6

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OFFICE OF PREVENTION PESTICIDES AND
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MEMORANDUM

SUBJECT: ***BROMUCONAZOLE*** - Report of the Hazard Identification Assessment Review Committee.

FROM: Meta Bonner, Toxicologist and Risk Assessor. Meta J. Bonner, 3-20-02
RAB3
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Mary Waller, PM Team 23
Fungicide Branch
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PC Code: 120503

On November 29, 2001 and on a revisit on December 13, 2001, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for Bromunconazole with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to Bromunconazole was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996. The conclusions drawn at these meetings are presented in this report.

Committee Members in Attendance

Members present were:	Jess Rowland, William Burnam, Pamela Hurley, Elizabeth Mendez, David Nixon, Ayaad Assaad, Jonathan Chen, Paula Deschamp, Virginia Fornillo, Elizabeth Doyle, John Liccione,
Member(s) in absentia:	Brenda Tarplee
Data evaluation prepared by:	Meta Bonner, Toxicologist, RAB3
Also in attendance were:	Amelia Acierto, Steve Dapson, Kelly O'Rourke, Clark Swentzel, all of RAB3, HED

Data Evaluation / Report Presentation

Meta J. Bonner 03-20-02
Meta Bonner
Toxicologist

1. INTRODUCTION

On November 29, 2001 and on December 13, 2001, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for Bromunconazole with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to Bromunconazole was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996.

2. HAZARD IDENTIFICATION

2.1.a Acute Reference Dose (RfD) - Subpopulation: Females 13-50

Study Selected: Developmental toxicity in the rat (oral). §83-3

MRID No.: 42937126 & 42937127

Executive Summary: Pregnant Sprague-Dawley rats (25/group) received LS860263 by gavage on gestational days (GDs) 6-15, inclusive, at dose levels of 0, 10, 70 or 500 mg/kg/day. Methyl cellulose (0.5%) was used as the control and vehicle for the test material. Maternal toxicity was observed at 500 mg/kg/day as evidenced by significantly increased water consumption during the dosing period (118-126% of controls), significantly decreased body weight gain during GDs 6-10 (75% of control) and significantly increased absolute and relative liver weights (110-113% of controls). One dam at 500 mg/kg/day was sacrificed of GD8 after exhibiting neurotoxic/ cholinergic-type clinical signs (hunched posture, abnormal gait, piloerection, brown staining on nose and around eyes, and weight loss) that had previously been noted in the range-finding study; this may also have been treatment related. Based on these results, the **Maternal Toxicity NOAEL = 70 mg/kg/day and the LOAEL was 500 mg/kg/day.**

Developmental toxicity was observed at 70 and 500 mg/kg/day as evidenced by dose-related and significant increases in placental weights (108% and 127%, respectively, of controls) and additional cervical ribs. Compound-related altered growth was apparent at 500 mg/kg/day; fetal body weight decreased significantly to 94% of control and there was delayed ossification in selected bones. Compound-related skeletal anomalies in the ribs were also evident at 500 mg/kg/day and included increased incidences of 13/14, 14/14 and additional cervical ribs. Based on increased placental weights and increased incidence of additional cervical ribs, the **Developmental Toxicity NOAEL = 10 mg/kg/day and the LOAEL = 70 mg/kg/day.**

Dose and Endpoint for Establishing RfD: Developmental NOAEL of 10 mg/kg/day, based on additional cervical ribs at 70 mg/kg/day.

Uncertainty Factor (UF): 100 (10 for interspecies extrapolation X 10 for intra-species variability).

Comments about Study/Endpoint/Uncertainty Factor: This endpoint is appropriate for acute RfD for females 13-50 years old, since the effect (additional cervical ribs) are presumed to occur after a single exposure.

Acute RfD = (Females 13-50 years old)	10 mg/kg (NOAEL) / 100 (UF)	=	**0.1 mg/kg**

2.1.b Acute RfD for General Population:

Study Selected: Developmental toxicity in the rat (oral). §83-3

MRID No.: 42937126 & 42937127

Executive Summary: (see summary above in Section 2.1.a)

Dose and Endpoint for Establishing RfD: Maternal toxicity NOAEL is 70 mg/kg/day based on clinical signs (hunched posture, abnormal gait, piloerection, brown staining on nose and around eyes at 500 mg/kg/day (LOAEL).

Uncertainty Factor(s): 100 (10 for interspecies extrapolation X 10 for intra-species variability).

Comments about Study/Endpoint/Uncertainty Factor(s): The clinical signs were seen two day (on GD 8) after treatment. Similar clinical signs were seen in the range-finding study.

Acute RfD = (General Population)	70 mg/kg (NOAEL) / 100 (UF)	=	**0.70 mg/kg**

2.2 Chronic Reference Dose (RfD)

Study Selected: Chronic/carcinogenicity toxicity study in rats. §83-5

MRID No.: 42937132

Executive Summary: Oral administration of Bromuconazole to F-344 rats occurred for 104 weeks for the toxicology phase via diet at dose levels of 20 [males 0.88/ females 1.09 mg/kg/day], 150 [males 6.48/females 8.27 mg/kg/day], 1000 [males 43.3/females 55.6

mg/kg/day], 2000 [males 87.2/females 114.2 mg/kg/day] ppm. There were significant decreases in body-weight gain during the first 13 weeks in females at the 1000 [94% of control] and 2000 [87% of control] ppm dose levels, during weeks 13-26 in males [89% of control] at 2000 ppm dose level, and overall [0-1-4 weeks] in females at 1000 ppm [89% of control] and in both sexes [males 92%/females 76% of control] at 2000 ppm. The main target organ is the liver. Liver weights, macroscopic lesions, and microscopic lesions [nodular hyperplasia, panacinar hepatocytic fatty vacuolation, focus of hepatocytic fatty vacuolation] were increased with increasing dose in males. High-dose [2000 ppm] males displayed an increased incidence [6.7%] of hepatocellular carcinomas compared to the concurrent [0%] and historical [0-6%] controls, although statistical significance was not attained. Females displayed a dose-related increase in microscopic, non-neoplastic lesions [fatty focal vacuolation, periacinar hepatocytic fatty vacuolation] in the liver at dose levels of 150 ppm and above. In males, the thyroid also appears to be a target organ. Thyroid weight and microscopic lesions [parafollicular hyperplasia and parafollicular cell adenomas] were increased with increasing dose in males. **Based on increased liver non-neoplastic lesions, the Chronic Toxicity NOAEL = 20 ppm (0.88 mg/kg/day) and the LOAEL = 150 ppm (6.48 mg/kg/day).**

Dose and Endpoint for Establishing RfD: A NOAEL of 0.88 mg/kg/day based on increased liver non-neoplastic lesions (fatty vacuolation) at 6.48 mg/kg/day.

Uncertainty Factor(s): 100 (10 for interspecies extrapolation X 10 for intra-species variability)

Comments about Study/Endpoint/Uncertainty Factor(s): The increased liver non-neoplastic lesions (fatty vacuolation) at the LOAEL (6.48 and 8.27 mg/kg/day in males and females, respectively), was also seen in the 90 Day rat and in the 2-generation studies. Hepatic toxicity seen with this triazole are consistent with other studies and other structurally related compounds.

Chronic RfD =	$\frac{\text{0.88 mg/kg/day (NOAEL)}}{\text{100 (UF)}}$ =	**0.009 mg/kg/day**

2.3 Occupational/Residential Exposure

2.3.1 Short-Term (1-30 Days) Incidental Oral Exposure

Study Selected: Developmental toxicity in the rat (oral). §83-3

MRID No.: 42937126 & 42937127

Executive Summary: Pregnant Sprague-Dawley rats (25/group) received LS860263 by gavage on gestational days (GD) 6-15, inclusive, at dose levels of 0, 10, 70 or 500 mg/kg/day. Methyl cellulose (0.5%) was used as the control and vehicle for the test

material. Maternal toxicity was observed at 500 mg/kg/day as evidenced by significantly increased water consumption during the dosing period (118-126% of controls), significantly decreased body weight gain during GDs 6-10 (75% of control) and significantly increased absolute and relative liver weights (110-113% of controls). One dam at 500 mg/kg/day was sacrificed of GD8 after exhibiting neurotoxic/cholinergic-type clinical signs (hunched posture, abnormal gait, piloerection, brown staining on nose and around eyes, and weight loss) that had previously been noted in the range-finding study; this may also have been treatment related. Based on these results, the **Maternal Toxicity NOAEL = 70 mg/kg/day and the LOAEL was 500 mg/kg/day.**

Developmental toxicity was observed at 70 and 500 mg/kg/day as evidenced by dose-related and significant increases in placental weights (108% and 127%, respectively, of controls) and additional cervical ribs. Compound-related altered growth was apparent at 500 mg/kg/day; fetal body weight decreased significantly to 94% of control and there was delayed ossification in selected bones. Compound-related skeletal anomalies in the ribs were also evident at 500 mg/kg/day and included increased incidences of 13/14, 14/14 and additional cervical ribs. Based on increased placental weights and increased incidence of additional cervical ribs, the **Developmental Toxicity NOAEL = 10 mg/kg/day and the LOAEL = 70 mg/kg/day.**

Dose and Endpoint for Risk Assessment: Maternal Toxicity NOAEL of 70 mg/kg/day based on the decreased body weights and clinical findings at dose 500 mg/kg/day.

Comments about Study/Endpoint: This end-point is appropriate for the population and duration of exposure of concern.

2.3.2 Intermediate-Term (1 to 6 Months) Incidental Oral Exposure

Study Selected: 90-Day oral toxicity - rats. §82-1a

MRID No.: 42937131

Executive Summary: Bromuconazole was administrated orally via the diet to Fisher 344 rats [10/sex/group] for 13 weeks at dose levels of 0, 40 ppm [males 2.72/females 3.04 mg/kg/day], 200 ppm [males 13.76 /females 15.08 mg/kg/day], 1000 ppm [males 68.1/ females 74.5 mg/kg/day], and 5000 ppm [males 342.5/females 381.2 mg/kg/day] resulted in signs of toxicity [decreased body weight at 13 weeks {males 94% and females 92% of control at the 5000 ppm dose level}, decreased overall body-weight gain {males 90% (5000 ppm) and females 87% (1000 ppm)/80% (5000 ppm) of control}, decreased food efficiency, changes in several hematology/blood chemistry parameters consistent with liver toxicity, increased adrenal weight {5000 ppm males} and liver weight {1000 ppm females/5000 ppm males & females}, and microscopic, treatment-related, changes in the

liver {increased incidence of hepatic fatty vacuolation}].

For the 90-Day oral toxicity in rats, the **NOAEL is 200 ppm [males 13.8/females 15.1 mg/kg/day] and the LOAEL is 1000 ppm [males 68.1/females 74.5 mg/kg/day],** based on decreased body-weight gains in females [87% and 80% of control at 1000 ppm and 5000 ppm, respectively], changes in hematology/blood chemistry parameters and in the incidence of microscopic lesions consistent with liver toxicity, and increased liver weight in both sexes.

Dose and Endpoint fir Risk Assessment: A NOAEL of 13.8 mg/kg/day, based on decreased body-weight gains in females, changes in hematology/blood chemistry parameters and in the incidence of microscopic lesions consistent with liver toxicity, and increased liver weight in both sexes.

Comments about Study/Endpoint: This end-point is appropriate for the population and duration of exposure of concern.

2.3.3 Dermal Absorption

Dermal Absorption Factor:

Study Selected: None available. §85-3

MRID No.: N/A

Executive Summary: N/A

Proposed Percentage (%) Dermal Absorption: Assume 100% (default).

Comments about Dermal Absorption: No dermal absorption studies are available. No dermal toxicity was seen at the highest dose tested in the dermal toxicity study in rabbits. However, developmental effects of concern were seen via the dermal route in a dermal developmental toxicity study in rats. Therefore, the HIARC determined that absorption by the inhalation route should be treated as equivalent to oral absorption..

2.3.4 Short-Term (1-30 Days) & Intermediate-Term (1- 6 Months) Dermal Exposure

Study Selected: Dermal developmental study in the rat. §83-3

MRID No.: 43018508

Executive Summary: In a developmental toxicity study, 25 Sprague-Dawley rats per

group received LS860263 (20% w/v a.i., note: dosages were calculated as LS860263 - the active ingredient, supplied as 20% active ingredient in Formulation 10064B) by the dermal route on gestational days (GDs) 6-15, inclusive, at dose levels of 0, 40, 133, or 400 mg/kg/day. Distilled water served as the control substance and vehicle for the test article. Analytical chemistry data indicated that the highest dose intended was not reached. Analyses conducted on the last week of the study revealed a concentration of 79% of 400 mg/kg/day. Therefore, the actual highest dose was judged by the reviewers to be 316 mg/kg/day.

Compound-related maternal toxicity was observed in a dose-related manner at 133 and 400 mg/kg/day as evidenced by increased clinical signs (ungroomed and unkempt coat). Consequently, the **Maternal Toxicity NOAEL was 40 mg/kg/day, and the Maternal Toxicity LOAEL was 133 mg/kg/day**.

Developmental toxicity was observed at 133 and 400 mg/kg/day as evidenced by increased fetal and litter incidences of skeletal abnormalities (one extra rib or a pair of extra ribs) compared to concurrent and historical controls. Consequently, the **Developmental Toxicity NOAEL was 40 mg/kg/day, and the Developmental Toxicity LOAEL was 133 mg/kg/day**.

Dose and Endpoint for Risk Assessment: Dermal developmental NOAEL of 40 mg/kg/day based on the increased incidences of skeletal anomaly (extra ribs) seen at 133 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The endpoint chosen for short- and intermediated-term dermal exposures is appropriate for the route and duration of concern because the developmental effects were seen following repeated dermal application in a developmental study. This dose/endpoint although based on in utero effects is applicable to dermal exposure to infants and children because the dose (70 mg) selected for incidental oral exposure would be protective of the effects of concern (decrease in body weights and clinical signs) for that exposure scenario.

2.3.5 Long-Term (Longer than 6 Months) Dermal Exposure

Study Selected: Chronic/carcinogenicity toxicity study in rats.

Dose and Endpoint for Risk Assessment: oral NOAEL of 0.88 mg/kg/day is based on the increased in liver non-neoplastic lesions (fatty vacuolation) at 6.48 mg/kg/day (LOAEL).

Comments about Study/Endpoint: This dose endpoint was selected for establishing the chronic RfD. Since an oral NOAEL was selected 100% dermal absorption factor should be used for route to route extrapolation.

2.3.6 Short (1-30 days) & Intermediate (1- 6 Months) Inhalation Exposure

Study Selected: Developmental rat study.

Dose and Endpoint for Risk Assessment: Oral developmental NOAEL of 10 mg/kg/day based on additional cervical ribs at 70 mg/kg/day (LOAEL).

Comments about Study/Endpoint: An oral study was selected because of the concern for development toxicity. Since an oral dose was selected 100% absorption factor should be used for route to route extrapolation.

2.3.7 Long-term Inhalation Exposure (Longer than 6 Months)

Study Selected: Chronic/carcinogenicity toxicity study in rats.

Dose and Endpoint for Risk Assessment: NOAEL of 0.88 mg/kg/day is based on the increased in liver non-neoplastic lesions (fatty vacuolation) at 6.48 mg/kg/day (LOAEL).

Comments about Study/Endpoint: This dose/study endpoint was used for establishing the chronic RfD. Since an oral dose was selected 100% absorption factor should be used for route to route extrapolation.

2.4 Margin of Exposure

An Margin of Exposure (MOE) of 100 is adequate for dermal and inhalation occupational exposure risk assessment. The MOE for residential exposure risk assessment will be determined by the FQPA Safety Factor Committee.

2.5 Recommendation for Aggregate Exposure Risk Assessments

The oral route cannot be combined with dermal and inhalation routes for short or intermediate aggregate risk assessments term because of the differences in the selected toxicity endpoints for short-term (maternal effects) and intermediate-term (systemic toxicity).

A common toxicity endpoint (increase in cervical ribs) was identical for the dermal and inhalation routes; therefore those routes can be aggregated for the short and intermediate exposure scenarios. A common toxicity endpoint (hepatic toxicity) was identified for the oral, dermal (oral equivalent) and inhalation (oral equivalent) routes. Therefore, these routes can be aggregated for the long-term exposure scenarios.

3. CLASSIFICATION OF CARCINOGENIC POTENTIAL

3.1 Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID No.: 42937132

Executive Summary: Oral administration of Bromuconazole to F-344 rats occurred for 104 weeks for the toxicology phase via diet at dose levels of 20 [males 0.88/ females 1.09 mg/kg/day], 150 [males 6.48/females 8.27 mg/kg/day], 1000 [males 43.3/females 55.6 mg/kg/day], 2000 [males 87.2/females 114.2 mg/kg/day] ppm. There were significant decreases in body-weight gain during the first 13 weeks in females at the 1000 [94% of control] and 2000 [87% of control] ppm dose levels, during weeks 13-26 in males [89% of control] at 2000 ppm dose level, and overall [0-1-4 weeks] in females at 1000 ppm [89% of control] and in both sexes [males 92%/females 76% of control] at 2000 ppm. The main target organ is the liver. Liver weights, macroscopic lesions, and microscopic lesions [nodular hyperplasia, panacinar hepatocytic fatty vacuolation, focus of hepatocytic fatty vacuolation] were increased with increasing dose in males. High-dose [2000 ppm] males displayed an increased incidence [6.7%] of hepatocellular carcinomas compared to the concurrent [0%] and historical [0-6%] controls, although statistical significance was not attained. Females displayed a dose-related increase in microscopic, non-neoplastic lesions [fatty focal vacuolation, periacinar hepatocytic fatty vacuolation] in the liver at dose levels of 150 ppm and above. In males, the thyroid also appears to be a target organ. Thyroid weight and microscopic lesions [parafollicular hyperplasia and parafollicular cell adenomas] were increased with increasing dose in males. **Based on increased liver non-neoplastic lesions, the Chronic Toxicity NOAEL = 20 ppm and the LOAEL = 150 ppm.**

Discussion of Tumor Data: High-dose [2000 ppm = 89.2 mg/kg/day] males displayed an increased incidence [6.7%] of hepatocellular carcinomas compared to the concurrent [0%] and historical [0-6%] controls, although statistical significance was not attained.

Adequacy of the Dose Levels Tested: Dose levels tested were adequate. Dietary levels for the study were selected based on the results of a 90-day range-finding study in rats in which overall body weight gain was significantly decreased in males and females of the mid-dose (1000 ppm) and high-dose (5000 ppm).

3.2 Carcinogenicity Study in Mice

MRID No.: 43018506 & 43018507

Executive Summary: The oral administration of Bromuconazole to CD-1 mice for 80 weeks [53 weeks interim sacrifice] via the diet at dose levels 100 [female 10.8/males 12.3 mg/kg/day], 1000 [females 112.9/males 134.5 mg/kg/day], and 3000 [females 369.9/males 248.7 mg/kg/day] ppm

resulted in a significant decrease in body-weight gain at the mid- and high-dose levels in both sexes. The target organ is the liver. Liver weights, macroscopic lesions, and microscopic lesions were increased with increasing dose in both sexes. In males, an increase in the incidence of microvesicular periacinar vacuolation was observed at all dose levels. High-dose males displayed an increased incidence of liver carcinomas compared to the concurrent and historical controls, although statistical significance was not attained. **The NOAEL for the effects other than micovesicular periacinar vacuolation can be set at 100 ppm [females 10.9/males 12.3 mg/kg/day] and LOAEL at 1000 ppm [females 134.5 mg/kg/day], based on decreased body-weight gains.**

Discussion of Tumor Data: The high-dose (3000 ppm = 428.7 mg/kg/day) males displayed an increased incidence of hepatocellular carcinomas (19.23%) compared to the concurrent (11.54%) and the historical (17.3%) controls, although statistical significance was not attained.

Adequacy of the Dose Levels Tested: Dose levels tested were adequate in the 80-week carcinogenicity study in mice (MRID No. 43018507) describe above. The previous 78-week carcinogenicity study in mice (MRID No. 43018506) established that the dose levels (20 [females 2.27/males 2.40 mg/kg/day], 100 [females 11.3/males 12.3 mg/kg/day], and 500 ppm [females 57.4/males 60.8 mg/kg/day]) utilized in that study were not adequate for assessing the carcinogenic potential of Bromuconazole. In the earlier study, dosing for 78 weeks did not produce an increase in the number of tumors or the type of tumors observed, but only produced slight decreases in body weight gain and food efficiency in the high-dose (60.8 mg/kg/day) males during the first 13-weeks of the study.

3.3 Classification of Carcinogenic Potential

On April 24, 1995 the RfD Committee classified Bromuconazole as a Group E chemical based on the lack of evidence for carcinogenicity in mice and rats. The HIARC concurs with this decision.

4. MUTAGENICITY

Acceptable mutagenicity studies available are Ames, micronucleus, in vitro cytogenetic, *in vivo* unscheduled DNA synthesis (UDS), and *in vitro* UDS assays. Acceptable assays satisfy pre-1991 mutagenicity guidelines.

4.1 In a *Salmonella*/microsome plate incorporation assay (MRID#43018509), strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to Bromuconazole at concentrations of 50, 158, 500, 1580, and 5000 ug/plate, with and without exogenous metabolic activation. Also, in a tryptophan reversion test, *Escherichia coli* strain WP2 (uvrA) was exposed to the same concentrations. Cytotoxicity was produced at 5000 ug/plate in all bacterial strains tested as evidenced by a reduction in the number of revertant colonies per plate and a thinning of the background lawn when compared to

solvent controls. There was **no evidence of induced revertant colonies over background at any dose tested, either with or without S9**. (Negative)

4.2 In an *in vitro* mammalian cell cytogenetic assay (MRID#42937136), Chinese hamster CHO-K1 cells were exposed to 10, 50, 100, and 250 ug/mL Bromuconazole with and without exogenous metabolic activation. Bromuconazole was tested to cytotoxic levels. **In the absence of S9 mix, statistically significant increases were seen in the mean percent of metaphases with chromosomal aberrations at 10 and 50 ug/mL when gaps were excluded from the determination. In the presence of S9 mix, statistically significant increases were seen at 10 and 50 ug/mL whether gaps were included or excluded from the determination.** While there were increases at 100 ug/mL, both with and without S9 mix, the increases were not statistically significant, likely due to reduced bioavailability of test material as mentioned with respect to cytotoxicity. (Positive)

4.3 In an *in vivo* mouse bone marrow micronucleus assay (MRID#42937137), five CD-1 mice/sex/dose/harvest time were each administered a single oral dose of Bromuconazole at 40, 200, or 1000 mg/kg body weight. One of two males given 2000 mg/kg Bromuconazole in the preliminary toxicity test died by five hours post-treatment and the remaining male and the two females given this dose were killed *in extremis*. Transient morbidity was seen at 1000 mg/kg in the preliminary toxicity test and also in the main micronucleus assay. Bromuconazole was, therefore, tested to an adequate top dose and **did not significantly increase the frequency of micronucleated polychromatic erythrocytes in mouse bone marrow** at any dose or sampling time tested. (Negative)

4.4 In two independently performed *in vitro* cytogenetic assays (MRID#43521101), human lymphocytes derived from a single donor were evaluated for chromosome damage 19 hours after continuous exposure to nonactivated Bromuconazole doses of 25, 50, or 100 ug/mL (both trials) and 43 hours after the continuous exposure to 50 ug/mL (initial trial only). Cytotoxicity was achieved at nonactivated doses >100 ug/mL and S9-activated doses >225 ug/mL. There was no evidence of a clastogenic response in the absence of S9 activation. However, it **was clastogenic in the presence of S9**, the S9-activated test material induced dose-related increases in the number and percentage of cells with structural aberrations over a concentration range of 100 to 225 ug/mL, significant at 225 ug/mL. (Positive)

4.5 In an *in vivo/in vitro* UDS in rat hepatocytes/mammalian cells procedure (MRID#42937138), Bromuconazole (i.e., LS860263), at doses of 189.7 and 600 mg/kg, was administered to six Wistar rats per test group by oral gavage. The mean net nuclear grain count was below zero for both treatment times indicating **no induction of UDS** as tested in this study. Bromuconaole was tested to a sufficiently high concentration as illustrated of lethargy and irregular breathing seen at 600 mg/kg in the range-finding study, although no mention was made of toxicity in the main UDS assay. (Negative)

4.6 In an *in vitro* UDS in primary rat hepatocytes/mammalian cells culture (MRID#43018510), Bromuconazole (i.e., LS860263), was applied at concentrations of 0.391, 1.24, 3.91, 12.4, and 39.1 ug/mL in one assay and at concentrations of 0.391, 1.24, 3.91, 12.4, 39.1, and 124 ug/mL in a second assay. Based on cell detachment and cell viability determinations in a preliminary cytotoxicity assay, the test material was tested to an acceptable high dose. There was **no evidence of UDS induction** at any tested concentration as measured by incorporation of tritiated thymidine into NDA. (Negative)

5. FQPA CONSIDERATIONS

5.1 Adequacy of the Data Base

No neurotoxicity studies are not available.

5.2 Neurotoxicity

Evidence of neurotoxicity from other oral toxicity studies: Some evidence of possible neurotoxicity occurred in the rat prenatal developmental study. Possible indication of neurotoxic cholinergic-type clinical signs in one female was found in this study and was also found in range-finding study (at 750 and 1000 mg/kg/day). This one female in the 500 mg/kg/day dose group was sacrificed moribund on GD 8. Prior to sacrifice this dam showed hunched posture, abnormal gait, piloerection, brown staining on nose and around eyes, and weight loss. From these findings it can not be determined at this time if treatment with Bromuconazole will result neurotoxicity.

5.3 Developmental Toxicity

Developmental Study in Rat (§83-3): In a developmental toxicity study (MRID # 42937127 & 42937126), pregnant Sprague-Dawley rats (25/group) received LS860263 by gavage on gestational days (GD) 6-15, inclusive, at dose levels of 0, 10, 70 or 500 mg/kg/day. Methyl cellulose (0.5%) was used as the control and vehicle for the test material. Maternal toxicity was observed at 500 mg/kg/day as evidenced by significantly increased water consumption during the dosing period (118-126% of controls), significantly decreased body weight gain during GDs 6-10 (75% of control) and significantly increased absolute and relative liver weights (110-113% of controls). One dam at 500 mg/kg/day was sacrificed of GD8 after exhibiting neurotoxic/cholinergic-type clinical signs that had previously been noted in the range-finding study; this may also have been treatment related. Based on these results, the **Maternal Toxicity NOAEL is 70 mg/kg/day and the LOAEL is 500 mg/kg/day.**

Developmental toxicity was observed at 70 and 500 mg/kg/day as evidenced by dose-related and significant increases in placental weights (108% and 127%, respectively, of controls) and additional cervical ribs. Compound-related altered growth was apparent at 500 mg/kg/day; fetal body weight decreased significantly to 94% of control and there was delayed ossification in

selected bones. Compound-related skeletal anomalies in the ribs were also evident at 500 mg/kg/day and included increased incidence of 13/14, 14/14 and additional cervical ribs. Based on increased placental weights and increased incidence of additional cervical ribs, the **Developmental Toxicity NOAEL is 10 mg/kg/day and the LOAEL is 70 mg/kg/day.**

Developmental Study in Rabbit (§83-3): In a developmental toxicity study (MRID 42937129 & 42937128), 18 New Zealand White rabbits per group received LS860263 (99.1% a.i.) by gavage on gestational days (Gds) 6-19, inclusive, at dose levels of 0, 12.5, 50, or 200 mg/kg/day. Aqueous methylcellulose (0.5%) served as a control substance and vehicle for the test article. Maternal toxicity was observed at 50 and 200 mg/kg/day. It was evidenced as increased clinical signs (lethargy/reluctance to move, increased respiration rate, ataxic/unsteady gait, aggression, lack of muscle tone and coordination, prone posture , and/or vocalization); decreased body weight gain during the dosing period (47% and 66% of control, respectively) and the entire gestation period (81% and 71% if control, respectively); and decreased food consumption during the dosing period (74%-92% of control). In addition at 200 mg/kg/day, the pregnancy rate decreased below control and mortality and abortion rates increased above control. Based on these results, the **Maternal Toxicity NOAEL is 12.5 mg/kg/day, and the Maternal toxicity LOAEL is 50 mg/kg/day**.

Developmental toxicity was observed at 200 mg/kg/day as evidenced by the decreased rate of live fetuses per doe (5.5 versus 9.3 in the control group). This was also demonstrated in the increased percentage of postimplantation loss (43% versus 13% in the control group). The rate of resorptions was also slightly increased at this dose level. The test compound did not have any effects upon growth and the rate of anomalies in those fetuses that survived. Based on these results, the **Developmental Toxicity NOAEL is 50 mg/kg/day. The Developmental Toxicity LOAEL is 200 mg/kg/day**.

5.4 Reproductive Toxicity

Reproductive Study in Rat (§83-4): In a two-generation reproduction study (MRID43029901 & 42937130), CD rats (30 per sex and group) received Bromuconazole (98.3% a.i.) in the diet at dose levels of 0, 20, 200, or 2000 ppm (0, 1.3, 13.8, or 141.2 mg/kg/day for F_0 males and 0, 1.6m 15.5, or 159.6 mg/kg/day for F_0 females, respectively, during pre-mating). Systemic toxicity was observed at 2000 ppm in both sexes and generations. It was manifested as decreased body weight gain (during pre-mating, 84%-88% of controls; during gestation for the females, 88%-93%), increased liver weights (120%-128% of controls), and increased incidences of hepatocytic fatty vacuolation. Based on these results, the **Paternal Systemic Toxicity NOAEL and LOAEL were 13.8 mg/kg/day and 141.2 mg/kg/day, respectively.**

Offspring toxicity was observed at 141.2 mg/kg/day and manifested as decreased pup viability in the first generation. At this dose 115 pups out of 26 litters died during Day 1-4 compared to 40 pups out of 25 litters in the controls (low and medium doses:16 pups/26 litters and 21 pups/25 litters for doses 1.3 and 13.8 mg/kg/day, respectively). The number of litters with dead pups

were 11 for controls, 10 for dose group 1.3 mg/kg/day, 11 for dose group 13.8 mg/kg/day, and 17 for dose group 141.2 mg/kg/day. Whole litters lost were 2 in the controls and 4 in the high dose group, none for the low and middle dose groups. In addition, decreased pup body weight gain during lactation was seen in both generations. Based on these results, the **Offspring Toxicity NOAEL and LOAEL were 13.8 mg/kg/day and 141.2 mg/kg/day, respectively.**

HIARC at the December 13, 2001 meeting concluded that the two-generation reproductive study in rats showed susceptibility based on pup death Day 1 - 4. HIARC conclusion was supported by the findings that deaths were stable over doses and that there was a steep increase at the high dose of litters with dead pups. The committee noted that the data presented qualitative evidence for susceptibility in pups because the effects could possibly be due to maternal toxicity, were not seen in the F2 generation, and could be due to in utero or lactation exposure.

5.5 Additional Information from Literature Sources (if available)

Searches were conducted (10/15/2001 & 11/14/2001). European Council reports indicate that Bromuconazole use was banned in Norway based on its high retention in soil.

5.6 Determination of Susceptibility

There is quantitative /qualitative evidence of increased susceptibility of rats fetuses to in utero exposure in the developmental study. Increased placental weights and increased incidence of additional cervical ribs were observed at dose (70 mg/kg/day) lower than the dose that induced maternal toxicity (500 mg/kg/day).

There is qualitative evidence of increased susceptibility in two-generation reproduction study in rats. The numbers of litters with pup deaths Day 1 - 4 in the F1 generation was greater in the high dose group (141.2 mg/kg/day) compared to the control group. Pup deaths were stable over doses except in the high dose group where there was a steep increase of high dose litters with dead pups. However, this effect was not seen in the F2 generation yet the mean size of litters were similar to the F1 generation. The pup death may be a possible maternal toxicity, due to an effect expressed in utero or in first few days of lactation, however there is not enough information to determined why the pup deaths occurred. Therefore, it was concluded that the offspring effects are of concern and indicate qualitative increased susceptibility.

There is no quantitative or qualitative evidence of increased susceptibility following in utero exposure to rabbits. Developmental effect was seen at a dose higher than that which caused maternal toxicity.

5.7 Recommendation for a Developmental Neurotoxicity Study

A developmental neurotoxicity study was not recommended since there is no evidence in the available studies to indicate the need for this study.

5.8 Evidence that suggest requiring a Developmental Neurotoxicity study:

- Quantitative /qualitative evidence of increased susceptibility of rats.
- Qualitative evidence of increased susceptibility in F1 offspring.

5.9 Evidence that do not support a need for a Developmental Neurotoxicity study:

- No evidence of neurotoxicity or neuropathology in the available studies.
- The effects seen in the developmental rat study (increase in cervical ribs) are not central nervous system malformations.

1. HAZARD CHARACTERIZATION

Bromuconazole is a low to mildly acute toxin that shows mild eye irritation, no dermal irritation or sensitization. In repeated dermal applications, rabbits did not show dermal toxicity and no dermal absorption studies are available in either rats or rabbits. Rats however in a dermal developmental study show increased fetal skeletal abnormalities (extra ribs). By the oral route, developmental exposure in rats also resulted in skeletal abnormalities (additional cervical ribs) as well as increased placental weights.

Subchronic and chronic studies in rats, mice, and dogs indicate that the liver is the target organ. Increases in liver weights were seen in all three species, hepatocytic fatty vacuolation was seen in rats and mice, and hepatocytic degeneration was noted in the dog. Effects seen in the liver are supported by the rat dermal developmental study increase in maternal liver weights and the rat reproductive study increase in parental liver weights and hepatocytic fatty vacuolation. Hepatic toxicity seen with this compound is comparable to findings of liver toxicity seen with other conazoles. Bromuconazole's carcinogenicity potential is classified as Group E based on lack of evidence of mice and rats tumors. Bromuconazole is mutagenic. There is evidence of increased susceptibility iin the rat developmental and two-generation reproductive studies. However, there is no increase susceptibility in the rabbit developmental study.

2. DATA GAPS

28-day inhalation study.

3. ACUTE TOXICITY

Table 1. Acute Toxicity of Bromuconazole – Technical

Guideline No.	Study Type & Species	MRID No. & % Active Ing.	Results & Study Acceptability	Toxicity Category
81-1	Acute Oral–Rat	42937121 95.7%✣	LD_{50} = 365 mg/kg (males and females, combined)	II
81-2	Acute Dermal–Rabbit	42937122 97.8%✚	LD_{50} > 2000 mg/kg (males and females, combined)	III
81-3	Acute Inhalation–Rat	42937123 98.4%	LC_{50} > 5.05 mg/L (males and females, combined)	IV
81-4	Primary Eye Irritation–Rabbit	43018501 95.7%	0.1 ml, mild irritant.	III
81-5	Primary Dermal Irritation–Rabbit	43018502 95.7%	Not an irritant.	IV
81-6	Dermal Sensitization –Guinea pig	43018503 95.7%	Not a dermal sensitizer.	NA

✣Bromuconazole Tech. Used in these studies is also known as LS860263 which is made of two isomers with the ratio of 55.5-56.5% LS850646 & 42.9-43.5% LS850647. ✚Isomer ratio not provided in the records.

4. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary F13-50	NOAEL= 10 UF = 100	based on increased incidences of in cervical ribs at 70 mg/kg/day	Developmental - rat
	Acute RfD = 0.1 mg/kg		
Acute Dietary general pop	NOAEL= 70 UF= 100	Clinical signs (hunched posture, abnormal gait, piloerection, brown staining on nose) at 500 mg/kg/day	Developmental - rat
		Acute RfD = 0.7 mg/kg	
Chronic Dietary	NOAEL = 0.88 UF = 100	Increased liver non-neoplastic lesions (fatty vacuolation) at 6.48 mg/kg/day	Chronic/ carcinogenicity - rat
		Chronic RfD = 0.009 mg/kg/day	
Short-Term Incidental Oral	NOAEL = 70	Decreased body weights and clinical signs at 500 mg/kg/day.	Developmental - rat
Intermediate-Term Incidental Oral	NOAEL = 13.8	Decreased body weight gains, changes in blood chemistry, increased liver lesions and weights at 68.1 mg/kg/day.	90-Day oral - rat
Dermal, Short- & Intermediate-Term	Dermal NOAEL= 40	based on increased incidences of skeletal anomalies (extra ribs) at 133 mg/kg/day .	Dermal developmental -rat
Dermal, Long-Term	Oral NOAEL= 0.88(a)	Increased liver non-neoplastic lesions (fatty vacuolation) at 6.48 mg/kg/day..	Chronic/ carcinogenicity - rat
Inhalation, Short- & Intermediate-Term	Oral NOAEL= 10(b)	Increased in cervical ribs at 70 mg/kg/day..	Developmental - rat
Inhalation, Long-Term	Oral NOAEL= 0.88(b)	Increased liver non-neoplastic lesions (fatty vacuolation) at 6.48 mg/kg/day.	Chronic/ carcinogenicity - rat

a = A 100 % dermal absorption should be used for dose to dose extrapolation.
b = A 100 % inhalation absorption should be used for dose to dose extrapolation.

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Chemical:	**1H-1,2,4-Triazole, 1-((4-bromo-2-(2,4-di**
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