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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: **May 12, 2003**

MEMORANDUM

TXR No.: 0050141

SUBJECT: **Lufenuron:** New Chemical Screen of Submitted Toxicology Studies and Exposure Potential.

FROM: William Greear, M.P.H., D.A.B.T., Toxicologist
and
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Registration Action Branch 3
Health Effects Division (7509C)

THROUGH: Stephen C. Dapson, Ph.D., Branch Senior Scientist
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TO: Cynthia Giles-Parker, Product Manager
PM-# 03
Registration Division (7505C)

DP Barcode:	D289048; D289065	**Submission:**	S631677; S631675
Chemical:	Lufenuron	**Case:**	065520; 065347
PC Code:	118205		

Action Requested: New Chemical Screen of the toxicology studies submitted and exposure potential for the tolerance petition of (Lufenuron) for use as a termiticide bait.

HED Response: The toxicology studies submitted to support the non-food use for the new chemical, lufenuron, have been screened for completeness and general acceptability. These studies are acceptable for review. The data requirements and acceptance criteria are attached. The acute "six-pack" with the technical grade active ingredient was not submitted with this action and was not considered in this screen. All acute toxicity studies are handled by RD. The acute oral toxicity study in the mouse is in a species not normally used for an acute oral data requirement.

No residential exposure is anticipated. While this product may be used in residential settings, it is only sold to trained pest control operators (PCOs) for installation and monitoring. The exposure potential for PCOs is believed to be relatively low, however, it is not clear from the label provided in the submission whether it is necessary for the PCO to touch the bait matrix in order to install it within the bait station. Regarding duration of exposure, because baited systems need to be inspected/serviced as frequently as 2- to 4-week intervals, and termite feeding may continue for many months, inhalation and dermal exposure is possible over short- and intermediate-term durations. At this time it is not known whether long-term exposure is likely (i.e., more than six months of consecutive workdays spent installing/inspecting active bait stations, as opposed to the inert monitoring devices).

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1. Toxicology Data Requirements (CFR 158.340) for the Non-Food Use of Lufenuron.

Instructions: In the "submitted" column, insert "Yes or No" to indicate if the study was submitted In the "MRID No." column, insert the MRID No. of the submitted study.

Guideline No.	Study Type	Technical		
		Required	Submitted	MRID No.
870.1100	Acute Oral Toxicity - Rat	Yes	No*	na
870.1100	Acute Oral Toxicity - Mouse	No	Yes	45853216
870.1200	Acute Dermal Toxicity	Yes	No*	na
870.1300	Acute Inhalation Toxicity	Yes	No*	na
870.2400	Acute Eye Irritation	Yes	No*	na
870.2500	Acute Dermal Irritation	Yes	No*	na
870.2600	Skin Sensitization	Yes	No*	na
870.3100	Subchronic (Oral) Toxicity - Rodent	x	Yes	45853217
870.3150	Subchronic (Oral) Toxicity - Non- Rodent ..	-	-	
870.3200	21/28-Day Dermal Toxicity	-	-	
870.3250	90-Day Dermal Toxicity	-	-	
870.3465	90-Day Inhalation Toxicity	-	-	
870.3700a	Prenatal Developmental Toxicity -Rodent ..	x	Yes	45853219
870.3700b	Prenatal Developmental Toxicity - Non-Rodent	-	Yes	45853218
870.3800	Reproduction and Fertility Effects	-	-	
870.4100a	Chronic (Oral) Toxicity - Rodent-	-	-	
870.4100b	Chronic (Oral) Toxicity - Non-Rodent	-	-	
870.4200a	Carcinogenicity -Rat	-	-	
870.4200b	Carcinogenicity - Mouse	-	-	
870.4300	Combined Chronic Toxicity /Carcinogenicity	-	-	
870.5100	Mutagenicity-Ames Test	x	Yes	45853220
870.5300	Mutagenicity-Point Mut. CHO.	-	Yes	45853221
870.5375	Mutagenicity-*In vitro* Chromos. Aberr	x	Yes	45853222
870.5395	Mutagenicity-*In vivo* Chromos. Aberr.	x	Yes	45853223
870.5550	Mutagenicity-UDS	-	Yes	45853224
870.6100a	Neurotoxicity - Acute Delayed Neurotox.- Hen	-	-	
870.6100b	Neurotoxicity - Subchronic - Hen	-	-	
870.6200a	Neurotoxicity Screening Battery - Acute - Rat	-	-	
870.6200b	Neurotoxicity Screening Battery - Subchronic - Rat	-	-	
870.6300	Developmental Neurotoxicity	-	-	

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* = The acute toxicity "six-pack" was not reviewed in this action and will be handled by RD.

2. Bibliography of the Submitted Toxicology Studies

81-1: 45853216 Hartmann, H. (1989) Acute Oral Toxicity in the Mouse: CGA-184699 Technical: Final Report: Lab Project Number: 881663: 244-88. Unpublished study prepared by Ciba-Geigy Limited. 19 p. {OPPTS 870.1100}

82-1: 45853217 Fankhauser, H. (1989) 3-Month Oral Toxicity in Rats (Administration in Food): CGA-184699 Technical: Final Report: Lab Project Number: 871736: 240-88. Unpublished study prepared by Ciba-Geigy Limited. 433 p. {OPPTS 870.3100}

83-3: 45853218 Meyer, L. (1989) A Teratology Study in New Zealand White Rabbits with CGA-184699 Technical: Final Report: Lab Project Number: F-00033: 352-89. Unpublished study prepared by Ciba-Geigy. 179 p. {OPPTS 870.3700}

83-3: 45853219 Gilles, P. (1989) A Teratology Study in CD Rats with CGA-184699 Technical: Final Report: Lab Project Number: F-00015: 353-89. Unpublished study prepared by Ciba-Geigy. 247 p. {OPPTS 870.3700}

84-2: 45853220 Deparade, E. (1988) Salmonella/Mammalian-Microsome Mutagenicity Test: CGA-184699 Technical: Final Report: Lab Project Number: 871717: 245-88. Unpublished study prepared by Ciba-Geigy Limited. 26 p. {OPPTS 870.5100}

84-2: 45853221 Dollenmeier, P. (1988) Point Mutation Test with Chinese Hamster Cells V79: CGA-184699 Technical: Final Report: Lab Project Number: 871719: 238-88. Unpublished study prepared by Ciba-Geigy Limited. 41 p. {OPPTS 870.5300}

84-2: 45853222 Strasser, F. (1989) Chromosome Studies on Chinese Hamster Ovary Cell Line CCL 61 In Vitro: CGA-184699 Technical: Final Report: Lab Project Number: 871718: 241-88. Unpublished study prepared by Ciba-Geigy Limited. 52 p. {OPPTS 870.5375}

84-2: 45853223 Meyer, A. (1989) Micronucleus Test, Mouse: CGA-184699 Technical: Final Report: Lab Project Number: 871714: 239-88. Unpublished study prepared by Ciba-Geigy Limited. 39 p.

84-2: 45853224 Ogorek, B. (2000) In Vivo/In Vitro Unscheduled DNA Synthesis in Rat Hepatocytes: CGA-184699 Technical: Final Report: Lab Project Number: 992048: 1315-99. Unpublished study prepared by Novartis Crop Protection AG. 78 p. {OPPTS 870.5550}

3. Acceptance Criteria

See the attached Acceptance Criteria.

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ATTACHMENT 1

Cover Page - Toxicology Acceptance Criteria

Chemical: Lufenuron PC Code: 118205 Food Use: _____ Non-Food Use: Yes

Title of study	Guideline No.	MRID No.	Comments
Acute Oral Toxicity - Mouse	870.100	45853216	Passes screen
Subchronic Oral - Rodent	870.3100	4583217	Passes screen
Subchronic Oral - Non-rodent	870.3100	No	Not required
21/28 Day Dermal	870.3200	No	Not required
Prenatal developmental - Rodent	870.3700	45853219	Passes screen
Prenatal developmental - Nonrodent	870.3700	45853218	Passes screen
Reproduction and Fertility	870.3800	No	Not required
Chronic Toxicity - Rodent	870.4100	No	Not required
Chronic Toxicity - Nonrodent	870.4100	No	Not required
Carcinogenicity - Rat	870.4200	No	Not required
Carcinogenicity - Mouse	870.4200	No	Not required
Combined chronic Toxicity Carcinogenicity	870.4300	No	Not required
Neurotoxicity Screening Battery - Acute	870.6200a	No	Not required
Neurotoxicity Screening Battery - Subchronic	870.6200b	No	Not required
Developmental Neurotoxicity	870.6300	No	Not required

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ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No. 45853216

870.1100 Acute Oral Testing

Does this study meet the following acceptance criteria?

1. Yes Study conducted under GLP (with statement).
2. Yes Technical form of the active ingredient used.
3. Yes[1] Full identification of the test material (physical state, color, percentage active).
4. No[2] Young (preferably 8-12 weeks old) adult rats used. If another species used, then justification should be given.
5. Yes Identification of the test animal strain and source.
6. Yes Animals fasted prior to substance administration (for rats, overnight)
7. Yes Oral (gavage) dosing. If a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. If necessary (particularly for solids) a vehicle (which should also be identified) may be used.
8. Yes Body weights reported (shortly before the test substance is administered and weekly thereafter, including just before terminal sacrifice which would usually be on day 14).
9. No Individual observations for at least 14 days, or until all test animals appear normal (whichever is longer).
10. Yes Gross necropsy performed on all animals dying during the test, as well as all others following terminal sacrifice
11. Yes Doses tested sufficient to determine a toxicity category or a limit dose (which may be 2000 or 5000 mg/kg).
12. No[3] Identification of methods used for calculation of LD50 (and 95% confidence limits, if appropriate).

[1] - color not stated.
[2] - mouse. No adequate justification provided. 4-5 weeks of age.
[3] - not required.

Comments:

The study is acceptable for review.

ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No. 45853217

870.3100 Subchronic 90-Day Oral Toxicity in Rodents

Does this study meet the following acceptance criteria?

No.	Yes/No	Criteria
1	**Yes**	Study conducted under GLP (with statement).
2	**Yes**	Technical form of the active ingredient used
3	**Yes**[1]	Full identification of the test material (physical state, color, batch or lot number, expiration date, percentage active).
4	**Yes**	Rat is preferred species, although other rodent species can be used.
5	**Yes**	Identification as to test animal strain and source.
6	**Yes**	Testing started with young healthy animals, no older than 8-9 weeks old for rats.
7	**Yes**	At least 10 animals/sex/dose level, with concurrent control group.
8	**Yes**	If interim sacrifices, number of animals/group should be increased accordingly.
9	**Yes**	Dosing duration of 90 days or 5 days/week for 13 weeks.
10	**Yes**	Doses tested include a NOAEL
11	**Yes**	Highest dose level produces indications of toxicity or is a limit dose (1000 mg/kg)
12	**Yes**[2]	Analyses for test material stability, homogeneity and concentration in dosing medium.
13	**Yes**	Individual daily observations.
14	**Yes**	Individual body weights (before administration, weekly thereafter, and at death).
15	**Yes**	Individual or cage food consumption.
16	**Yes**	Ophthalmoscopic examination (pretest & term) for at least control and high dose.
17	**No**	Assessment of motor activity, grip strength, reactivity to sensory stimuli (not earlier than week 11).
18	**Yes**	Hematology and clinical chemistry at termination.

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ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No. 45853217

870.3100 Subchronic 90-Day Oral Toxicity in Rodents

19		**Hematology** x Erythrocyte count x Leukocyte count x Differential count x Hemoglobin x Hematocrit x Mean corpuscular hemoglobin. x Mean corpuscular volume ___ Mean corpuscular hemoglobin. concentration x Platelet count x Prothrombin time or activated partial thromboplastin time.
20		**Clinical Chemistry** x Potassium x Urea nitrogen x Bilirubin x Sodium x Creatinine x Globulin x Alanine aminotransferase x A/G x Aspartate aminotransferase x Ca x Alkaline phosphatase x Cl x Glucose x Total protein ___ PO_4 x Albumin x Total cholesterol ___ Cholinesterases (if appropriate.)
21*	No	**Urinalysis** (optional; during last week: parameters include appearance, volume, osmolality or specific gravity, pH, protein, glucose, and blood or blood cells.
22	**Yes**	Individual gross necropsy of all animals
23	**Yes**	**Organ weights:** x Liver x Ovaries x Spleen x Kidneys ___ Uterus x Brain x Adrenals x Thymus x Heart x Testes (~~with Epididymides~~)
24	**Yes**[3]	Full histopathology of the following tissues from at least all control and high-dose animals, and all rodents that died or were killed during the study, all gross lesions in all animals, and target tissues in all animals.

ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No. 45853217

870.3100 Subchronic 90-Day Oral Toxicity in Rodents

25		x adrenals	x jejunum	x pituitary
		x aorta	x kidneys	prostate
		x bone marrow	larynx	rectum
		x brain (3 regions)	x liver	x salivary glands
		x cecum	x lungs	seminal vesicle
		x colon	x lymph nodes	skin
		x duodenum	musculature§	spinal cord (3X)
		x heart	mammary gland x	spleen
		epididymides	nose	x stomach
		x esophagus	x ovaries x testes	
		eyes	oviduct§	x thymus
		gallbladder (if present)	x pancreas	x thyroid
			parathyroids	x trachea
		x heart	x peripheral nerve	x urinary bladder
		x ileum	pharynx	x uterus
		x bone	x gross lesions	

§Not indicated as required in 1998 OPPTS Harmonized Test Guidelines

[1]- color not provided.
[2]- homogeneity stated to have been conducted and reported in FAR 513/88, dated 2/17/1988; Test No. 87 1738. No data or tables or description of sampling were provided. [The homogeneity results should be submitted.]
[3] - list of tissues is somewhat below current standards, but sufficient.

Comments:

The study is acceptable for review.

NOTE:

In this study, neurotoxicity signs in the form of tonic/clonic convulsions were observed in animals in the top two dose levels of 1500 ppm (104.8 mg/kg/day - 1/10 females) and 15,000 ppm (1010 mg/kg/day - 9/20 males; 1052 mg/kg/day - 8/20 females). In addition, one female in the 15,000 ppm group exhibited a motor disorder. The seizures did not occur until after 10 weeks of dietary administration of the test material-indicating a possible cumulative effect; however, there was no evidence of the mechanism. The clinical signs of toxicity, tonic/clonic convulsions, did not appear to be agonal.

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ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No: 45853219

870.3700a Prenatal Developmental Toxicity Study

Does this study meet the following acceptance criteria?

1	**Yes**	Study conducted under GLP (with statement).
2	**Yes**	Technical form of the active ingredient used
3	**Yes**[1]	Full identification of the test material (chemical identification, percentage active batch or lot number, physical properties purity/impurities, expiration date, vehicle used, if any). It is preferable to use one lot throughout the study
4*	**Yes**[2]	Analyses for test material stability, homogeneity and concentration in dosing medium.
5	**Yes**	Preferred species are rat (rodent) and rabbit (nonrodent).
6	**Yes**	Identification as to test animal strain and source.
7	**Yes**	Young adult animals should be used; females should be nulliparous, and the should be mated with males of the same species and strain, avoiding the mating of siblings, if parentage is known.
8	**Yes**	Normally (except when a limit dose of 1000 mg/kg/day shows no effects) there should three dose levels and a concurrent control. Dose levels should be spaced to produce a gradation of toxic effects.
9	**Yes**	At the highest dose level, there should be significant maternal toxicity (but mortality should not exceed 10%), or a limit dose should be achieved (1000 mg/kg/day by the oral or dermal exposure routes)
10	**Yes**	The lowest dose level should not produce any evidence of either maternal or developmental toxicity.
11	**Yes**	The test substance should be administered daily from implantation to the day before cesarean section (one day prior to the expected day of parturition). If preliminary studies indicate a low potential for preimplantation loss, treatment may be from fertilization to 1 day prior to the expected day of termination
12	**Yes**	Each test and control group should contain a sufficient number of animals to yield approximately 20 animals with implantation sites at necropsy. [Note: if rabbit is used and the study was initiated or completed before January 2000 then 12 animals/dose level may be acceptable)
13	**Yes**	Individual daily observations.
14	**Yes**	Individual body weights (on day 0, at termination, and at least at 3-day intervals during the dosing periods.

ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No: 45853219

870.3700a Prenatal Developmental Toxicity Study

15	**Yes**	Individual food consumption (on at least 3-day intervals, preferably on days when body weights are recorded).
16	**Yes**	Reporting of gravid uterine weights, as well as body weight changes adjusted for gravid uterine weights
17	**Yes**	Individual uterine examination for implantation data, number of viable (by sex) and dead fetuses, and early and late resorptions. Uteri that appear to be non-gravid should be examined by a technique (such as ammonium sulfide staining) to confirm nonpregnant status.
18	**Yes**	All ovaries examined to determine number of corpora lutea.
19	**Yes**	Sex and body weight of each fetus determined. Report should include fetal body weight data, preferably by sex and with sexes combined
20	**Yes**	Each fetus examined for external anomalies.
21	**Yes**	For rodents, approximately one-half of each litter prepared by standard techniques and examined for skeletal alterations. Remainder appropriately prepared and examined for soft tissue anomalies. Also acceptable: examination of all fetuses by careful dissection for soft tissue anomalies followed by examination for skeletal anomalies.
22	--------	For rabbits, all fetuses examined for both soft tissue and skeletal alterations. An adequate evaluation of the internal structures of the head, including the eye, brain, nasal passages, and tongue, should be conducted on at least half the fetuses.
23	**Yes**	Historical control data with litter incidence and fetal incidence within litter[usually from the performing laboratory], including dates of studies, strain and source of animals, and the vehicle(s) and route(s) of administration, when appropriate to enhance the interpretation of study results.

*Preferred, but not specified in the OPPTS Harmonized Test Guidelines.

[1]- no physical properties, impurities, expiration date.
[2] - stability data absent, but, reported to have been previously conducted. Results given, but not referenced.

Comments:

The study is acceptable for review.

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ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No: 45853219

870.3700b Prenatal Developmental Toxicity Study

Does this study meet the following acceptance criteria?

1	**Yes**	Study conducted under GLP (with statement).
2	**Yes**	Technical form of the active ingredient used
3	**Yes**[1]	Full identification of the test material (chemical identification, percentage active batch or lot number, physical properties purity/impurities, expiration date, vehicle used, if any). It is preferable to use one lot throughout the study
4*	**Yes**	Analyses for test material stability, homogeneity and concentration in dosing medium.
5	**Yes**	Preferred species are rat (rodent) and rabbit (nonrodent).
6	**Yes**	Identification as to test animal strain and source.
7	**Yes**	Young adult animals should be used; females should be nulliparous, and the should be mated with males of the same species and strain, avoiding the mating of siblings, if parentage is known.
8	**Yes**	Normally (except when a limit dose of 1000 mg/kg/day shows no effects) there should three dose levels and a concurrent control. Dose levels should be spaced to produce a gradation of toxic effects.
9	**Yes**	At the highest dose level, there should be significant maternal toxicity (but mortality should not exceed 10%), or a limit dose should be achieved (1000 mg/kg/day by the oral or dermal exposure routes)
10	**Yes**	The lowest dose level should not produce any evidence of either maternal or developmental toxicity.
11	**Yes**	The test substance should be administered daily from implantation to the day before cesarean section (one day prior to the expected day of parturition). If preliminary studies indicate a low potential for preimplantation loss, treatment may be from fertilization to 1 day prior to the expected day of termination
12	**Yes**	Each test and control group should contain a sufficient number of animals to yield approximately 20 animals with implantation sites at necropsy. [Note: if rabbit is used and the study was initiated or completed before January 2000 then 12 animals/dose level may be acceptable)
13	**Yes**	Individual daily observations.

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ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No: 45853219

870.3700b Prenatal Developmental Toxicity Study

14	**Yes**	Individual body weights (on day 0, at termination, and at least at 3-day intervals during the dosing periods.
15	**Yes**	Individual food consumption (on at least 3-day intervals, preferably on days when body weights are recorded).
16	**Yes**	Reporting of gravid uterine weights, as well as body weight changes adjusted for gravid uterine weights
17	**Yes**	Individual uterine examination for implantation data, number of viable (by sex) and dead fetuses, and early and late resorptions. Uteri that appear to be non-gravid should be examined by a technique (such as ammonium sulfide staining) to confirm nonpregnant status.
18	**Yes**	All ovaries examined to determine number of corpora lutea.
19	**Yes**	Sex and body weight of each fetus determined. Report should include fetal body weight data, preferably by sex and with sexes combined
20	**Yes**	Each fetus examined for external anomalies.
21	---------	For rodents, approximately one-half of each litter prepared by standard techniques and examined for skeletal alterations. Remainder appropriately prepared and examined for soft tissue anomalies. Also acceptable: examination of all fetuses by careful dissection for soft tissue anomalies followed by examination for skeletal anomalies.
22	**Yes**	For rabbits, all fetuses examined for both soft tissue and skeletal alterations. An adequate evaluation of the internal structures of the head, including the eye, brain, nasal passages, and tongue, should be conducted on at least half the fetuses.
23	**Yes**	Historical control data with litter incidence and fetal incidence within litter [usually from the performing laboratory], including dates of studies, strain and source of animals, and the vehicle(s) and route(s) of administration, when appropriate to enhance the interpretation of study results.

*Preferred, but not specified in the OPPTS Harmonized Test Guidelines.

[1]- impurities not addressed; expiration date not given.

Comments:

The study is acceptable for review.

ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No. 45853220

870.5100 Bacterial Reverse Mutation Test
(Ames Assay)

Does this study meet the following acceptance criteria?

1. Yes Study conducted under GLP (with statement).
2. Yes Technical form of the active ingredient used.
3. Yes[1] Full identification of the test material (chemical identification, percentage active, batch or lot number, physical properties, purity/impurities, expiration date, vehicle used, if any).
4. No Five strains of bacteria, including at least four from *Salmonella typhimurium*:
   X TA1535 X TA98 X TA100
   X TA1537 (or _____ TA97a or _____ TA97)
   _____ TA102 (or one of the *E. coli* strains indicated below)
   If TA102 is not used, then one of the following *E. coli* strains:
   _____ WP2 *uvrA* _____ WP2 *uvrA* (pKM101)
5. No Amino acid requirement for growth (histidine for the *S. typhimurium* strains; tryptophan for *E. coli* strains) demonstrated for each frozen stock culture.
6. Yes Phenotypic characteristics checked (ampicillin resistance in strains TA98, TA100, TA97a or TA97, WP2 *uvrA* and WP2 *uvrA* (pKM101) and ampicillin + tetracycline resistance in strain TA102); presence of characteristic mutations (rfa mutation in *S. typhimurium* through sensitivity to crystal violet, and *uvrA* mutation in *E. coli* or *uvrB* mutation in *S. typhimurium* through sensitivity to UV light).
7. Yes Strains should yield spontaneous revertant colony plate counts within the frequency ranges expected from the laboratory's historical control data, and preferably within ranges reported in the literature.
8. ? Fresh cultures of bacteria in late exponential or early stationary growth phase should be used (approximately 10^9 cells/mL)
9. Yes Bacteria exposed to test substance both in the presence and absence of a metabolic activation system (usually S9 prepared from livers of rodents dosed with Aroclor 1254 or a combination of phenobarbitone and beta-naphthoflavone).
10.* Yes Post-mitochondrial fraction used at concentrations ranging from 5 to 30% v/v in the S9 mix.
11. Yes Solid test substance dissolved or suspended in appropriate solvent or vehicle; liquid test substances added directly to test system and/or diluted prior to treatment.
12. ? Fresh preparations tested unless stability data demonstrate the acceptability of storage.
13. Yes Maximum dose based on either cytotoxicity (significant reduction in number of revertant colonies, clearing or dimunition of background lawn, or degree of survival of treated cultures) or insolubility of test material (precipitation of test material under test conditions) or a limit dose (5 mg or 5 µL/plate).

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870.5100 Bacterial Reverse Mutation Test

Ames Assay
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14.* Yes Plate incorporation or preincubation method used.
15. Yes Five dose levels, negative and positive controls (both for activated and nonactivated conditions) used.
16. Yes Incubation at 37°C for 48-72 hrs.
17. Yes Report includes individual plate counts, mean numbers of revertant colonies/plate/strain/dose and standard deviations, concurrent negative (solvent/vehicle) and positive control data, with ranges, means and standard deviations.
18. Yes Report includes historical negative (solvent/control) and positive control data (strain and activation [S9] specific), with ranges, means and standard deviations.

[1] - Physical properties, impurities and expiration date were not provided.

Comments:

The study is acceptable for review.

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ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No. 45853221

870.5300 In vitro Mammalian Gene Mutation Test

Does this study meet the following acceptance criteria?

1. Yes Study conducted under GLP (with statement).
2. Yes Technical form of the active ingredient used.
3. Yes[1] Full identification of the test material (chemical identification, percentage active, batch or lot number, physical properties, purity/impurities, expiration date, vehicle used, if any).
4. ? Fresh preparations used unless stability data demonstrate acceptability of storage.
5.* Yes Data on pH and osmolality of treatment medium.
6. Yes Several suitable cell lines, including the following:
   - ____ L5178Y mouse lymphoma cells
   - x CH V79 cell line
   - ____ CHO AS52 cell line
   - ____ TK6 human lymphoblastoid cells
   - ____ Other (CHO - KI - BH4)
7. Yes Cell line or tissue source identified as to source and/or subclone, and have a high cloning efficiency, and a stable spontaneous mutant frequency.
8. Yes Cell line is routinely checked for *Mycoplasma* contamination.
9. No Cultures cleansed of pre-existing mutant cells prior to test.
10. Yes Proliferating cells exposed to test substance both with and without metabolic activation (S9).
11. Yes Exposure is for a suitable period of time (usually 3-6 hrs is effective) but may be extended .
12.* Yes S9 prepared from livers of rodents treated with Aroclor 1254 or a mixture of phenobarbitone and Beta-naphthoflavone.
13. Yes Cytotoxicity determined with and without metabolic activation.
14. Yes At least four analyzable concentrations should be used.
15. Yes If highest concentration is based on cytotoxicity then at that dosage there should be approximately 10-20% (not less than 10%) relative survival (relative cloning efficiency) or relative total growth.
16. Yes Highest concentration, in the absence of cytotoxicity and precipitation, should be 5 µL/mL, 5 mg/mL or 0.01M, whichever is lowest.
17. Yes Relatively insoluble substances tested up to or beyond their limit of solubility under culture conditions. Any precipitation should not interfere with scoring.
18. Yes Concurrent positive and negative controls should be used, both with and without metabolic activation.

    Without metabolic activation:
    - x Ethylmethanesulfonate
    - ____ Ethylnitrosourea
    - ____ Methylmethanesulfonate
    - ____ Other

    With metabolic activation:
    - ____ 3-Methylcholanthrene
    - ____ Cyclophosphamide
    - ____ N-Nitrosodimethylamine
    - ____ Benzo(a)pyrene
    - ____ 7,12-Dimethylbenzanthracene
    - x Other

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870.5300 In vitro Mammalian Gene Mutation Test
(Page 2)

19. __Yes__ Either duplicate or single treated cultures may be used at each concentration tested. When single cultures are used, the number of concentrations should be increased to ensure an adequate number of cultures for analysis (e.g., at least eight analyzable concentrations). Duplicate negative (solvent) controls used.
20. __Yes__ At the end of the exposure period, cells are washed and cultured to allow for expression of the mutant phenotype.
21. __----__ HPRT and XPRT require at least 6-8 days for phenotypic expression; TK requires at least 2 days.
22. __----__ If test substance is positive in the L5178Y TK+/- test, colony sizing should be performed on at least the highest positive concentration, and on negative and positive controls. If the test substance is negative in this test, colony sizing should be done on the negative and positive controls.
23. __Yes__ Reporting should include survival (relative cloning efficiencies), cytotoxicity and/or viability determination, colony counts and mutant frequencies (expressed as number of mutants/surviving cells) for the treated and control cultures.
24. __Yes__ Individual culture data should be provided.

[1] - Physical properties, impurities and expiration date were not provided-not needed.

Comments:

The study is acceptable for review.

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ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No. 45853222

870.5375 In vitro Mammalian Chromosome Aberration Test

Does this study meet the following acceptance criteria?

1. Yes Study conducted under GLP (with statement).
2. Yes Technical form of the active ingredient used.
3. Yes[1] Full identification of the test material (chemical identification, percentage active, batch or lot number, physical properties, purity/impurities, expiration date, vehicle used, if any).
4. ? Fresh preparations used unless stability data demonstrate acceptability of storage.
5.* Yes Data on pH and osmolality of treatment medium.
6. Yes A variety of mammalian cell lines can be used, including the following:
   - ____ Chinese hamster fibroblasts
   - X Chinese hamster ovary cells
   - ____ Other
   - ____ Human leukocytes
   - ____ Rat bone marrow cells
7. Yes Cell line or tissue source identified as to species, origin, tissue type etc.
8. No If an established cell line is used, should be checked routinely for modal chromosomal number and absence of *Mycoplasma* contamination. The normal cell-cycle time for the cells under the culture conditions used should be known.
9. Yes Cell cultures exposed to test substance both with and without metabolic activation (S9).
10.* Yes S9 prepared from livers of rodents treated with Aroclor 1254 or a mixture of phenobarbitone and Beta-naphthoflavone.
11. Yes Cytotoxicity determined with and without metabolic activation.
12. Yes At least three analyzable concentrations should be used.
13. Yes Highest concentration, in the absence of cytotoxicity, should be 5 µL/mL, 5 mg/mL or 0.01M, whichever is lowest.
14. Yes If the maximum concentration is below these values, then the highest concentration should show a significant reduction (>50%) in confluency, cell count or mitotic index, or there should be precipitation of test material in system.
15. Yes Concurrent positive and negative controls should be used, both with and without metabolic activation.

    Without metabolic activation:
    - ____ Ethylmethanesulfonate
    - ____ Methylmethanesulfonate
    - ____ 4-Nitroquinoline-N-Oxide
    - ____ Ethylnitrosourea
    - x Mitomycin C
    - ____ Other

    With metabolic activation:
    - ____ Benzo(a)pyrene
    - ____ Other
    - x Cyclophosphamide
16. Yes Duplicate cultures used at each concentration of the test substance, both with and without metabolic activation.

17. __Yes__ In first experiment, cells should be exposed to the test substance ± metabolic activation for 3-6 hours, and sampled at a time equivalent to about 1.5x normal cell-cycle length; if results are negative, an additional experiment without activation should be done, with continuous exposure until sampling at a time equivalent to about 1.5x normal cell-cycle length.
18. __Yes__ Negative results with metabolic activation in first experiment need to be confirmed, or justification must be provided for not confirming results.
19. __Yes__ Cell cultures treated with Colcemid or colchicine for 1-3 hours before processing.
20. __Yes__ All slides, including those from positive and negative controls, should be coded before microscopic analysis, and read "blind."
21. __Yes__ Cells scored should contain a number of centromeres equal to the modal number ±2 of chromosomes for all cell types.
22. __Yes__ At least 200 well-spread metaphases should be scored per concentration and control, equally divided among the duplicate cultures.
23. __Yes__ Polyploidy and endoreduplication recorded when observed.
24. __Yes__ Definition for aberrations, including gaps.
25. __Yes__ Reporting of number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture.
26. __Yes__ Report includes concurrent, as well as historical negative (solvent/control) and positive control data, with ranges, means and standard deviations.

Note: For item 13 Guidelines state the maximum concentration for relatively non-cytotoxic compounds should be 5 µg/mL, 5 mg/mL or 0.01M, whichever is lowest. The 5 µg/mL is a misprint, and should be 5 µL/mL.

[1] -Physical properties, impurities and expiration date were not provided-not needed.

Comments:

The study is acceptable for review.

ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No. 45853223

870.5395 Mammalian Erythrocyte Micronucleus Test

Does this study meet the following acceptance criteria?

1. Yes Study conducted under GLP (with statement).
2. Yes Technical form of the active ingredient used.
3. Yes[1] Full identification of the test material (chemical identification, percentage active, batch or lot number, physical properties, purity/impurities, expiration date, vehicle used, if any).
4.* Yes If there is evidence that the test substance or a reactive metabolite will not reach the target tissue, it is not appropriate to use this test.
5. ? Fresh preparations (solids dissolved or suspended in appropriate solvents) used unless stability data demonstrate acceptability of storage.
6. Yes Commonly used strains of mice or rats generally used, although any mammalian species can be used as long as the level of micronuclei is low (<1-2%) or it is one in which the spleen does not remove micronucleated erythrocytes or unless it is relatively insensitive to agents which cause structural or numerical chromosomal aberrations:
   _____ Rat X Mouse_____ Other
7. Yes Each treated and control group should include at least five analyzable animals per sex/sacrifice time. If previous data demonstrate no substantial differences between sexes in toxicity, then testing in a single sex will be sufficient.
8. Yes Concurrent positive and negative (solvent/vehicle) controls should be included for each sex. Positive controls may be the following:
   _____ Ethylmethanesulfonate _____ Ethylnitrosourea
   _____ Mitomycin C _____ Cyclophosphamide
   _____ Other
9. Yes Negative controls included for every sampling time, unless adequate historical control data are provided.
10. Yes For one-day-dosing or short-term studies (14 days or less) highest [daily] dose is a limit dose (2,000 mg/kg/day) given either as a single dose or as two doses on the same day, or causes toxicity such that higher doses would be expected to produce lethality. Alternatively, the highest dose may produce some indication of toxicity in the bone marrow (such as a reduction in the proportion of immature erythrocytes among total erythrocytes in bone marrow or peripheral blood). Limit dose for studies >14 days is 1,000 mg/kg/day.
11. Yes Samples of bone marrow (or peripheral blood) taken at least twice, starting not earlier than 24 hrs after last treatment, but not extending beyond 48 hrs after last treatment.

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870.5395 Mammalian Erythrocyte Micronucleus Test
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12. Yes If there is toxicity, three dose levels should be used for the first sampling time.
13. Yes At the later sampling time only the highest dose needs to be used.
14.* Yes Dose administered orally by gavage or by intraperitoneal injection. Volume (by either route) should not exceed 2 mL/100g body weight, unless justification is provided.

[1] - Physical properties, purity/impurities, expiration date not provided. Not necessary.

Comments:

The study is acceptable for review.

JB

ACCEPTANCE CRITERIA

Chemical: Lufenuron PC Code: 118205 MRID No. 45853224

870.5550 Unscheduled DNA Synthesis Test in Rat Hepatocytes

Does this study meet the following acceptance criteria?

No 'new" Acceptance Criteria exits for this test. However, according to I. Mauer, the study would pass the "old" Acceptance Criteria as well as meet current standards for testing of this type of study.

Comments:

The study is acceptable for review.