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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

PC 128831 3

EPA Reviewer: Pamela M. Hurley, PhD Pamela M Hurley, Date 2/13/2001
Registration Action Branch 2 (7509C)

EPA Secondary Reviewer: John Whalan, Date 2-13-01
Registration Action Branch 2 (7509C)

DATA EVALUATION RECORD
Supplement to DER for MRID No.: 43393401 Cyfluthrin: [Inhalation Developmental Toxicity Study] **This supplement includes an updated executive summary.**

STUDY TYPE: Inhalation Developmental Toxicity - Rat
OPPTS Number: 870.3700 OPP Guideline Number: §83-3

DP BARCODE: N/A SUBMISSION CODE: N/A
P.C. CODE: 128831 TOX. CHEM. NO.: 266E

TEST MATERIAL (PURITY): Cyfluthrin Technical; Cyano(fluoro-3-phenoxyphenyl)methyl-3-(2,2-dichloroethenyl)-2.2-dimethyl-cyclopropanecarboxylate (96.2% a.i.)

SYNONYMS: FCR 1272, Baythroid™

CITATION: Holzum, B.; Schmidt, U.; Pauluhn, J.; et al. (1994) FCR 1275: Explanatory Report on Results and Mechanistic Studies for Embryotoxic Effects in Rats After Inhalation: Lab Project Number: 106369: 23219: 22581. Unpublished study prepared by Bayer Ag. MRID 43393401.

SPONSOR: Miles Inc., Agriculture Division, 8400 Hawthorn Road, Box 4913, Kansas City, MI 64120-0013

EXECUTIVE SUMMARY: FCR 1272 (technical cyfluthrin (96.2% a.i.) (MRID 43393401) was administered by inhalation to 25 pregnant female Wistar rats/group at the following analytically determined concentrations: air control, vehicle control (polyethylene glycol-400: 50% ethanol), 0.00046, 0.00255, 0.0119 or 0.0128 mg/L plus 39% oxygen for gestational days 6 through 15 in a dynamic nose-only inhalation chamber. The rats were exposed to the test material 6 hours/day, 7 days/week. The MMAD ± geometric standard deviation was 1.1 ± 1.5 µm for all the groups. Additional satellite rats (5/group) were exposed similarly from gestational day 6 through day 13 to determine the effect of cyfluthrin exposure on body temperature, ventilation rate and plasma cyfluthrin levels.

Maternal body weight was statistically significantly decreased at ≥ 0.00046 mg/L for the interval gestational days 6 to 15 (≥ 26% from the vehicle control) and gestational days 0 to 20 (≥ 16% from the vehicle control). The relative efficiency of food utilization appeared to be decreased at

≥ 0.00046 mg/L (≥ 9% of the vehicle control). Increases in untidy and ruffled fur and retarded breathing were observed at 0.0119 and 0.0128 (with O_2) mg/L. Labored and irregular breathing, hypoactivity, high-stepping gait, salivation and reduced stool were observed only at 0.0119 mg/L. The satellite animals showed a decreased (but relatively flat dose-response) breathing rate/minute in the treated animals versus the air and vehicle control groups. Minimum volume (ml/min, ml/min/kg and as % of vehicle control) were decreased in a dose-related manner (bradypnea). Rectal temperatures were slightly decreased in the treated animals.

Placental weights (≥ 7% from the vehicle control) and fetal weights (≥11% from the vehicle control) were decreased at ≥ 0.00255 mg/L. Developmental toxicity was also expressed in the form of retarded skeletal ossification in the phalanx, metacarpals, cervical vertebrae, sacral and caudal arches at ≥ 0.00255 mg/L (Table G). Dose-related total fetal malformations such as microphthalmia and skeletal dysplasia were evident at 0.0119 mg/L (8.8% in fetuses/43% in letters vs. vehicle controls with 1.0% in fetuses/14% in litters).

The weight of the evidence would suggest that the cyfluthrin exposure at 0.0119 and 0.0128 mg/L caused the developmental toxicity partly through the bradypnea in dams. While the bradypnea in dams at 0.00255 mg/L may have caused the reduced fetal and placental weight and retarded ossification, the data presented were insufficient to draw this conclusion. The potential mechanism is discussed further at the end of this review under the Discussion.

The maternal LOAEL is 0.00046 mg/L based on decreased body weight gain and reduced relative food efficiency. The NOAEL is not determined. The developmental NOAEL is 0.00046 mg/L and the developmental LOAEL is 0.00255 mg/L based on reduced fetal and placental weights and reduced ossification in the phalanx, metacarpals and vertebrae.

This study is classified as **acceptable guideline** and satisfies the guideline requirements for a developmental toxicity study (**§83-3, 870.3700**) in the rat via inhalation.

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

#266E

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

Subject: ID# 128831: Cyfluthrin; DER on the Inhalation Developmental Toxicity Study in Rats (MRID# 43393401).

Barcode No.: D208938.
Submission No.: S476252.
Case No.: 003677.
Company No.: 003125.

ToxChem No.: 266E
PC No.: 128831.
MRID No.: 43393401.

From: David G Anderson, PhD 2/14/95
Toxicologist,
Section 3, Toxicology Branch-1
HED (7509C)

To: George LaRocca/John Hebert PM 13
Reregistration Branch
SRRD (7508W)

Thru: Karen Hamernik, PhD 3/18/95
Section 3 Head, Toxicology Branch-1
HED (7509C)

The registrant submitted a developmental toxicity study in rats via inhalation.

B Holzum and J Pauluhn (8/12/94) Explanatory Report on Results and Mechanistic Studies for Embryotoxic Effects in Rats after Inhalation. Study conducted by Bayer AG for Miles Lab. Study No. Main Report 23219; Suppl. No. 1, T3041008, report no. 22581; Suppl. No. 2: T3041008, report no. 22726; Suppl. No. 3: T4041207/T1041006, report no. 21865; Suppl. No. 4: T3034510, report no. 19852. MRID# 43393401. 3 Volumes.

The developmental effects occurring in this study may be secondary to the bradypnea in dams at the highest exposure levels or all exposure levels. Thus, this bradypnea should be taken into account in any risk assessment.

EXECUTIVE SUMMARY: FCR 1272 (cyfluthrin) was administered by inhalation to 25 female Wistar rats per group for air control, vehicle control (polyethylene glycol-400: 50% ethanol), 0.46, 2.55, 11.9 or 12.8 mg/m^3 plus 39% oxygen (analytically determined) exposure levels for gestational days 6 through 15 in

a nose only inhalation chamber. The rats were exposed to the test material 6 hours per day, 7 days per week. The particle sizes in the inhalation chambers had a MMAD ± geometric standard deviation of 1.1 ± 1.5 μm for the all groups. Additional satellite rats (5 per group) were exposed similarly from gestational day 6 through day 13 to determine the effect of cyfluthrin exposure on body temperature, ventilation rate and plasma cyfluthrin levels.

Maternal body weight was statistically significantly decreased at ≥ 0.46 mg/m^3 for the interval gestational day 6 to 15 (≥ 26% from the vehicle control) and gestational day 0 to 20 (≥ 16% from the vehicle control). The relative efficiency of food utilization appeared to be decreased at ≥ 0.46 mg/m^3 (≥ 9% of the vehicle control).

Placental (≥ 7% from the vehicle control) and fetal weights (≥ 11% from the vehicle control) were decreased at ≥ 2.55 mg/m^3. Developmental toxicity was also expressed in the form of retarded skeletal ossification in the phalanx, metacarpals, cervical vertebrae, sacral and caudal arches at ≥ 2.55 mg/m^3 (Table G). Dose related total fetal malformations such as microphthalmia and skeletal dysplasia were evident at 11.9 mg/m^3 (8.8% in fetuses/43% in litters vs. vehicle controls with 1.0% in fetuses/ 14% in litters).

The weight of the evidence would suggest that the cyfluthrin exposure at 11.9 and 12.8 mg/m^3 caused the developmental toxicity indirectly through the bradypnea in dams. While the bradypnea in dams at 2.55 mg/m^3 may have caused the reduced fetal and placental weight and retard ossification, the data presented was insufficient to draw this conclusion. However, this reviewer believes that the data presented are sufficient to raise questions about the appropriateness of this study for risk assessment for potential human developmental effects from low level cyfluthrin exposure via inhalation. The potential mechanism is discussed further at end of this review under Discussion.

The maternal NOEL/LOEL were < 0.46/< 0.46 mg/m^3 based on decreased body weight gain and reduced relative food efficiency. The developmental NOEL/LOEL were 0.46/2.55 mg/m^3 based on reduced fetal and placental weight, reduced ossification in the phalanx, metacarpals and vertebrae.

Core classification: Guideline. The study (MRID# 433934-01) is acceptable under guideline 83-3 for a developmental toxicity in rats via inhalation.

Primary reviewer: David G Anderson, PhD. David G Anderson 2/14/95
Section 2, Tox. Branch-1 (7509C).
Secondary reviewer: Karen Hamernik, PhD.
Section 2, Tox. Branch-1 (7509C).

DATA EVALUATION REPORT

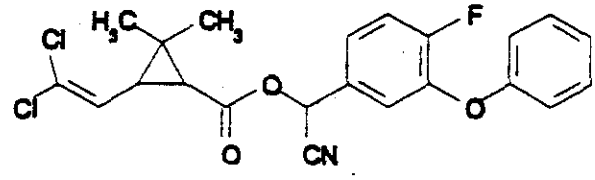
STUDY TYPE: Inhalation Developmental Toxicity (Suppl. study no. 1, 2, 3 & 4).

DP Barcode No.: D208938. Submission No.: S476252.
ToxChem. No.: 266E. MRID No.: 433934-01.
PC No.: 128831. Action Code: 405 6(a)(2).
Cas No.: 68359-37-5. Case: 003677.

TEST MATERIAL: Cyfluthrin, technical. Cyano(fluoro-3-phenoxyphenyl)methyl-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylate.

SYNONYMS: FCR 1272. Baythroid™, a pyrethroid.

STRUCTURE:



SPONSOR: Miles Inc., Agriculture Division, 8400 Hawthorn Road, Box 4913, Kansas City, MI 64120-0013.

TESTING FACILITY: Bayer AG, Fachbereich Toxikologie, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal.

STUDY NO.: Main report No. 23219;
Suppl. study No. 1: T3041008, report no. 22581.
Suppl. study No. 2: T3041008, report no. 22726
Suppl. study No. 3: T4041207/T1041006, report no. 21865
Suppl. study No. 4: T3034510, report no. 19852
Other reported no. 106369.

REPORT TITLE: FCR 1272 (Cyfluthrin); Explanatory Report on Results and Mechanistic Studies for Embryotoxic Effects in Rats after Inhalation and an Inhalation Study for Embryotoxic Effects in Rats.

AUTHOR(S): B Holzum and J Pauluhn.

REPORT ISSUED: Main report: August 12, 1994; No.1, October 5, 1993; No. 2, December 2, 1992; No. 3, November 24, 1992 and No. 4, January 11, 1991.

EXECUTIVE SUMMARY: FCR 1272 (cyfluthrin) was administered by inhalation to 25 female Wistar rats per group for air control, vehicle control (polyethylene glycol-400: 50% ethanol), 0.46, 2.55, 11.9 or 12.8 mg/m^3 plus 39% oxygen (analytically determined) exposure levels for gestational days 6 through 15 in a nose only inhalation chamber. The rats were exposed to the

test material 6 hours per day, 7 days per week. The particle sizes in the inhalation chambers had a MMAD ± geometric standard deviation of 1.1 ± 1.5 μm for the all groups. Additional satellite rats (5 per group) were exposed similarly from gestational day 6 through day 13 to determine the effect of cyfluthrin exposure on body temperature, ventilation rate and plasma cyfluthrin levels.

Maternal body weight was statistically significantly decreased at ≤ 0.46 mg/m^3 for the interval gestational day 6 to 15 (≥ 26% from the vehicle control) and gestational day 0 to 20 (≥ 16% from the vehicle control). The relative efficiency of food utilization appeared to be decreased at ≤ 0.46 mg/m^3 (≥ 9% of the vehicle control).

Placental (≥ 7% from the vehicle control) and fetal weights (≥ 11% from the vehicle control) were decreased at ≥ 2.55 mg/m^3. Developmental toxicity was also expressed in the form of retarded skeletal ossification in the phalanx, metacarpals, cervical vertebrae, sacral and caudal arches at ≥ 2.55 mg/m^3 (Table G). Dose related total fetal malformations such as microphthalmia and skeletal dysplasia were evident at 11.9 mg/m^3 (8.8% in fetuses/43% in litters vs. vehicle controls with 1.0% in fetuses/ 14% in litters).

The weight of the evidence would suggest that the cyfluthrin exposure at 11.9 and 12.8 mg/m^3 caused the developmental toxicity indirectly through the bradypnea in dams. While the bradypnea in dams at 2.55 mg/m^3 may have caused the reduced fetal and placental weight and retard ossification, the data presented was insufficient to draw this conclusion. The potential mechanism is discussed further at end of this review under Discussion.

The maternal NOEL/LOEL were < 0.46/< 0.46 mg/m^3 based on decreased body weight gain and reduced relative food efficiency. The developmental NOEL/LOEL were 0.46/2.55 mg/m^3 based on reduced fetal and placental weight, reduced ossification in the phalanx, metacarpals and vertebrae.

Core classification: Guideline. The study (MRID# 433934-01) is acceptable under guideline 83-3 for a developmental toxicity in rats via inhalation.

A. MATERIALS:

1. Test compound: Test material: FCR 1272; technical grade cyfluthrin, a pyrethroid; Description, yellow brown, solidified mass, clear yellow-brown oil above 50°C. Batch # 238005176, purity - 96.2%. Molar mass: 434.3 g/mole. Empirical formula: $C_{22}H_{18}FNO_3$. FCR 1272 is a synthetic pyrethroid composed of 4 diastereomers: isomer I cis (23.9%), isomer II cis (18.8%), isomer III trans (34.4%) and isomer IV trans (23.0%); total = 95.2%. The vehicle used to nebulize the test material was polyethylene glycol 400:ethanol = 1:1 [The ethanol vaporizes during aerosol generation and promotes smaller particle sizes. A 4-week inhalation study with rats indicate that there were no

toxicological differences between this vehicle (exposure concentration unspecified) and air.]

2. Test animals: Species: Rat, Strain: Wistar (Bor:WISW; SPF Cpb), Age: ≈ ?, Weight: Males > 300 g and females 186 to 244 g at day 0 post coitus (p.c.), Source: F. Winkelmann Co., Borchen. Acclimatization was 7 days.

3. Environmental Conditions: Animals were housed individually in room 546, building 514 in Makrolon™ Type II cages with low dust wood shavings as nesting material (analysis on file at Bayer). Room temperature 22.5 ± 0.5°C and the humidity was 50 ± 10%. Light:dark = 6AM:6PM. Air was changed 10 times per hour. The animal rooms were disinfected weekly with aqueous Rapidosept™ or Zephirol™. Cages were initially washed with hot water.

4. Food and Water: Food was standard diet (Altromin™ 1324 from Altromin Co. in Lage) and tap water. Both food and water were provided *ad-libitum* and analyzed and records are on file at Bayer AG.

B. **STUDY DESIGN**: Mating was natural after which the animals were randomly assigned. In the main study dams were exposed on gestational day (gd) 6 through 15 and satellite animals were exposed day 6 through gd 12. Presumed pregnant female rats (25 per group) were exposed for 6 hours daily gd 6 through 15 (10 exposures) via nose only inhalation. In addition, satellite groups of 5 pregnant females were exposed similarly (but for 8 exposures only) and the maternal parameters were investigated such as reflexes, rectal temperature, lung function tests (including respiration rate and respiration minute volumes) and plasma levels of FCR 1272. Chamber temperature was 22°C±2°C and humidity was < 14%. Gross necropsy was conducted on the dams. Fetuses were examined viscerally by a modified Wilson technique and skeletally by the Dawson technique. A description of the inhalation procedures, specifications and the special studies on the satellite animals are described in more detail in the Appendix to this DER.

1. Stability Studies - Cyfluthrin is stable in the stock solution (3.15% nominal concentration) in vehicle for at least 72 hours and in the diluted spray solutions for at least 6.5 hours and each diastereomer of cyfluthrin was stable for 6 days in the stock solution.

2. Animal Assignment - Animals were assigned to cages by random numbers.

Test group	Nominal dose in mg/m^3	Number of animals Female	Mean analytical conc. of FCR 1272-vehicle (standard deviation) (% by volume)
1. Vehicle		25	0
2. Cont.	Air	25	0
3. Low (LDT)	0.5	25	0.46 (0.134)
4. Mid (MDT)	2.5	25	2.55 (0.183)
5. High (HDT)	12.5	25	11.9 (1.45)
6. High(HDT)	12.5+40% O_2	25	12.8 (1.06) + 39% O_2

The vehicle was 50:50 Polyethylene glycol 400: ethanol; Nebulizer airflow was 10 Lpm.

3. Statistics - The following procedures were utilized in analyzing the numerical data: 1. Fisher's exact significance test at $p \leq 0.05$ and $p \leq 0.01$ (two tailed) was used for Fertility and gestation rate. 2. The F-test, and t-test or Welch t-test were used for the food intake, body weight gains, corpora lutea per dam, implantations per dam, live fetuses per dam, fetal and placental weight per dam and malformed fetuses per dam. 3. Yates-corrected CHI^2 was used for fetuses or litters with malformation, fetuses or litters with deviations (variations including retardation), pre- and post implantation loss.

4. A quality assurance statement was signed 10/4/93 by H. Lehn of QA. The study was inspected 4/22,5/15,5/18,5/19,5/25,6/3/1992 and 1/26 and 2/24/1993.

C. **METHODS AND RESULTS**: Exposure chamber conditions and axillary apparatus are more completely described in the Appendix.

1. Exposures - The report stated for all groups 98% of the mass fraction of the particles had a aerodynamic diameter $\leq 3\mu m$ and that for all groups the MMAD = 1.1 μm; GSD = 1.5 (p 754) (Table A).

The ratio of the diastereomers in the stock and spray solutions was determined by gas chromatography and flame ionization detector. The system computer controlled and maintained very stable exposure conditions.

Selected particle analyses were performed with Berner cascade impactors (Type I and II). The individual impactor stages were gravimetrically evaluated.

(a) The bases of the exposure levels

The exposure concentrations selected were based on a preliminary 4 week subacute inhalation study at 0.44, 6.0 and 46.6 mg/m^3 for 6 hours per day, 5 days per week to 10 Wistar rats per sex per group. No gender specific changes were noted at any dose level. Bradypnea, piloerection and hyperactivity were noted at 46.6 mg/m^3. No specific body weight changes were noted in female rats up to 46.6 mg/m^3, but toxicologically and

statistically significant body weight changes were noted in males ≥ 6 mg/m^3. Transient reflexively induced slight bradypnea and body temperature decrements were induced by cyfluthrin at ≥ 6 mg/m^3. Relative decreases in thymic weights and marginal increases in adrenal weights were attributed to the stress associated with sensorial irritation. Based on the results from this study 0.5, 2.5 and 12.5 mg/m^3 were selected for the developmental toxicity study.

(b) Studies with the satellite animals

These studies were conducted because it is desirable to document the respiratory disturbances caused by the test material under conditions similar to those in the developmental toxicity study without disturbing the results from the main study.

Lung function tests were conducted under quasi-isobar and isothermal conditions in a whole-body plethysmography in 4 satellite animals per group (page 755 of the submitted report). The animals were adapted for about 10-30 minutes followed by basal lung function parameters for approximately 15 minutes. The animals were exposed to air only during this period, which was termed the control period in the tables on the lung function parameters in the submitted report. A 4-5 hour exposure coupled with measurement of lung function followed the control period. Due to these closed systems, 4-5 hours measurement is the maximum feasible. After lung function tests, nose only exposure was conducted such that actual daily exposure was about 6 hours.

Lung function tests were conduced on the first day of exposure (gd 6), only for satellite animals (p785 of the submitted report). If other lung function studies were conducted on additional days in the satellite animals, I could not find the data. However, the location of some of data was not always presented in the table of contents and frequently tables in the report did not adequately identify for which study the data were generated.

The discussion in submitted report (p810) indicates that the 4-week rang-finding study showed no evidence of reflex bradypnea receptor damage, desensitization, i.e., no evidence that multiple exposures lead to an return toward normal breathing rates in terms of ml/min/kg. It should be noted that rectal temperatures after multiple exposures appeared to return toward normal (data discussed below).

Rectal temperatures were collected on all satellite animals immediately following exposure on gd 6 and gd 12 using a digimed H 11 digital thermometer with a F2 probe.

Plasma levels of FCR 1272 were determined on satellite animals exposed at 11.9 and 12.8 mg/m^3 + 39%O_2.

Results - Table A shows examples of the exposure concentrations during the main developmental toxicity study and for the special studies conducted on the 5 satellite animals exposed similarly, but for shorter time period. Only selected determinations on an average basis are presented in Table A, however, daily concentrations were determined and presented in the submitted report.

Exposure levels in the main study and satellite animals are presented in Table C. The average dosage in mg/kg/day associated with the nominal exposure levels of 0.5, 2.5, 12.5 mg/m^3 (analytical exposure levels of 0.46, 2.55, 11.9 and 12.8 mg/m^3 + 39% O_2) are calculated below.

To calculate the average dose level in mg/kg/day, the data from Table C were used. From the minute volumes (1202, 1099, 706 and 650 ml/minute/kg, Table C) determined in the satellite animals at the exposure levels, it can be calculated that for the 0.46 mg/m^3 exposure level,

Dose in mg/day/kg = (1202 ml/min/kg) X (l/1000 ml) X 0.46 mg/m^3 X (m^3/1000 l) X (60 min/hr) X (6 hr/day) = 0.199 mg/kg/day.

Similarly the dose levels in mg/kg/day can be calculated for the remaining exposure levels (Table A & C). These calculations assume 100% lung absorption of the cyfluthrin; in most inhalation studies only a fraction of the dose is absorbed.

Table A: Summary of daily mean mass aerodynamic diameter (MMAD), standard deviations and range in MMAD in micrometers (μm) during the study. GSD = Geometric standard deviation and dose levels in mg/kg/day.

Parameter	Vehicle	0.46 mg/m^3	2.55 mg/m^3	11.9 mg/m^3	12.8 +O_2 mg/m^3
Dose level (mg/kg/day)	-	0.199	1.02	3.02	3.00
	MMAD ± GSD	MMAD ± GSD	MMAD ± GSD	MMAD ± GSD	MMAD ± GSD
Minimum	1.0 ± 1.8	1.06 ± 1.97	0.98 ± 1.96	1.05 ± 2.02	1.01 ± 1.95
Maximum	1.17 ± 1.37	1.9 ± 1.84	1.20 ± 1.39	1.22 ± 1.89	1.20 ± 1.38
Mean	1.10 ± 1.47	1.12 ± 1.49	1.12 ± 1.50	1.14 ± 1.51	1.15 ± 1.50
Standard deviation (SD of GSD)	0.05 (0.24)	0.033 (0.26)	0.056 (0.25)	0.046 (0.27)	0.065 (0.26)

The mean mass aerodynamic diameter of the exposure aerosol ± GSD for the main study animals and satellite animals are presented in Table A.

The ventilation rates (ml/minute/kg) in these satellite animals were decreased compared with the air and vehicle control, respectively: 21% and 29% at 0.46 mg/m^3, 28% and 37% at 2.55 mg/m^3, 54% and 58% at 11.9 mg/m^3 and 57% and 61% at 12.8 mg/m^3 plus 39% O_2 (Table C).

The data on the rectal temperature was variable, but it generally supported a decreased temperature in animals breathing cyfluthrin at ≥2.55 mg/m^3 (Table B & C). The temperature decrement in animals exposed on the first day was generally greater than in animals exposed for 6 hours after 6 days of exposure. No data were submitted for the possible temperature

decreases on other days, but it could be assumed that it was less than on gd 6, the first day of exposure.

Table B: Temperature deficit after and before exposure 6 hour for the 6th exposure for satellite animals (p799 and 974-975,978-979 of the submitted report).

Exposure levels	Air control	Vehicle control	0.46 mg/m^3	2.55 mg/m^3	11.9 mg/m^3	12.8 mg/m^3 +39%O_2
Individual animal	Temperature change in °C after a 6 hour exposure on gestational 6.					
1	+0.6[a]	-0.20	-2.20	-2.20	-3.90	-5.10
2	0.0	-1.10	-1.20	-5.90	-5.60	-4.30
3	-0.80	-1.40	-1.40	-4.60	-6.10	-5.60
4	+0.30	+0.40	-2.90	-2.80	-4.00	-6.30
5	+0.70	-0.40	-2.40	-1.50	-4.20	-4.50
Mean	+0.16[a] (+0.48)[b]	-0.54[a] (+0.70)[b]	-2.02	-3.40	-4.76	-5.16
Individual animal	Temperature change in °C after a 6 hour exposure on day 12.					
1	-0.20[a]	+1.20	+0.90	-0.40	-1.70	-1.00
2	+0.10	+0.70	-0.10	-0.10	-3.60	-0.60
3	+0.30	+1.20	+1.30	-0.80	-4.30	-2.50
4	0.0	+0.40	+0.10	-0.50	-1.10	-0.80
5	+0.10	+1.10	+0.30	0.0	-2.50	-0.80
Mean	+0.14	+0.92	+0.54	-0.36	-2.64	-1.14

[a] = Data calculated from the individual animal data (p974, 975, 978 & 979 of the submitted report). [b] = Data from the summary table on p799 in the submitted report.

The variation in temperature in individual animals was large (p974-5 of the submitted report). The maximum temperature among all the individual satellite animals on gd 6 before exposure at 2.55 mg/m^3 was 38.1°C and the minimum temperature after 6 hours exposure on gd 6 was 32.1°C (a difference of 6°C)(data not shown). The maximum temperature in individual satellite animals before the day 12 exposure at 2.55 mg/m^3 was 37.6 and minimum temperature after 6 hours of exposure on gd 12 was 36.7°C (a difference of 1°C). Thus, animals in the main study may have also varied by larger amounts than would be indicated by data from the satellite animals.

In the satellite females the mean rectal temperature deficit was -4.8°C at 11.9 mg/m^3 and -5.2°C at 12.8 mg/m^3 (Table B) after a 6 hour exposure on gd 6 with other tests being conducted during the 6 hour exposure. Whereas, in the satellite females the mean rectal temperature was -2.7°C at 11.9 mg/m^3 and -1.1°C at 12.8

mg/m^3 (Table B) after 6 days of 6 hour exposures on gd 12 with other tests being conducted during the 6 hour exposure. Other special studies [See the Appendix, (c) special study on 13.2 mg cyfluthrin/m^3 in air (Table L)] showed 4.8°C decreases in temperature after 4 hours of exposure..

The range in temperature change among the 5 satellite animals on gd 12 at 2.55 mg/m^3 was 0.0 to -0.8°C, at 11.9 mg/m^3, the range was -1.1 to -4.3°C, at 12.8 mg/m^3 + 39% O_2, the range was -0.60 to -2.5°C (Table B). If the temperature deficit in the main study was as small as that indicated from satellite animals after multiple exposures at 2.55 mg/m^3 indicates, then additional mechanisms probably would be needed to explain the delayed development at this exposure level, such as other results of the bradypnea. The cause of the developmental effects at 2.55 mg/m^3 is unknown. Even at the 11.9 and 12.8 mg/m^3 exposure levels, the temperature decreases of the magnitudes shown in the satellite animals have not been proven to cause the retarded development seen at these dose levels, although these temperature deficits are possibly responsible.

The temperature deficit in the satellite animals after more than one 6 hour exposure was small and there are only equivocal data or evidence of a rectal temperature reduction that could cause the developmental effects in animals at 2.55 mg/m^3. However, data on the effect of decreased blood glucose and/or other energy sources on development and in the cyfluthrin exposed animals may indicate the indirect effects of bradypnea. This reviewer believes that these effects could be profound, but will not **assume** that they occur in the current study.

Plasma levels of cyfluthrin were determined in satellite animals exposed to 11.9 mg/m^3 (plasma levels = 19.0±13.3 [CV=70%] pmol cyfluthrin/ml of plasma) and those exposed to 12.8 mg/m^3 + 39% O_2 (plasma levels = 14.7±4.4 [CV=30%] pmol of cyfluthrin/ml of plasma) (page 1099 of the submitted report). These plasma levels were determined after the 7th day of treatment in 5 satellite animals dosed at 11.9 and 12.8 mg/m^3 during the main developmental toxicity study and are not statistically significantly different from each other. However, because of the extremely high CVs involved, in the opinion of the current reviewer, these values have been demonstrated to be similar, only.

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Table C: Summary of mean concentrations and selected exposure conditions showing equipment parameters for the main study and the satellite animals exposed similarly. Data collected on the special studies on 5 satellite females per group after exposure are also shown. Rectal temperatures can be found on page 799 and 800 and 974 and 989 of the submitted report. Lung function summaries p804 (data p1024-1085 of the submitted report).

Exposure concentrations, calculated dosage and exposure parameters for females in the developmental toxicity study and for satellite females						
Nominal exposure concentration (mg/m^3)	Air	Vehicle	0.5	2.5	12.5	12.5 + O_2
Mean analytical concentration (mg/m^3)	-	-	0.458	2.55	11.883	12.832
Calculated dosage (mg/kg/day)	-	-	0.199	1.01	3.02	3.00
Chamber volume (l)	7.6	7.6	7.6	7.6	7.6	7.6
Air flow -main (l/min) -through generator -total -exhaust -air changes/hr -nominal conc. (mg/m^3)	15.0 15.0 30.0 27.0 237 0	15.0 15.0 30.0 27.0 237 0	15.0 15.0 30.0 27.0 237 3.3	15.0 15.0 30.0 27.0 237 11.0	15.0 15.0 30.0 27.0 237 60.0	15.0 15.0 30.0 27.0 237 60.0
Generation test atmosphere -dispersion pressure -vehicle (µl/30 l/min) -spray sol'n (%)	 600 0 0	 600 100 0	 600 100 0.1	 600 100 0.33	 600 100 1.8	 600 100 1.8
Chamber temp. (°C)	22.5	22.4	22.8	22.8	22.9	23.5
Chamber rel. hum. (%)	3.9	4.1	11.6	5.0	6.9	9.7
O2 conc. (%)	20.5	20.5	20.4	20.5	20.5	39.2
CO2 conc. (%)	0.33	0.370	0.27	0.23	0.22	0.13
Parameters (special) on the 5 satellite dams per dose level exposed in the same inhalation apparatus	5	5	5	5	5	5
Mean breathing rate/min.	143	148	115	107	111	89
Min. vol. (ml/min)	382	411	298	258	164	155
Min. vol. (ml/min/kg)	1524	1682	1202	1099	706	650
Min. vol. as % of vehicle control	90.6	100	71.5	65.3	42.0	38.6
Rectal temperature before exposure on day 0 ± SD	37.5± 0.20	37.6± 0.30	38.0± 0.45	37.8± 0.29	37.7± 0.18	37.7± 0.47
Rectal temperature after 6 hours of exposure ± SD	37.6± 0.72	37.0± 0.70	36.0± 0.36	34.4± 1.68	32.9± 1.07	32.6± 0.58
Rectal temperature before exposure on day 6 ± SD	37.4± 0.32	37.6± 0.30	37.5± 0.29	37.5± 0.08	37.4± 0.23	37.2± 0.21
Rectal temperature after exposure on day 6 ± SD	37.6± 0.30	38.5± 0.14	38.0± 0.53	37.2± 0.38	34.7± 1.29	36.1± 0.85

2. Observations - Animals were inspected daily after exposure for signs of toxicity and mortality and before and after the inhalation exposure. In addition to the clinical observations, observations on 5 animals per group were studied after 7 days of exposure for visual placing response, grip strength, tonus, corneal-reflex, light-reflex, pinna reflex, startle response-sound (no reaction, normal reaction), startle response-touch (no reaction, turn around, freezes, aggressive reaction), tail pinch (no reaction, indifferent turn around, freezing+vocalization, exaggerated response+vocalization), righting, righting-dorsal drop 30 cm.

Results - Toxicity - Dose related increases in observations occurred at 11.9 and 12.8 mg/m^3 + O_2, such as untidy fur (8 & 9, respectively), ruffled fur (19 & 21, respectively), retarded, labored and/or irregular breathing (18 & 10, respectively). High stepping gait and hypoactivity were seen only at 11.9 mg/m^3 (Table D) in 5 of the animals with one or more of the breathing problems. Only 1 animal demonstrated the hypoactivity with retarded breathing and ruffled fur at 12.8 mg/m^3 + O_2 (Table D).

A few observations in controls, 0.46 and 2.55 mg/m^3 groups were unrelated to dose. There were 4 incidences of untidy fur in controls, 6 at 0.46 mg/m^3 and 2 at 2.55 mg/m^3 (Table D). In addition, bloody mouth, ruffled fur (and untidy fur) and untidy fur were each seen in 3 different animals at 2.55 mg/m^3 (Table D).

In addition to the clinical observations, behavioral/reflex observations were made on satellite dams after 7 days of exposure. Although, a few parameters on the behavior/reflex observations were statistically significantly different from the control reaction, the authors believed there was no test material related or pathodiagnostic differences. The data indicated no reaction occurred in 4/5 females associated with tail pinch at 0.46, 11.9 and 12.9 mg/m^3 (only 2/5 showed a positive response at 2.55 mg/m^3). These reactions appeared to indicate a lack feeling in the tail (page 915 to 919 of the submitted report). However, since the methods were not reported, no positive control data was reported and only 2/5 responded at 2.55 mg/m^3, the findings may not be meaningful (Data not otherwise shown).

Mortality (Survival) - One animal died at 2.55 mg/m^3 and the death was considered incidental to the study.

Table D: Clinical Observations in rats after exposure to cyfluthrin. Some animals may have been counted more than once due to multiple observations.

Symptoms and Observations	Analytically determined exposure atmosphere concentration (mg/m^3)					
	Control (air)	Vehicle control	0.46	2.55	11.9	12.8 + O_2
Untidy fur	4	-	6	2	8	9
Ruffled fur	-	-	-	1	19	21
Hair loss, reddening at abdomen	-	-	-	-	1	-
Hair loss	-	-	-	-	-	3
Retarded breathing	-	-	-	-	17	10
Labored breathing	-	-	-	-	5	-
Irregular breathing	-	-	-	-	1	-
Hypoactivity	-	-	-	-	5	1
High-stepping gait	-	-	-	-	5	-
Salivation	-	-	-	-	1	-
Reduced stool	-	-	-	-	1	-

3. Body Weight - Body weight was determined daily from post coital (pc) day 0 to day 15 pc and at day 20 pc.

Results - Body weight gain was statistically significantly decreased at ≥ 0.46 mg/m^3 for the interval 6 to 15 (≥26% from the vehicle control) day 0 to 20 (≥14% from the vehicle control). The carcass weight was decreased (≥16% from the vehicle control) at ≥ 2.55 mg/m^3 (Table E).

Food consumption was statistically significantly decreased at pc day 6-11, 11-16 and 16-20 (6%, 3% and 7%, respectively from the vehicle control)(Table E) at 0.46 mg/m^3. A slight decrease in relative food efficiency as indicated by a greater percentage decease in body weight gain than in food consumption also supports toxicity at ≤ 0.46 mg/m^3 (Table E) and especially at the two HDT. However, all food consumption was within the historical control range following: study means pc day 6-11 = 16.3 to 18.9 g/animal/day, study means pc day 11-16 = 18.6 to 20.9 g/animal/day and study means pc day 16-20 = 19.2 to 23.2 (Data not otherwise shown).

5. Maternal Gross Findings - The only findings at gross necropsy of dams were helminths in 3/20, 8/20, 1/20, 7/20, 5/20 and 8/20 dams in air controls, vehicle controls, 0.46, 2.55, 11.9 and 12.8 mg/m^3, respectively.

Table E: Body weights (BWt)(p162-167), body weight gain (p58) and food consumption (p57) at selected intervals during exposure to cyfluthrin and pregnancy. The data were taken from tables in the report and some rounding errors may have occurred.

Female Group	Air control	Vehicle control	0.46 mg/m^3	2.55 mg/m^3	11.9 mg/m^3	12.8 mg/m^3 + O_2
Number of animals	21	22	23	23	23	23
BWt at sacrifice (day 20)	294.9±13.4	297.7±16.0	281.3±25.6	281.0±15.0	264.6±21.3	268.3±15.0
BWt at day 15	245.6±9.5	244.8±10.8	237.7±12.5	233.2±11.3	226.3±11.0	225.7±12.3
BWt at day 6	233.5±11.7	231.5±13.3	227.9±9.6	230.6±11.2	230.7±9.8	228.3±12.2
BWt gain day 6-15	12.0±6.4	13.3±5.6	9.8±7.3*	2.6±4.5*** ‡‡	-4.3±5.6*** ‡‡	-2.6±6.2*** ‡‡
BWt gain day 0-20	83.6±11.8	88.8±8.9	76.8±21.7*	74.7±8.9*** ‡‡	58.7±18.8*** ‡‡	62.3±14.2*** ‡‡
BWt gain day 0-20 (corrected for uterine Wt)	20.0±9.6	23.0±8.2	19.8±7.5	19.3±6.1*	13.6±8.7*** †	12.5±4.9*** ‡‡
Food consumption at selected intervals (g/animal/day)						
Food consumption day 0-6	19.6±1.1	19.9±1.7	19.5±1.2	20.1±1.0	19.8±1.4	19.7±1.3
Food consumption day 6-11	17.5±1.5	17.4±1.1	16.4±1.0** ‡‡	14.5±1.6*** ‡‡	13.0±2.5*** ‡‡	12.6±1.5*** ‡‡
Food consumption day 11-16	20.2±1.1	19.9±1.1	19.3±1.4 †	18.1±1.8*** ‡‡	16.3±2.1*** ‡‡	16.3±2.0*** ‡‡
Food consumption day 16-20	22.8±2.3	23.5±1.5	21.9±2.2**	22.8±1.4	22.2±2.0**	21.6±1.2*** †
Food consumption day 0-20	19.9±1.0	20.0±1.1	19.1±1.1** ‡‡	18.7±1.2*** ‡‡	17.7±1.6*** ‡‡	17.4±1.1*** ‡‡
Relative efficiency of food utilization (change in weight g)/(change in food consumption over the same time period)						
Body weight gain day 0 to 20	4.20	4.44	4.02	3.99	3.32	3.58
Uterine corrected body weight gain day 0 to 20	1.00	1.15	1.04	1.03	0.768	0.718

*, **, ***, †, ‡‡ & ‡‡‡ = Statistically significantly different from the vehicle control and air control, respectively at $p \leq 0.05$, ≤ 0.01 & ≤ 0.0001, respectively.

6. Maternal Examination and Reproduction Data -

Results - The placental weights (≥7% from vehicle controls) and fetal weights (≥11% from vehicle controls) were statistically significantly decreased at ≥ 2.55 mg/m^3 (Table F). There was a increase in pre-implantation loss at ≥ 0.46 mg/m^3 (165% of the vehicle control and 116% of the air control) that may have been due to toxicity or the stress of the inhalation procedure at this

dose level, since maternal weight gain and food consumption were also reduced (Table F). This pre-implantation loss was within the historical control values (The historical control range was 10.7% to 28.6% of corpora lutea in 5 studies or 115 litters) in all groups. No changes occurred in the number of corpora lutea (CL), resorptions or % implantations of CL, but live fetuses/dam were lower at 11.9 and 12.8 mg/m^3 (both 87% of the vehicle control).

The changes in ventilation, hypothermia, blood CO_2 and other similar parameters are discussed in the Appendix along with the special studies conducted with cyfluthrin.

Table F: Maternal reproduction data and placental and fetal weight.

	Exposure concentration (mg/m^3)					
Dose level	Air control	Vehicle control	0.46	2.55	11.9	12.8 + O_2
# dams with implantations # dams with viable fetuses Parameter	21 21	22 22	24 23	24 23	23 23	23 23
Corpora lutea (CL)/dam	14.3	14.2	13.6	13.7	13.9	13.5
Implantations % of CL/dam	86.0	90.1	83.7	83.2	81.5	83.5
Pre-implantation loss % of CL	14.0	9.9	16.3*	16.8*	18.5**	16.5*
Live fetuses/dam	11.6	12.0	10.7	10.9	10.4*	10.4*
Resorptions/dam (% of implantations/dam)	0.8	0.8	1.2	0.7	0.9	0.8
Early resorptions (% of implantations/dam)	0.4	0.3	0.8*	0.5	0.1	0.3
Late resorptions (% of implantations/dam)	0.4	0.5	0.4	0.2	0.8	0.6
Placental weights/dam	0.61	0.60	0.62	0.56*	0.46***	0.49***
Fetal weights/dam ♂ & ♀ ♂ ♀	3.41±0.2 3.49±0.2 3.36±0.2	3.50±0.2 3.57±0.3 3.43±0.2	3.48±0.4 3.48±0.4 3.34±0.3	3.13±0.3*** 3.19±0.3*** 3.06±0.3***	2.48±0.5*** 2.47±0.4*** 2.41±0.5***	2.83±0.2*** 2.91±0.2*** 2.76±0.2***

*, **, *** = Statistically significantly different from the vehicle control at $p \leq 0.05$, 0.001 & 0.0001, respectively.

7. Fetal examination - About half the fetuses were examined viscerally by a modification of the Wilson method and the remaining fetuses were examined skeletally by the Dawson technique. No heart malformations were found in spite of the finding of heart malformations in rats deprived of oxygen, however no visceral variations (indicative of effects on the heart) were reported and may not have been conducted. Haring noted that rats exposed to 6% CO_2 and 10% O_2 during gestation developed heart malformations [Haring, OM (1966) Circ. Res., 19:544-551].

Results on malformations, external, visceral and skeletal - (Table 6, page 48, pages 642-646 of the submitted report.) The most frequent malformation seen was microphthalmia at the 11.9 mg/m^3 and 12.8 mg/m^3 + O_2 dose levels. At these 2 dose levels the elevations in microphthalmia and total malformations appeared to biologically significantly elevated. Although not statistically significant in litters in these 2 groups, in fetuses it was statistically significant at 11.9 mg/m^3 (13 fetuses in 8 litters vs. 2 fetuses in 2 litters in the vehicle control)(Table G). Total malformations were similarly statistically significantly elevated in fetuses, but not in litters at 11.9 mg/m^3 (3 fetuses in 3 litters in the vehicle control vs, 21 fetuses in 10 litters at 11.9 mg/m^3), only (Table G). Thus, the number litters with malformations at 11.9 and 12.8 were 44% and 30%, respectively, which were just outside of and at the high end of the range. Historical control data on litters with malformations in individual studies range from 4.2% to 30%.

Results of visceral examination for variations - (pages 146 and 147 of the submitted report. Only selected data were extracted for the current review.)

Incidence of dilated renal pelves, undescended testes, a heart slightly dislocated to the left, an elevated liver and an eye with reduced lense size were scattered through the controls and dose groups with no dose relationship. These variations were considered incidental to the study. Historical control data was submitted with the study and where the data was comparable to the current study, it is reported with the effect. However, some of the data was reported differently from the current study and could not be compared, i.e., only total retarded ossifications were reported by (fetuses only) sternum, vertebral column, ribs, limbs, skull, hyoid bone and 14th rib (individual sites included were not given), making it impossible to compare with the individual sites of ossification as seen in Table H. Retarded ossification in litter data was reported only for 1 other study. In these cases statistically significant effects in fetuses and litters was considered indicative of effects.

Results of skeletal examination - These results are summarized for fetal and litter data. Only selected data were extracted from the submitted report. Pertinent pages in the submitted report are 74-110 (fetal data) and 111-145 (litter data).

Increased retarded ossification were seen at ≥2.55 mg/m^3 (Table H). The % affected fetuses and % affected litters with incomplete ossification and unossified phalanx in the fore and hind limbs at the 11.9 and 12.8 mg/m^3 +39%O2 exposure levels are presented in Table H. In a few parameters these reduced ossifications were demonstrated as low as 2.55 mg/m^3 in litters and fetuses (Table H).

Table G: Malformations noted on external and visceral examination (p 28 and p662 of the submitted report).

Dose level	Analytical concentration in mg/m^3					
Malformation	Air control	Vehicle control	0.46	2.55	11.9	12.8+02
Fetuses/litters examined	243/21	263/22	245/23	251/23	239/23	240/23
# fetuses/# litters affected						
Microphthalmia	1/1	2/2	1/1	3/2	13▼/8	7/5
Anophthalmia					1/1	1/1
Hydrocephalus internus	1/1					
Skeletal dysplasia (legs)		1/1	1/1	4/2	1/1	3/2
Filiform tail				1/1		
Spinal (displaced vertebrae					2/1	
Rib (fusion, supernumerary)					1/1	
Exoccipital bone and cervical vertebral arches malformed					3/1	
Dysplastic exoccipital bone					1/1	
Umbilical hernia					1/1	
Malformed fetuses/litters	3/2	3/3	2/2	9/4	21▲/10	10/7
% fetuses/% litters	1.24/9.5	1.14/13.6	0.82/8.7	3.19/17.4	8.70/43.5	4.17/30.4

▲, ▼ = Statistically significant at $p \leq 0.001$ and 0.01, respectively.

Table H: Selected fetal and litter data on skeletal variations. These data were extracted from the reported data on pages 74 through 145. Only those data that were statistically significant for litters at 2.55 mg/m^3 and above are presented here. The remaining data can be found in the submitted report.

Dose levels	Analytical exposure concentration (mg/m^3)					
Percentage of Findings (% fetuses affected/%litters affected)	Air control	Vehicle control	0.46	2.55	11.9	12.8 + O_2
# fetuses examined/litters examined	126/21	138/22	128/23	133/23	124/23	126/23
Proximal F phalanx incompletely ossified	22.2/52.4	31.2/68.2	23.4/47.8	3.0⊕/17.4▼	1.6⊕/8.7⊕	0.8⊕/4.3⊕
R 3rd	18.3/47.6	26.1/59.1	19.5/43.5	1.5⊕/ 8.7▼	1.6⊕/8.7▼	0.0⊕/0.0⊕
R 4th	18.3/47.6	24.6/59.1	21.1/43.5	3.8⊕/21.7⊕	1.6⊕/8.7▼	0.0⊕/0.0⊕
L 3rd	15.9/47.6	20.3/50.0	15.6/34.8	3.0⊕/17.4⊕	0.8⊕/4.3▼	0.0⊕/0.0⊕
L 4th						
Metacarpals unossified						
R 5th	8.7/38.1	4.3/27.3	3.9/21.7	33.8⊕/73.9▼	84.7⊕/91.3⊕	72.2⊕/91.3⊕
L 5th	7.1/28.6	4.3/27.3	3.9/21.7	33.1⊕/69.7⊕	85.5⊕/87.0⊕	70.6⊕/91.3⊕
Wavy ribs, 9th	2.4/9.5	2.9/13.6	7.8/26.1	15.8⊕/47.8⊕	2.4/13.0	15.9⊕/56.5▼
Cervical vertebrae -present	12.7/52.4	18.1/68.2	18.1/56.5	6.8▼ /30.4⊕	1.6⊕ /8.7⊕	3.2⊕/17.4▼
7th body -incompletely ossified	11.9/38.1	15.9/50.0	9.4/39.1	2.3⊕ 4.3▼	0.8⊕ /4.3▼	0.0⊕ /0.0⊕
5th body	14.3/47.6	30.4/90.9	24.2/69.6	9.0⊕ /43.5⊕	0.8⊕ /4.3⊕	2.4⊕ /13.0⊕
6th body						
Sacral vertebrae -incompletely ossified arches						
R 4th	5.6/28.6	6.5/27.3	6.3/21.7	22.6⊕/73.9▼	41.1⊕/82.6⊕	27.0⊕/78.3▼
L 4th	7.1/33.3	6.5/31.8	6.3/34.8	23.3⊕/69.6▼	40.3⊕/82.6▼	31.7⊕/82.6▼
Caudal vertebrae -present arches						
R 3rd	8.7/42.9	10.1/45.5	4.7 /17.4	1.5▼ /4.3▼	0.8▼ /4.3▼	1.6▼ /8.7⊕
-present						
L 5th body	59.5/100	55.8/90.9	39.1▼/87.0	16.5⊕/39.1⊕	5.6▼ /21.7⊕	6.3⊕ /30.4⊕

⊕,▼,⊕ = Statistically significant at $p \leq 0.001$, 0.01 & 0.05, respectively, compared with the vehicle control and for % affected fetuses and % affected litters, respectively, compared with the vehicle control.

The addition of 39% O_2 to the 12.8 mg/m^3 exposure level reduced the incidence of retarded skeletal ossification in some but not all end points. Some endpoints showed statistical significant increase in delayed ossification at 11.9 and not at 12.8 mg/m^3. In some end points retard ossification was greater and statistical significance at 12.8 mg/m^3 + 39%O_2 than at 11.8 mg/kg. Table I is an attempt to shows the comparative frequency with which ossification delays were exhibited at 12.8 and 11.9 mg/m^3.

Thus, addition of oxygen to the exposure atmosphere slightly

reduces the frequency and severity of the some of the skeletal variations (Table I). Of the 319 parameters studied, 94 were statistically significantly different from control values either at 11.9 mg/m or/and at 12.8 mg/m^3 + 39%O_2 exposure levels, 85 were statistically significant at the 11.9 mg/m^3 and 63 at the 12.8 mg/m^3 + 39%O_2. Of the 94 parameters, 38/94 were statistically significant at the 11.9 mg/m^3 only, 15/94 were statistically significant at the 12.8 mg/m^3 + 39%O_2 dose level only while 20/94 were statistically significant at a probability greater in the 11.9 mg/m^3 exposure group than in the 12.9 mg/m^3 + 39%O_2 exposure group when both exposure groups were statistically significant (Table I).

Table I: Summary litter data on skeletal variations at 11.9 mg/m^3 and 12.8 mg/m^3 + 39% O_2. These data were extracted from the reported data on pages 74 through 145. The data can be found in the data copied from the submitted report. Since these data included more endpoints than are include in Table H, the incidence reported in Table H can not be compared with incidence reported in Table I.

Dose level	Analytical dose levels mg/m^3	
Number of parameters significantly different than control values	11.9	12.8 + 39% O_2
Number fetuses examined/litters examined	124/23	126/23
Number of parameters studied	319	319
Number of parameters that statistically significant in 1 or both dose levels	94	94
Number of parameters that were statistically significant	85	63
Number of parameters that were statistically significant at 1 dose level only	38	15
When both dose levels were statistically significant: Number of parameters with a statistical significance at p value less than that for 12.8 mg/m^3 + 39% O_2.	20	

D. DISCUSSION: (especially of the mechanism)

The registrant claims that the developmental effects in the study were secondary to the reflex bradypnea induced by sensory irritation. This bradypnea causes decreased alveolar ventilation, hypertension (resulting from peripheral vasoconstriction) and decreased lung perfusion resulting in hypoxia, hypothermia, and decreased metabolism, reduced glucose and reduced lactate production. All of these could have a serious impact on the energy consuming rapidly developing embryo/fetus (Shepard et al., 1970 and Smoak et al., 1991).

This contention is not without merit, unfortunately, no data were submitted indicating the quantitative skeletal effects that

could be expected from decreased plasma glucose, metabolic slow down, reduced temperature or from the bradypnea, making it difficult to estimate and distinguish among the effects from these sources and possible direct chemical toxicity. No quantitative data were submitted on metabolic slow down, lactate production or reduced glucose (changes in lactate production or reduced glucose were assumed by the authors of the submitted report, because there was no supporting data) on the possible indirect or on direct effects of cyfluthrin exposure on developmental toxicity.

The registrant reasonably contends that this bradypnea resulting from cyfluthrin exposure is tolerated by mothers but not by fetuses. It was implied that the reduced placental and fetal weight and reduced ossification would not have occurred in the absence of the bradypnea in mothers and indeed no such effects were noted in fetuses in two oral studies conducted at exposure levels 10 and 30 times those that occurred in the inhalation study.

In conclusion, it may be inappropriate to attribute the developmental effects seen in this inhalation study to the direct or indirect effects of cyfluthrin on development, at least at exposure levels of 2.55 mg/m^3, the LOEL. It is even possible that all the developmental effects seen at the HDT in this inhalation study are secondary to the results of the bradypnea in dams.

A. <u>Factors which tend to lend support that the bradypnea indirectly caused the developmental delays seen at necropsy in the main study.</u>

(1) According to Smoak and Sadler, 1991, a temperature decrement of -5°C and -2.0°C caused growth retardation and decreased lactate production in day 9 embryos but only decreased lactate production in day 10 embryos.

(2) The temperature deficit at 11.9 and 12.8 mg/m^3 + 39% O_2 may have caused some or all the development effects at these 2 exposure levels. These temperature deficits were large from gd 6 to gd 12 (gd 6 = -4.8 and 5.2°C and gd 12 = -2.6 and -1.1, respectively), and may have continued to the end of exposure on gd 15.

(3) Other factors such as reduced oxygen, reduced metabolic rate or reduced fetal glucose levels may have resulted in the effects at this exposure level. The registrant indicates that plasma glucose levels and urine pH may be lowered by bradypnea, but no data was submitted supporting these reduced parameters, except the bradypnea at 2.55 mg/m^3 (reduced minute volume from the vehicle control by 34%) and higher at higher exposure levels.

(4) It was noted in the 4 week range-finding study on males and non pregnant females that no decreases in body weight were noted

in females up to 46 mg/m^3 and transient bradypnea was noted at ≥ 6 mg/m^3. This would indicate that pregnancy increased the severity of the effects in dams, which again may be due to the effect on the conceptus. It is suggested that the extra energy requirement of the developing conceptus caused the maternal body weight decrement at ≥ 0.46 mg/m^3 in the main study.

(5) It should be noted that oral developmental toxicity studies in the same stain of rat indicated that no effects on skeletal parameters or any other parameters were shown to occur up to 10 mg/kg/day (HDT) (HED Doc# 005362, 1986). In a earlier developmental toxicity study of 1983, rats bred at Bayer AG (BAY:FB30) showed neurotoxic signs at ataxia and decreased motility, but no averse effect on weight gain up to 30 mg/kg/day (HDT) or fetal effects (the lack of fetal effects were stated in the review, but tables of skeletal parameters studied with potential effects were not presented in the review) or placental weight (HED Doc# 004285, 1985).

(6) The addition of 39% O_2 to the 12.8 mg/m^3 dose level was only partially successful in reversing the effects of the bradypnea, but the fact that the 39% O_2 decreased the developmental effects suggests that the bradypnea caused at least a slight oxygen deficiency. In addition, it may be expecting too much to believe that 39% O_2 supplementation would reverse the effects of the bradypnea on the developing fetal tissue, especially with the constellation of effects proposed resulting from the bradypnea.

B. <u>Factors that tend to not support bradypnea as the indirect cause of the developmental effects, especially at 2.55 mg/m^3.</u>

(1) According to Smoak and Sadler, 1991, a temperature decrement of -5°C and -2.0°C caused growth retardation and decreased lactate production in day 9 embryos but only decreased lactate production in day 10 embryos.

(2) The scientific merit of extrapolating temperature effects in a mouse embryo culture study to temperature effects in a rat developmental toxicity study is doubtful. Ordinarily effects in a developmental toxicity study are shown to be similar to effects in an *in vitro* study without a strain or species difference before conclusions can be drawn about possible mechanisms.

(3) These temperature decrements occurring at 2.55 mg/m^3 were marginal in causing developmental effects (Smoak and Sadler, 1991) in cultured day 9 and 10 mouse embryos. At gestational days between gd 6 and 12, the temperature decrement is assumed to be intermediate between -3.40 and -0.36°C, however these temperature deficits could be larger or smaller in the main study. If a linear decrement is assumed, this may mean a temperature decrement of -3.40 on gd 6, -2.89 on gd 7, -2.39 on gd 8, -1.87 on gd 9, -1.36 on gd 10, -0.85 on gd 11 and -0.36 on

gd 12. These temperature deficits may be even protective of the effects of metabolic slow down. Smoak and Sadler, 1991, suggest that slight temperature decrements may be protective of the cultured embryo under conditions of slight hypoglycemia.

(4) Calculations of the relative efficiency of food utilization would tend to support the hypothesis that reduced weight was due to toxicity at the 11.9 and 12.8 mg/m^3 + 39%O_2. Reduced relative efficiency of food utilization appears to decrease at the 11.9 and 12.8 mg/m^3 +39%O_2 exposure level. At 0.46 and 2.55 mg/m^3 exposure levels, food efficiency is nominally lower than controls, but the decrement is only slight and may not be biologically significant.

(5) No data are available on the potential decreased in serum glucose levels or fetal lactate production that may have occurred from the bradypnea.

References:

1. Shepard, TH, T Tanimura and MA Robkin (1970) Energy metabolism in early mammalian embryos. Dev. Biol., Suppl. 4: 42-48.
2. Smith, AU (1957) The effect on foetal development of freezing hamster embryos. J. Embyol. Exp. Morphol. 5: 311-323.
3. Smoak, IW and TW Sadler (1991) Hypothermia: Teratogenic and protective effects on the development of the mouse *in vitro*. Teratology 43: 635-641.
4. Schardein, JL (1985) Chemically Induced Birth Defects, Markel Dekker, Inc., NY and Basel.

B. APPENDIX:

(1) Description of the Inhalation procedures: The main developmental toxicity study, the studies on the satellite animals and the special studies all used some of the same equipment and some of same monitoring devices to control exposures except for the atmospheres and some of the special monitoring equipment indicated under the appropriate studies.

The test material was nebulized into a cylindrical chamber 50 cm X 14 cm internal diameter (7.6 l internal volume), equipped with baffles and ports for nose only exposure. The generated aerosol with diluted with 15 l of air per minute. Thus, about 237 air exchanges per hour were generated. Analytical determinations were conducted using a HP liquid chromatography with a UV detector on samples obtained from the breathing zone of the rats. Samples were collected at steady state, mid exposure and near the end of the exposure.

The aerosol was generated by a binary nozzle developed at

Bayer AG and purified and conditioned compressed air at a rate of 100 µL/15 liters of air per minute. The aerosol diluted with 15 liters of air per minute was then passed into the inhalation chamber. The aerosol generation conditions ensure about 237 air exchanges per hour and operate at a steady state in less than a minute ($t_{95\%}$ = 3 X [chamber volume/air flow] (McFarland, 1976). Chamber temperature (23±1°C) and humidity (<10%) were controlled.

Nominal concentration = [(V X Ci)/100] mg/m^3 air.
V = Volume of vehicle sprayed per m^3 air (3333µL)
Ci = Concentration of sprayed solution.

The analytical concentration of FCR 1272 was determined HPLC equipped with a UV detector. The ratio of diastereomers in the stock and spray solutions was determined by GC and flame ionization detector. Samples from the breathing zone of the rats were collected using Florisil-packed glass tubes and eluted quantitatively with acetonitrile. Samples were collected at the beginning of test (at steady state), one at the mid point and one shortly before the end of the test period. The results are expressed as mg of 96.2% FCR 1272 per m^3 and are not adjusted to 100% a.i.

Selected particle analyses were performed with Berner cascade impactors (Type I and II). The individual impactor stages were gravimetrically evaluated. The particle analyses were mainly performed using an aerodynamic particle sizer with laser velocimeter (TSI APS 3300). The parameters unequivocally characterizing the numerical aerosol particle distribution (NMAD and GSD) were determined from the probit-transformed numerical cumulative frequency distribution (y) and ECD (effective cutoff diameter) logarithms (x) of the individual measurement channels of the APS by linear regression. The NAME (TSI instrument) was converted to the MMAD using the following equation.

$$\ln(\text{MMAD}) = \ln(\text{NMAD} \times \text{density}) + 3(\ln[\text{GSD}])^2$$

Density = 1.134 g/ml (density of polyethylene glycol)

The parameters unequivocally characterizing the mass-related aerosol particle distribution (MMAD and GSD) were determined from the probit-transformed, mass-related cumulative frequency distribution (y) and the ECD logarithms (x) of the individual impactor stages (manufacture's instrument specification, Hauke Co., A-4810 Gmunden, Austria) by linear regression.

(2) Special studies:

Since cyfluthrin also causes reduced ventilation rates in rats, additional animals were added (5 dams per dose level) to the main study. The studies on these 5 dams per dose level were used to characterize and document the body temperature, respiration rates and plasma levels of cyfluthrin. In addition,

other studies were conducted on arterial blood gases and elevated CO_2 levels in other animals.

In the tables of data on these special studies, ApO_2 = Alveolar O_2 partial pressure, mmHg, = $pIO_2 - apCO_2/R$ X (1 - FIO_2(1 - R), where pIO_2 is the oxygen partial pressure in the inspiration air on water vapor saturation at body temperature, $apCO_2$ is the arterial carbon dioxide partial pressure, R = 0.8 (because of post-operation stress R was assumed to have deviated from 0.8), FIO_2 is the oxygen fraction in the breathing air (FIO_2 = 0.20). A - apO_2 = ApO_2 - apO_2. Qs/Qt = ($Cc'O_2$ - CaO_2)/($Cc"O_2$ - CvO_2), where $Cc"O_2$ = capillary oxygen concentration and CvO_2 is venous oxygen concentration. The venous admixture (Qs/Qt) is calculated from the ApO_2. It was assumed that ApO_2 and capillary pO_2 are equal. The arterial oxygen concentration (CaO_2) is obtained from the arterial oxygen partial pressure (apO_2), with Hb-oxygen saturation calculated according to Kelman (1966). The venous oxygen concentration (CvO_2) was calculated according to Jones (1987). The authors assumed an arteriovenous oxygen difference of 5ml/100ml. In all calculations they assumed the hemoglobin - oxygen saturation was 1.34 ml oxygen/g Hb.

An acute oral study at 125, 250 or 500 mg/kg show no decrease in body temperature where as inhalation studies have shown a decreased in body temperature of 2.2 to 4.8°C Table E, K and L). In fact a temperature elevation of 0.96°C at 500 mg/kg and 1.8°C at 125 mg/kg occurred, but these elevations were considered to be within the range of the variances of the study (page 1321 to 1366 of the submitted report).

(a) Special study on exposure of rats to CO_2 (multiple concentrations) and blood gas analysis - Rats (4 males; the 4 males were exposed to various concentrations of CO_2 in sequence, staring with air only) were exposed[1] (nose only) to CO_2 concentrations of 0%, 1%, 2%, 4% or 10% for 30 minutes and blood gases determined on retro-orbital blood samples immediately after cessation of exposure. Respiration rate (plethysmography), rectal temperature were also determined.

Results - (page 1138 to 1139 of the submitted report) Tidal volume increased increasing CO_2 concentration, but no significant increase in respiratory frequency occurred up to 10% CO_2 (Table J). Blood gases indicated a slight acidosis, hypercapnia, as well as a reduction in venous O_2 partial pressure in CO_2 exposed animals. No relevance is attributed to the arterio-alveolar O_2 difference and the shunt blood calculation, due to the venous admixture used (Table K).

The decreased hemoglobin concentration in Table K was

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[1] The same apparatus with conditions similar (except the exposure atmosphere) to the main developmental toxicity were used.

believed to be related to repeated bleedings in these animals.

Table J: Summary of plethysmography results on lung function from exposure to 0, 1%, 4%,or 10% CO_2 consecutively.

Concentration	Relative changes - mean values vs. air control (%) after 30 minutes exposure.				
Concentration	Tidal vol. (l)	Respiration rate (sec^{-1})	Minute vol. (l/min)	Inspiratory time (sec.)	Expiratory time (sec.)
1% CO_2	0	0	0	0	0
2% CO_2	+22	< 10	+22	< 10	< 10
4% CO_2	+51	< 10	+51	< 10	< 10
10% CO_2	+101	< 10	+101	< 10	< 10

(b) Special study on exposure to 4% CO_2 and blood gas analysis (page 1140 of the submitted report) - Rats (4 males per group) were exposed to air and 4% CO_2 for 4 hours and blood gases and lung function tests were conducted 30 minutes after exposure. Rectal temperatures were taken. Exposure conditions except for concentration of CO_2 were similar to the special study under (a).
Results - (page 1140 to 1143 of the submitted report) The blood gases equilibrated very fast such that no effects were demonstrated. The minute volume was decreased around 50% and the rats exhibited a lower temperature, but the expected effects on blood gases were not seen because of the rapid re-equilibration time for these gases.

No data were extracted from the submitted data because it demonstrated effects only on the minute volume and on the rectal temperature. These effects have been adequately demonstrated in the Tables reported for Special Studies (a) and (c).

Table K: Summary of measured blood gas parameters

	Blood gases- mean values 2-4.5 minutes after exposure								
Time	Temp. (C°)	Hb	P50 (O_2 1/2 saturation, mmHg)	HbO_2 (oxyhemoglobin %)	$HbCO_2$ (Carboxyhemoglobin, mmHg)	MetHB (Methemoglobin, %)	pO_2 (apO_2 concentration	pCO_2 ($apCO_2$ concentration, mmHg)	SAETT% (O_2 saturation, %)
Before exposure	38.9	15.3	34.4	62.6	1.5	0.5	50.2	45.9	83.9
After exposure	36.7**	13.4**	32.5	60.9	1.4*	0.6	42.3*	49.2	82.2
Time	pH	HCO_2 (Bicarbonate conc., mmol/l)	TCO_2 (Total CO_2 conc., mmol/l)	ABE (Actual base excess, mmol/l)	SBE (Standard base excess, mmol/l)	SBIC (Standard bicarbonate, mmol/l)	$ApCO_2$ (Alveolar O_2 conc., mmHg)	A-apO_2 (Arteno-alveolar O_2 difference, mmHg)	Qs/Qt (Venous blood, %)
Before exposure	7.38	24.0	25.3	-1.0	-0.6	22.7	89.0	38.8	57.2
After exposure	7.32	24.9	26.4	-1.6	-0.9	22.3	86.1	43.8	55.6

*, ** = Statistically significant at $p \leq 0.05$, $p \leq 0.01$, respectively.

(c) Special study on exposure to cyfluthrin (13 mg/m^3) and blood gas analysis - Rats (2 male and 1 female; although 21 rats were catheterized, due to technical difficulties results were obtained from only 3 rats) were exposed to 0 and 13.2 mg cyfluthrin in air for 4 hours. Blood gases were determined from samples obtained by a intraarterial carotid catheter. Samples and analyses were

obtained and determined 20 minutes before exposure, after 30, 60, 120, 180 and 240 minutes of exposer to the cyfluthrin. Exposure conditions except for the exposure time and the various CO_2% were similar to that indicated under (a).

Results - (page 1142 to 1143 of the submitted report) Exposure of the rats to the 13.2 mg cyfluthrin/m_3 resulted in a time dependent progressive increase in arterial pH from pH 7.37 to 7.46, which was statistically significant after 3 (pH=7.44) and 4 hours (pH=7.46) of exposure (Table L) . In addition, CO_2 partial pressure was reduced slightly and was consistent with the increase in pH. The negative values for A-apCO_2 (arterio-alveolar oxygen difference was stated to be an artifact. Calculation of this value was stated to be based on a respiratory quotient of R = 0.8. However, postoperative stress was assumed to altered the R = 0.8, what would have resulted in a change in the Qs/Qt value.

Table L: Summary of blood gas analysis by intraarterial carotid artery catheter blood sampling, plethysmography results on lung function and rectal temperature from exposure to 0, 13.2 mg cyfluthrin in air/m^3 for 4 hours.

	Blood gases- mean values before and 30, 60, 120 and 240 minutes after exposure								
Time	Temp. (C°)	Hb	P50 (O_2 1/2 saturation, mmHg)	HbO_2 (oxyhemaglobin %)	$HbCO_2$ (Carboxyhemoglobin, mmHg)	MetHB (Methemoglobin,%)	pO_2 (a-pO_2 concentration	pCO_2 (a-pCO_2 concentration, mmHg)	SAETT% (O_2 saturation, %)
-20 min.	37.9	14.3	-	92.4	1.5	0.7	106.7	38.5	94.5
30 min.	36.9	13.6	-	92.6	1.6	0.7	97.7	34.8	94.7
60 min.	36.3	12.3	-	94.0	1.0	0.7	108.6	35.4	95.6
120 min.	35.3*	12.1	-	95.0	1.7	0.7	105.9	35.5	97.3
180 min.	34.2	11.7	-	95.3	1.8	0.9	106.6	35.0	97.9
240 min.	33.1	10.9	-	96.1	1.9	0.9	112.9	30.0	98.9
Time	pH	HCO_3 (Bicarbonate conc., mmol/l)	TCO_2 (Total CO_2 conc., mmol/l)	ABE (Actual base excess, mmol/l)	SBE (Standard base excess, mmol/l)	SBIC (Standard bicarbonate, mmol/l)	ApCO_2 (Alveolar O_2 conc., mmHg)	A-apO_2 (Arterioalveolar O_2 difference, mmHg)	Qs/Qt (Venous blood, %)
-20 min	7.37	21.8	22.9	-2.5	-2.7	22.3	94.9	-11.8	7.7
30 min.	7.40	21.4	22.5	-2.5	-2.8	22.3	100.5	2.8	10.1
60 min.	7.41	22.4	23.5	-1.8	-2.0	22.9	100.0	-8.6	6.7
120 min.	7.42	23.0	24.2	-1.4	-1.4	23.2	100.3	-5.6	3.0
180 min.	7.44*	24.4	25.6	-0.1	-0.1	24.4	101.4	-5.2	1.4
240 min.	7.46**	22.5	23.6	-1.6	-1.7	23.1	108.0	-4.9	0.7

*, ** = Statistically significant at $p \leq 0.05$, $p \leq 0.01$, respectively. - = Not determined.
A-apO_2 = pO_2 - ApO_2; ApO_2 = Alveolar O_2 partial pressure, mmHg = pIO_2 -apCO_2/R X (1 - FIO_2(1 - R), pIO_2 is the oxygen partial pressure in the inspiration air on water vapor saturation at body temperature;

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Chemical:	**Cyfluthrin**
PC Code:	**128831**
HED File Code	**13000 Tox Reviews**
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