilutions were used to prepare diets. For example, to obtain a concentration of 2400 ppm in feed, 1 mL of 2.4 mg/mL stock solution was added to 1 g of feed (see Table below). Therefore, the test diet reflected levels of sodium acifluorfen and not the diluted aqueous solution. [Note: In the BASF's comments, errors were made in units. Therefore, both 2.4 mg/L and 0.24 mg/L were corrected as 2.4 mg/mL and 0.24 mg/mL, respectively in the above paragraph.]

Concentration of Stock solution (mg acifluorfen/mL)	**Amount of Stock solution added (mL)**	**Amount of Feed (g)**	**Concentration of acifluorfen in Feed (mg acifluorfen/g)**	**Concentration of acifluorfen in Feed (ppm)**
2.4	**1**	**1**	**2.4**	**2400**

When analytical verifications were made, the feed levels were compared against the standards above and the reported results reflected actual sodium acifluorfen levels. Therefore, the dose levels as given in the report (625, 1250 and 2500 ppm) should be used as actual sodium acifluorfen. As given in the RESULTS section of the report, these feed concentrations resulted in average sodium acifluorfen intakes of 119, 259 and 655 mg/kg/day from the low to high dose males, respectively. These values should be used for the dose levels for Q1* calculations and no adjustment was needed. When the above higher daily intake values were used, the Q1* calculation resulted in a lower value, 1.4×10^{-2} $(mg/kg/day)^{-1}$.

HED Response:

A. BASF's petition entitled "**Sodium Acifluorfen: Request for reassessment of current cancer classification considering peroxisome proliferation associated mode of action**" dated Feb. 2, 2001 was submitted to EPA. The data on proposed mechanism of action were considered at the preliminary meeting of the Mechanism of Toxicity Assessment Review Committee (MTARC), HED on Aug. 23, 2001 to see if a full meeting for acifluorfen is warranted.

There were, however, weaknesses in the database. For example, primary rat hepatocytes exposed to acifluorfen did not indicate an increased peroxisome number by transmission electron microscopy (TEM). The Committee concluded that peroxisome proliferation may be a possible mode of action for acifluorfen-induced liver tumors in rats and mice. However, because of shortcomings in the database, the available information was insufficient to support this proposed non-genotoxic mode of action for acifluorfen. Therefore, MTARC concluded that a new mechanism study involving TEM analysis would be valuable to evaluate the possible **peroxisome proliferation associated mode of action** for acifluorfen. BASF was informed on the deficiency of the database to support MOE approach (e-mail to R. Hawks from B. H. Chin, dated 9/12/01). BASF agreed to initiate a new study to resolve this issue. Therefore, MTARC **this issue will be considered when the data from the new mechanism study with acifluorfen become available.**

B. This reviewer re-evaluated the chemical analysis section of the study report and found the registrant's comments on the dose levels were valid. It was recommended that the dose levels of 119, 259 and 655 mg/kg/day for male mice be used for Q1* calculation.

4. FQPA Safety Factor (Pages 3 and 4)

BASF Comments:

References are made to the fact that acifluorfen demonstrated increased susceptibility to offspring in the rat teratology study. However, as discussed above under Point 2 and below under "Toxicology Chapter," Point 7, the results of this study and other toxicology studies indicate that maternal toxicity was likely understated and the developmental toxicity was likely overstated in this study. In addition, the developmental effects are likely secondary to growth delays observed in fetuses, as evidenced by reduced body weights, and do not represent frank developmental toxicity... Therefore, there is no evidence of increased susceptibility to offspring. Based on the lack of susceptibility to offspring, the FQPA safety factor should be removed for both chronic and acute risk assessments.

HED Response:

The current thinking is that when adverse effects are seen at the same dose level in the fetuses/offspring as in the parental animals and the effects in the fetuses/offspring are different from those seen in the parental animals and/or considered to be more severe, the result is described as **qualitatively increased susceptibility** (*Current Approach to Recommending the FQPA Safety Factor for Use in Human Health Risk Assessment;* Brenda S. Tarplee and Edward Zager; HED, OPP, US EPA; Presented at the California Pesticide Residue Workshop, March 2000*).* In this study, the developmental toxicity (decreased fetal body weight and increase in anatomical variations including dilated lateral ventricles of the brain) was seen in the presence of **minimal maternal toxicity** (clinical signs including excessive salivation and piloerection) at the same dose. According to the current policy, HIARC concluded that there is qualitative evidence of increased susceptibility following *in utero* exposure to acifluorfen in the prenatal developmental toxicity study in rats. **The HIARC can not discount the increased incidence of dilated lateral ventricles of the brain because historical data for the incidence were not available.**

Subsequently, the FQPA Safety Factor committee recommended a 10X safety factor be retained when assessing acute dietary and short/intermediate-term residential (non-occupational) exposures for females 13-50. Since there was a datagap for a developmental neurotoxicity study, the FQPA safety factor was reduced to 3X when assessing chronic dietary and long-term residential (non-occupational) exposures for females 13-50 and infants and children subgroups.

5. Safety factor and values of acute population adjusted dose (aPAD) and of chronic population adjusted dose (cPAD) (Page 4)

BASF Comments:

A. Based on the discussion above, the acute population adjusted (aPAD) should use only a 100 X safety factor, resulting in a value of 0.2 mg/kg/day.

B. As discussed in Point 8 under the Toxicology Chapter, a more supportable NOAEL for the chronic population adjusted dose (cPAD) would be 7.5 mg/kg/day from the chronic dog study. No FQPA safety factor is needed, so the cPAD would be 0.08 mg/kg/day.

HED Response:

A. As discussed above, there were data to suggest that there was a qualitative increase in susceptibility and the FQPA safety factor should be retained at 10X when assessing acute dietary and short/intermediate-term residential (non-occupational) exposures for females 13-50. The value of aPAD (0.02 mg/kg/day) was calculated accordingly.

B. The Agency disagree that a more supportable NOAEL for the chronic population adjusted dose would be 7.5 mg/kg/day from the chronic dog study.

The parental NOAEL from the two-generation rat reproduction study is 1.25 mg/kg/day based on kidney lesions, characterized predominantly by dilatation of tubules in the outer medulla, in females of both generations.

As a matter of science policy of the HIARC, HIARC concluded that **1.25 mg/kg/day is considered the lowest NOAEL in the most sensitive species** (HIARC report, page 6)**,** considering all multiple dose oral studies with acifluorfen.

Therefore, NOAEL for the chronic population adjusted dose is 1.25 mg/kg/day from the 2-generation reproduction study in rats. No FQPA safety factor is needed, so the cPAD would be 0.013 mg/kg/day.

6. Q1* value. (Page 4)

BASF Comments:

It is BASF's position that a quantitative low dose extrapolation is not appropriate for acifluorfen...there appears to be errors in the Agency's calculation (see Point 3 above).

HED Response:

See point 3 above.

7. Two-Generation Reproduction Study (Page 10, Table 2)

BASF Comments:

For the two-generation reproduction study, the NOAEL for offspring toxicity is given as 1.25 mg/kg/day. The NOAEL in this study should be 50 mg/kg/day as discussed in detail under "Toxicology Chapter" Point 5.

HED Response:

See Point 5 under "Toxicology Chapter"

8. aPAD and cPAD (Page 11, Table 3)

BASF Comments:

The acute and chronic PAD's should be adjusted as discussed above. The NOAEL used for short-term and intermediate-term dermal exposure should be 300 mg/kg/day from the 21-day dermal toxicity study in rabbits. This is discussed in detail under "Toxicology Chapter" Point 8.

HED Response:

See Point 8 under "Toxicology Chapter".

9. Dietary Risk for Water (Pages 21 and 22)

BASF Comments:

DWLOC values should be recalculated with a new Q1* and should be compared to new estimated environmental concentrations (EECs) in ground water.

HED Response:

DWLOC values will be recalculated using a Q1* value of 1.27×10^{-2} in human equivalents (memo from Lori Brunsman, 11/8/01, Doc 0050263. The EECs will be provided by the Environmental Fate and Effects Division.

Toxicology Chapter

1. Ames assays (Page 4)

BASF Comments:

An Ames assay using the pre-incubation procedure is requested. This study was recently submitted and been assigned MRID No. 45393902. Additionally, two other Ames assays were submitted with this study and have been assigned MRID Nos. 45323501 and 45393901).

HED Response:

All three Ames assays (MRID 45393902, 45323501 and 45393901) have been reviewed.

See Point 1 of this document under Health Effects Division Chapter for the Reregistration Eligibility Decision Document section.

2. Oral LD50 (Page 5, Table 1)

BASF Comments:

The acute oral toxicity in rats is given as 1540 mg/kg for the 40% a.i. This value contradicts the value presented in the "HED Chapter for the Reregistration Eligibility Decision" document (page 7) which gives the rat oral LD50 for the 20.2-23.35% a.i. material as 2025 mg/kg (males) and 1370 mg/kg (females). The latter study is more recent and gives both male and female data. It is suggested that the LD50 data on Tackle (20.2-23.25% a.i.) be used consistently in

both documents. This would also be consistent with the use of Tackle for the remainder of the acute toxicity testing categories.

HED Response:

The Agency agrees with the registrant. The LD50 value in the Toxicology Chapter will be replaced with the value presented in the "HED Chapter for the Reregistration Eligibility Decision" document (page 7) which gives the rat oral LD50 for the 20.2-23.35% a.i. material as 2025 mg/kg (males) and 1370 mg/kg (females).

3. Subchronic Toxicity in Mice (Page 8)

BASF Comments:

Subchronic toxicity study in mice (Accession No. 00252826; MRID No. is not assigned) is classified as "Unacceptable/guideline but upgradeable". No reason is given for the "unacceptable" classification.

HED Response:

The subchronic toxicity study in mice (Accession No. 00252826; MRID No. is not assigned) is classified as "Unacceptable/guideline but upgradeable". The unacceptable classification is due to lack of the individual histopathology data as described in Tox Review (TXR) No. 003963 dated 9/17/87. The missing data was not submitted to the Agency. Until the individual animal histopathology has been submitted and evaluated, this study remains as "Unacceptable/guideline but upgradeable".

4. Mouse Oncogenicity Study (MRID No. 00122732) (Pages 10-11)

BASF Comments:

A. It should be added to the review that the high dose (2500 ppm) in this study exceeded the MTD for both male and female animals. In males there was a statistically significant increase in mortality and a body weight decrease of 25% compared to controls at week 79. In females, there was a body weight decrease compared to controls of 34% at week 79. Mortality is certainly an indication that the dosing was too high, and body weight differences of greater than 20% exceed the MTD criteria.

B. It should be also noted that the stomach papillomas observed in males and females were significantly increased over control only at the high dose. Although stomach papillomas can ben induced in rodents, they are not found in humans because of anatomical differences between the stomach of the human and the rodent. Therefore, the stomach papillomas should not be considered in risk assessments for acifluorfen. This topic is discussed in a position paper that was submitted to the Agency on

February 2, 2001 (BASF Reg. Doc. 2001/5000878) and is also discussed in Appendix 2 in the phase 3 comments.

C. The liver tumors in this study are likely due to a threshold, non-linear MOA....

HED Response:

A. The highest dose level tested (2500 ppm) in males appeared to be slightly excessive; however, the highest dose tested in females was considered to be adequate in the evaluation of carcinogenic potential of the test chemical. Based on this observation, Cancer Peer Review Committee concluded that Acifluorfen was associated with statistically significant positive trends for liver tumors (adenomas, carcinomas, and adenomas/carcinomas combined) and stomach tumors (papillomas) in both sexes (see the Carcinogenicity Peer Review Committee Report, TXR No. 007698, dated March 17, 1988).

B. A relatively high incidence of uncommonly occurring benign stomach papillomas were observed in B6C3F1 mice of both sexes at the highest dose level tested. However, the Agency did not consider the stomach tumor observed in the mice in the risk assessments for acifluorfen. The calculation of the Q* for acifluorfen was based on the liver tumors in mice.

C. See Point 3 under Health Effects Division Chapter for the Reregistration Eligibility Decision Document

5. Reproductive Toxicity Study in Rats (MRID 00155548) (Pages 15 and 16)

BASF Comments:

A. In the HIARC report (page 13) and the HED RED Chapter (page 10) the doses for this study are described as being 0, 1.25, 25 and 125 mg/kg/day. The Registrant believes the doses as described in the Toxicology Chapter (0, 2.5, 50 and 250 mg/kg/day) are correct.

B. It is stated that in the F2 generation, the incidence of pups dying between lactation days 1 and 4 was significantly increased for the mid and high dose groups when compared to controls. However, the difference from control at the mid dose (500 ppm) is considered spurious and not related to treatment. Pup mortality from day 1 to 4 for the mid dose group was 3% compared to the control value of 1%. While this increase was statistically significant, the Registrant does not believe that an increase from 1 to 3% is biologically significant and may only represent random variation. This point of view is supported by the fact that a 3% incidence is similar to the test facility historical control data. The LOAEL is also based on dilated renal pelvis at this dose. Pup/litter incidence of dilated renal pelvis (control to high dose): 3/2; 4/4; 8/3; 29/13.... As there were no treatment-related effects in offspring at the mid dose of 500 ppm in this study(MRID 00155548), 500 ppm should be considered the NOAEL for offspring toxicity for this study.

C. The registrant encourages the Agency to also consider data from a separate three-generation study that has been submitted to EPA (MRID 00122745)...

HED Response:

A. In this 2-generation reproduction study (MRID No.00155548), Tackle "2S" (21.1-21.6% a.i.) was administered to Crl:COBS CD (SD) BR Rats (35/sex/dose) in the diet at levels of 0, 25, 500 or 2500 ppm. The doses as described in the Toxicology Chapter (0, 2.5, 50 and 250 mg/kg/day) are based on a conversion factor of 1 ppm = 0.1 mg/kg/day. Since the Agency has been using the conversion factor of 1 ppm in food equals 0.05 mg/kg/day for the adult rats, the correct doses for this reproduction study are 0, 1.25, 25 and 125 mg/kg/day (based on a conversion factor of 1 ppm = 0.05 mg/kg/day) as shown in the HIARC report (page 13) and the HED RED Chapter (page 10).

B. In the second generation, the incidence of pups dying between days 1 and 4 of lactation was **significantly increased ($p < 0.01$)** for the mid- and high-dose groups when compared to the control group. Since pup mortality from day 1 to 4 for the mid dose group was 3% compared to the **concurrent control value** of 1%, it is considered biologically significant even though 3% incidence is similar to the test facility historical control data. In addition, increased incidence of kidney lesions (dilated renal pelvis) was observed at the mid dose. Therefore, the NOAEL for offspring toxicity for this study should be 25 ppm (1.25 mg/kg/day) and not 500 ppm (25 mg/kg/day) as suggested by BASF.

C. The Agency did not consider data from a separate three-generation reproduction study that has been submitted to EPA (MRID 00122745; Gulf South Research, Project No. 413-987-41, dated 4/29/83). The Agency review (see TXR No. 003723, dated 8/11/87) stated that the study was found to be "INVALID" because numerous deficiencies were found. Throughout the report, numerous reporting errors, inconsistencies, and omissions were noted. In addition, reproductive performance of the control animals was so poor during the first two generations of the study there was no baseline data available for comparisons to the treated animals. Also an extremely high incidence of cannibalization among control and treated animals were reported. These problems had an adverse impact on the interpretation of this study and the data were not reliable.

6. Mutagenicity Study (Page 17)

BASF Comments:

The report states that acceptable genetic toxicology studies indicate that sodium acifluorfen was weakly positive in a few assays and negative in the remainder. BASF believes that when all the genotoxicity data collected for acifluorfen are considered, the weight of the evidence indicates that the compound is not genotoxic. BASF has presented a position paper on this issue (BASF Reg. Doc. 2001/5000878) and has also recently submitted three Ames assays

(MRID 45393902, 45323501 and 45393901). Additionally, information requested by the Agency to upgrade MRID 00148272/00122740 has been also been submitted (MRID not assigned).

HED Response:

Genetic toxicology studies indicate that sodium acifluorfen was weakly positive in a few assays and negative in the remainder. Based on the weight-of-the-evidence analysis the Cancer Review Committee concluded that the acifluorfen was positive for mutagenicity activity in tests in insects (MRID 00122737; TXR 003556) and yeast (MRID 00148272; TXR 003556) (see the Carcinogenicity Peer Review Committee Report, TXR No. 007698, dated March 17, 1988).

All three Ames assays (MRID 45393902, 45323501 and 45393901) have been reviewed.
See Point 1 under Health Effects Division Chapter for the Reregistration Eligibility Decision Document

7. FQPA Considerations/Uncertainty Factor (Page 20)

BASF Comments:

A. ...However, BASF believes that the factor should be reduced to 3X for assessing the chronic dietary and long-term residential (non-occupational) exposures for the Females 13-50 and the Infants and Children subgroups.

B. Thus, there is no evidence of increased sensitivity for offspring and no need for an additional safety factor. The FQPA safety factor should be removed for both short-term and chronic assessments.

HED Response:

A. As described on page 20 of the Toxicology Chapter, the factor was already reduced to 3X for assessing the chronic dietary and long-term residential (non-occupational) exposures for the Females 13-50 and the Infants and Children subgroups.

B. See Point 4 under Health Effects Division Chapter for the Reregistration Eligibility Decision Document

8. Table of Doses and Toxicological Endpoints Selected for Various Exposure Scenarios (Page 21, Table 2)

BASF Comments:

A. The chronic dietary non-carcinogenic NOAEL is given as 1.25 mg/kg/day from the two-generation rat reproduction study... In the chronic toxicity study in rats, the NOAEL was 25 mg/kg/day... Considering all multiple dose oral studies with acifluorfen, the NOAEL of 7.5 mg/kg/day in the chronic dog study should be used for the chronic RfD.

B. The Agency has calculated a Q1* value for acifluorfen of 5.33×10^{-2} based on male mouse liver tumors. ..the actual Q1* based on these tumors is 1.4×10^{-2}...MOE approach should be used for cancer risk assessment...

C. For dermal risk assessment considerations, it is more appropriate to use a route to route comparison. The NOAEL for systemic toxicity in a 21-day dermal toxicity study in rabbits was 300 mg/kg/day. This should be used for dermal risk assessments.

HED Response:

A. The Agency disagree that the NOAEL of 7.5 mg/kg/day in the chronic dog study should be used for the chronic RfD. **The parental NOAEL from the two-generation rat reproduction study is 1.25 mg/kg/day based on** kidney lesions, characterized predominantly by dilatation of tubules in the outer medulla, in females of both generations. This toxicity endpoint **is considered the lowest NOAEL in the most sensitive species** (HIARC report, page 6)**,** considering all multiple dose oral studies with acifluorfen.

B. See Point 3 under **Health Effects Division Chapter for the Reregistration Eligibility Decision Document**

C. A 21-day dermal toxicity study in rats (MRID No. 00122731) is available. However, the HIARC selected the oral developmental toxicity study because the concern for the developmental effects were also seen in the rats. In addition, the fetal effects are not evaluated in the dermal toxicity study and thus the consequences of these effects of concern cannot be ascertained with the dermal route of exposure. Also, the developmental effects are presumed to occur after a single or multiple doses during the dosing period (12 days) which is appropriate for this exposure period of concern (1-7 days).

EXECUTIVE SUMMARY:

In this 4-week subchronic oral toxicity study (MRID 45323503), acifluorfen (83.9% a.i., Lot/batch # 347-77-12) was administered continuously in the diet for 4 weeks to 10 B6C3F1 mice/sex/group at doses of 0, 59, 181, 522, 1286, 2471, or 4983 ppm (equivalent to 0, 8.85, 27.15, 78.3, 192.9, 370.65, and 747.45 mg/kg/day, calculated by the reviewers). An additional satellite group of 10 mice/sex was included for baseline determinations of hematology, clinical chemistry, and urinalysis parameters; however, no adverse findings were noted in this group.

No animals died prior to scheduled sacrifice. Urinalysis results were compromised by insufficient sample collection. Clinical signs of toxicity were limited to thin appearance in the 4983 ppm group during Weeks 3 and 4. Decreases ($p \leq 0.05$ or 0.01) in mean body weight, were observed throughout the study in the 4983 ppm group (↓20-36%) and beginning at Week 2 in the 2471 ppm males (↓12-27%). Overall (Weeks 0-4) body weight gains (calculated by reviewers) were decreased in the 1286, 2471, and 4983 ppm groups (↓33-266%). Food consumption was generally decreased at 2471 and 4983 ppm (↓6-44%).

The liver was considered to be the target organ of toxicity. At 522 ppm, increases ($p \leq 0.05$ or 0.01) in absolute, relative (to body), and relative (to brain) liver weights were observed in males. In addition, these increases in liver weight were noted at ≥1286 ppm (in males and females). The following histopathological changes were observed in the liver of both males and females: (I) mild to moderate diffuse acidophilia at ≥2471; (ii) slight to moderate multifocal acidophilia at 59, 181, 522 and 1286 ppm; (iii) diffuse cell disassociation at ≥1286 ppm in males and at ≥2471 ppm in females; (iv) multifocal single cell necrosis at ≥2471 ppm; (v) diffuse hepatocellular hypertrophy at ≥1286 ppm; and (vi) multifocal cell disassociation, multifocal hepatocellular hypertrophy at 522 and 1286 ppm, and coagulative necrosis at 1286 ppm. Additionally, focal fibrosis was observed in the 1286 ppm males.

The following differences ($p \leq 0.05$ or 0.01) in clinical chemistry parameters were observed: (I) alanine amino-transferase (ALT) was increased at 4983 ppm; (ii) aspartate amino-transferase (AST) was increased at ≥2471 ppm; (iii) cholesterol was increased at ≥1286 ppm; and (iv) glucose was decreased at 2471 ppm (males only) and 4983 ppm.

Decreased ($p \leq 0.01$) absolute and relative (to brain) kidney weights were observed in the males at ≥2471 ppm. In the females, absolute kidney weights were decreased ($p \leq 0.01$) at 4983 ppm. Microscopically in the kidneys of the males, mitotic increase was observed at 4983 ppm, and nuclear enlargement occurred at ≥2471 ppm.

The following observations were of equivocal toxicological importance because of the lack of corroborating evidence: decreased ($p \leq 0.05$ or 0.01) spleen (in males and females) and uterus weights (absolute, relative (to body), and relative (to brain)) at 1286, 2471, and 4983 ppm; and decreased leukocytes in the 2471 and 4983 ppm males.

The LOAEL is 522 ppm (78.3 mg/kg/day), based on increased absolute and relative (to body and to brain) liver weights in males and histological changes in the liver (slight to moderate multifocal acidophilia, multifocal cell disassociation, multifocal hepatocellular hypertrophy in males and females, and diffuse hepatocellular hypertrophy in females). The NOAEL is 181 ppm (27.15 mg/kg/day).

The submitted study is classified as **acceptable/non-guideline** and satisfies the purpose for which it was intended, as a range-finding study.

EXECUTIVE SUMMARY: In a hepatocyte proliferation assay (MRID 45323502), 12 male B6C3F1 mice per dose group were exposed orally by gavage to one dose of Acifluorfen (lot no. B27234), in corn oil, at dose levels of 200 or 1000 mg/kg body weight. Additional 10-12 mice were exposed by gavage to single doses of Tackle (lot no. 25300D02201) or Blazer (lot no. not reported), dissolved in water, at dose levels of 200, 700, or 1000 mg/kg body weight. Corn oil or water alone (vehicle controls) and a positive control (carbon tetrachloride) were also administered by gavage to 3 male mice each. At 24 and 48 hours post-treatment, primary hepatocytes isolated from the treated animals were cultured in the presence of ^{3}H-thymidine and then cells in S-phase were counted.

The results indicated that 48-hours following exposure of mice to Acifluorfen, Tackle, and Blazer at 200 mg/kg body weight, there were increases in the percent of cells in S-phase when compared to the vehicle controls. Mortality was observed at ≥700 and 1000 mg/kg for Blazer and Tackle, respectively; no mortality was observed for Acifluorfen.

This study is classified as **unacceptable/non-guideline**. The study can be upgradable if the following information is provided: chemical purities, criteria for a positive response, historical control values, dose analyses, and the no. of animals/group/sampling time.

EXECUTIVE SUMMARY:

In a single microbial mutagenicity test (MRID 45393901), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 were exposed to acifluorfen (99.5% a.i.; Lot No. 39/141-1) in dimethylsulfoxide (DMSO) at concentrations of 20, 100, 500, 2500, or 5000 µg/plate in the presence and absence of exogenous metabolic activation (±S9). The standard plate incorporation method was used. S9 homogenates for metabolic activation were derived from Aroclor-induced rat liver. Standard strain-specific mutagens served as positive controls.

Acifluorfen was tested up to the cytotoxic dose levels and the limit dose (5000 µg/plate) (±S9). Cytotoxicity was observed at 5000 µg/plate in TA98, TA100, TA1537 (+S9) and TA1535 (-S9). No increases in the number of revertants/plate were observed in any bacterial strain at any dose level of acifluorfen in the presence or absence of S9-activation, compared to vehicle controls. The efficacy of the S9-mix and the sensitivity of the test system to detect mutagenic agents was adequately demonstrated by the responses obtained with the non-activated and S9-activated positive controls. Under the conditions of this study, **acifluorfen is considered not to be mutagenic in *S. typhimurium* when tested up to 5000 µg/plate, with or without exogenous metabolic activation.**

This study is classified as **acceptable/guideline (§84-2)** and satisfies the FIFRA Test requirements for *in vitro* mutagenicity (bacterial reverse gene mutation) study in four strains of *S. typhimurium* (*S. typhimurium* TA 102 or *E. Coli* WP2 was not included in the study).

CITATION :

Engelhardt, G. and Hoffmann, H.D. (1990) Report on the Study of Blazer (Rohm and Haas) in the Ames Test (Standard Plate Test and Preincubation Test with Salmonella typhimurium TA100). BASF AKTIENGESELLSCHAFT, Department of Toxicology, Ludwigshafen/Rhein, Germany. BASF Registration Document No. 90/0040. January 20, 1990. MRID 45393902. Unpublished.

EXECUTIVE SUMMARY: In two independent microbial mutagenicity tests (MRID 45393902), *Salmonella typhimurium* strain TA100 was exposed to acifluorfen (46% a.i.; Lot No. 88-0020) in water at concentrations of 100, 500, 2500, 5000, 7500, 10000, or 12500 µg/plate in the presence and absence of exogenous metabolic activation (±S9). The standard plate incorporation test and a preincubation test were performed in the first mutagenicity assay (±S9). The confirmatory assay was performed using only S9-activated cultures (derived from rat or mouse livers) in a standard plate incorporation test. S9 homogenates for metabolic activation were derived from Aroclor-induced rat or mouse livers. 2-Aminoanthracene (+S9) and N-methyl-N'-nitro-N-nitroso-guanidine (-S9) served as positive controls.

Acifluorfen was tested up to 12500 µg/plate (±S9), above the limit dose (5000 µg/plate). Cytotoxicity was only observed at 10000 µg/plate (±S9) in the preincubation test. In the first mutagenicity assay, slightly increased revertant colonies was observed at ≥2500 µg/plate in the presence of S9-activation. However, in the confirmatory test, reproducible increases in revertant colonies (1.5-1.8x vehicle control) were only observed at ≥10000 µg/plate (+S9). The reproducible increases in revertant colony counts did not meet the criteria (2x vehicle control value) for a positive mutagenic response and were only observed at ≥2x the limit dose. The results of this study support the findings of the cited Rohm and Haas study of slightly enhanced colony numbers (<2x) at ≥4000 µg/plate in the presence of S9-activation. The efficacy of the S9-mix and the sensitivity of the test system to detect mutagenic agents was adequately demonstrated in the current study by the responses obtained with the non-activated and S9-activated positive controls. Under the conditions of this study, **acifluorfen is considered not to be mutagenic in *S. typhimurium* TA100 when tested up to the limit dose (5000 µg/plate), with or without exogenous metabolic activation.**

The study is classified as **unacceptable** and does not satisfy the FIFRA Test Guideline requirements for an *in vitro* mutagenicity (bacterial reverse gene mutation) data **(§84-2)**, because strains TA98, TA1535 and TA1537 were not tested. However, the results confirmed finding in another study which tested acifluorfen (99.5% a.i.) at the concentrations up to 5000 ug/plate (MRID 45393901) where no compound related increase in revertant colony was seen.

EXECUTIVE SUMMARY:

In two independent microbial mutagenicity tests (MRID 45323501), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 were exposed to Blazer (25.5% a.i.; Lot No. 156 9009) in water at concentrations of 20, 100, 500, 2500, or 5000 µg/plate in the presence and absence of exogenous metabolic activation (±S9). The standard plate incorporation method was used in the first mutagenicity test and a 20-minute pre-incubation step was added for the confirmatory test. S9 homogenates for metabolic activation were derived from Aroclor-induced rat liver. Standard strain-specific mutagens served as positive controls.

Blazer was tested up to the limit dose (5000 µg/plate) (±S9). No cytotoxicity was observed at any dose level in either test. No increases in the number of revertants/plate were observed in any bacterial strain at any dose level of Blazer in the presence or absence of S9-activation, compared to vehicle controls. The efficacy of the S9-mix and the sensitivity of the test system to detect mutagenic agents was adequately demonstrated by the responses obtained with the non-activated and S9-activated positive controls. Under the conditions of this study, **Blazer is considered not to be mutagenic in *S. typhimurium* when tested up to 5000 µg/plate, with or without exogenous metabolic activation.**

This study is classified as **acceptable (§84-2)** and satisfies the FIFRA Test Guideline requirements for *in vitro* mutagenicity (bacterial reverse gene mutation) study with S. typhimurium strains TA 95, TA 100, TA 1535 and TA 1337.