

US EPA ARCHIVE DOCUMENT

DD-1056
THR-964

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Section 29 - Vol. IV of VII

Subacute Inhalation Toxicity To The Rat Of
DU 112307 W.P. 25% Insecticide Powder

(Evaluation of Methemoglobinemia)

PDR 197/741013

Test Compound: DU 112307 Technical - Batched # 405093 and #309181

Test Specie: Albino Rat - Sprague Dawley CD1 (Grade IV)

Number of Rats: males and twenty females
 Five males and five females per dose level
 Five males and five females controls

Route of Administration: Inhalation

Dose and Duration of Exposure: Exposure periods 1 hour
 Five days per week
 Three weeks
 Nominal concentrations 0.5, 5 and 50
 mg/liter air

Testing Laboratory: Huntingdon Research Center
 Huntingdon, England

Sponsor: N. V. Philips-Duphar, Weesp, Holland

Petitioner: Thompson Hayward Chemical Company

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Methodology: A Wright dust generator and/or a Timbrell dust generator was used to aerosolize DU 112307 w.p. 25% into chambers at a rate to offer nominal concentrations of 0.5, 5.0 and 50 mg of test material per liter of air. The dust was generated into chambers for one hour per day, five days per week for three weeks. The chamber contained one group of ten rats per exposure period, with wire mesh separating each rat. Control groups were treated only with air. Actual concentrations of dust were calculated by gravimetric sampling. Actual mean gravimetric finding for the three dose levels were 0.121 mg/liter air, 0.866 and 1.85 mg/liter respectively for the nominal concentrations of 0.5, 5 and 50 mg/liter. Particle sizes were within respirable range. Chamber temperatures were 24°C* 2°C and humidity 35%* 3%.

Results: This experiment designed to detect methemoglobinemia showed that there was an increase in methemoglobinemia in Sprague Dawley rats inhaling DU 112307 25% w.p. at the estimated concentrations of 0.121 mg/liter air, 0.866 mg/liter and 1.85 mg/liter for one hour each day, five days per week for three weeks. The results were statistically significant in the 0.121 mg/liter and 0.866 mg/liter range in males and in all three dose levels in females. No effects were noted in reticulocyte counts post exposure. All other parameters appeared within normal range.



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Comments: No spleen weights were recorded in this experiment notwithstanding the increase in spleen weights in previous experiment using 25% wettable powder. No histology done on tissues.

Conclusion: There is no indication in this experiment on the pathogenesis of methemoglobinemia or whether methemoglobinemia is transitory or permanent, only that it is present under the above stated conditions.

Validation: Core-Guidelines



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Effect of DU 112307 On Pregnancy of the Rat

PDR 192/74978

Test Compound: DU 112308 Batch # 309181

Test Specie: Pathogen Free Charles River Rats

Number of Rats: Eighty female rats - 20/dose level

Route of Administration: Intragastric Intubation

Dose: Group 1 - controls

Group 2 - 1 mg/kg/day

Group 3 - 2 mg/kg/day in 0.5% gum tragacanth

Group 4 - 4 mg/kg/day

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: N.V. Philips-Duphar, Weesp, Holland

Petitioner: Thompson Hayward Chemical Company

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Teratology, rat

Methodology: Specific pathogen free Charles River female rats 20/test level, 20 controls were mated and then treated with DU 112307 suspended in 0.5% gum tragacanth from the 6 to 15th day of pregnancy at 1 mg/kg, 2 mg/kg and 4 mg/kg respectively for groups 2, 3 and 4. All animals had tap water "ad lib" and Spratts Laboratory Diet #1.

Animals were examined for toxicity and weighed on 1, 3, 6, 10, 14 and 17 and 20. The animals were killed by CO₂ euthanasia. Ovaries and uterine contents were examined immediately to determine number corpora lutea, viable young, resorption sites, litter weight (to obtain mean pup weight) and foetal abnormalities. The method of Wilson was used to determine soft tissue abnormalities and skeletons were examined using alizarin stain.

Results: Female rats were not affected by the administration of test material at 1, 2, and 4 mg/kd p.o. when administered from day 6 to 15th. of gestation as reflected by signs of toxicity, body weight and pregnancy rate. Litter parameters were not affected as measured by litter size, foetal loss, and litter size. Embryonic and foetal development appeared within normal limits from the data made available and in comparison to gestation history of 1,960 control animals tested in this laboratory.

Comment: Pup weights were presented as mean pup weight per day. No individual pup weight data was presented. Test material needs to be completely identified in this report.

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Validation: Core - guidelines - tentative.

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Effect of DU 112307 on Pregnancy Of The
New Zealand White Rabbit

PDR 193/74937

Test Compound: DU 112307 (Batch # 309181)

Test Specie: New Zealand White Rabbit

Number of Rabbits: Thirteen per group - total 52 Rabbits

Route of Administration: Intragastric intubation

Dose: Group 1 controls

Group 2 - 1 mg/kg/day

Group 3 - 2 mg/kg/day

Group 4 - 4 mg/kg/day

} in 0.5% gum tragacanth

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: N. V. Philips-Duphar, Weesp, Holland

Petitioner: Thompson Hayward Chemical Company

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Methodology: DU 112307 was administered to mature pregnant New Zealand white rabbits at 3 dose levels, 1, 2, and 4 mg/kg intragastrically during days 6 to 18 of gestation. There were 13 animals per test group. Doses were injected with luteinizing hormone to assist ovulation. Rabbits were identified by ear tags. Test volume were always 1 ml/kg. Vehicle chosen was 0.5% gum tragacanth. Diet chosen was BOCM Coney (351) and tap water.

Toxic signs were recorded daily and weights were taken on days 1, 6, 10, 14, 21 and 28. On day 29, animals were sacrificed by cervical dislocation and dissected to ascertain number of young, uterine disposition, resorption sites, and corpora lutea. All young were examined for soft tissue and skeletal abnormalities.

Results: Test material DU 112307 administered to 3 groups of female rabbits at 13 per group dosed at 1, 2 and 4 mg/kg p. o. respectively from day 6 to 18 of gestation did not produce effects substantially different from the control group of 13 rabbits. The effects did not appear substantially different from laboratory standard values of 4,292 New Zealand white rabbits assayed at Huntingdon Research Center, Huntingdon, England.

Comment: There are no individual pup weights but only mean pup weight for each dam. Test material needs complete identification in this report.

Validation: Core-Guidelines - Tentative

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Tumorigenicity of DU 112307 To Mice

PDR/75685

Test Compound: DU 112307 Technical - Batch # P7227

Test Species: DELP Swiss Mice - Carworth - Europe

Number of Mice: 52 males and 52 females per group, five groups

Route of Administration: Dietary

Duration of Study: 80 Weeks

Dietary Levels: 0 (Controls)
4 ppm
8 ppm
16 ppm
50 ppm

Note: The report states that the dietary levels were chosen by the sponsor:

Testing Laboratory: Huntingdon Research Center
Huntingdon, Cambridgeshire, England

Sponsor: B. V. Philips-Duphar, Weesp, Holland

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Methodology: Mice of known litter origin were randomly distributed in the five test groups. All animals were identified by earmarks. Randomization was done according to body weights. Mice were housed four to each polypropylene cage with sifted sawdust as litter. Room temperature was 21* 20 C. Lighting was 12 hours illumination, 12 hours dark. Cages were also randomly distributed, in regards to spatial distribution. Mice had tap water "ad lib" and powdered laboratory food (Spatt's Laboratory Animal Diet No. 2). DU 112307 was incorporated into the diet to supply the above indicated dose levels. All animals were examined daily for signs of ill health, toxicity and behavioral changes. Food, water consumption were recorded. At end of test, surviving mice were killed by CO₂ asphyxiation. Gross pathology was done on all animals. All abnormalities were recorded including appearance and size of gonads, adrenals, thyroids, intra-abdominal lymph nodes and accessory reproductive organs. Adhesions, invasion between presumptive neoplasia and adjacent structures were noted.

Microscopic examination was routinely performed on adrenals, thyroids, ovaries, liver, spleen, lymph nodes, and pituitary glands and all macroscopically observed lesions suggestive of neoplasia from every animal. Blood and bone marrow smears were also made. Lung and liver tumors were classified according to Walters, 1966 (Brit. J. Cancer 20, 148-160) and lymphoreticular tumors were classified according to Dunn, 1954, (J. Natn. Cancer Inst. 14,

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1281-1432). Tissues preserved but not processed in this oncog-
nity study were:

heart,	pancreas	seminal vesicle
lungs	kidney	skin
thymus (where present)	urinary bladder	stomach glandular and non-glandular)
salivary gland	testis	brain (medullary, cerebellar, cortical)
trachea	tongue	skeletal muscle
oesophagus	mammary gland	bone
aorta	jejunum	gall bladder
eyes	mid-colon	
sciatic nerve	prostrate	

Statistics: The report mentions.

"Where the data suggested evidence of a response to treatment, the Student's "t" test was used to determine differences between groups.

The dose levels of DU 112307 in the diet were 0, 4, 8, 16 and 50 ppm. The actual intake of DU 112307, based upon food consumption data was 0 mg/kg day for controls, 0.34 mg/kg/day, 0.67 mg/kg/day, 1.39 mg/kg/day, and 4/30 mg/kg/day respectively for males and 0 mg/kg/day, 0.42 mg/kg/day, 0.80 mg/kg/day, 1.58 mg/kg/day, and 4/87 mg/kg/day respectively for females.

Results: No clinical signs of toxicity were reported that could be related to the administration of test material. Mortalities recorded during the experimental period did not appear to be dose related. No treatment related effects were noted for food consumption, water intake, body weight, or growth rate changes. Microscopic pathology did not reveal changes unusual for this strain and age of animal.

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Tumors

The histopathology data which can be evaluated for carcinogenicity are in those seven tissues which were routinely examined microscopically from all animals. These seven tissues are lymph nodes, spleen, liver, thyroids, ovaries, adrenals, and pituitary glands.

Male Mice

Of the 260 male mice on study, one pituitary adenoma was found at the 4 ppm dose level. Two thyroid papillary cystadenoma were found, one in controls and one at the 8 ppm dose level. One adrenal pheochromocytoma was found at the 4 ppm dose level. No tumors were reported in spleens. *3 granular cell tumors at 50 ppm*

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11/22/68
60 2000*

It is necessary to distinguish between those animals which survived the whole test period and were therefore maximally exposed to test material from those animals which died during the test period, when the data suggest a difference in tumorigenic effects.

Liver tumors

The percent incidences of single liver tumors in male mice in the group that both died during the experimental period and those that were sacrificed at termination of the experiment were 16% (8/50) at the control level 0 ppm, 14% (7/49) at 4 ppm, 18% (9/51) at 8 ppm, 22% (10/45) at 16 ppm, and 22% (11/50) at 50 ppm.



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The percent incidences of single liver tumors in male mice that survived the test period and were killed at termination of the experiment (maximum time exposure) were 16% (6/38) at 0 ppm, 15% (6/39) at 4 ppm, 16% (6/38) at 8 ppm, 26.7% (8/30) at 16 ppm and 30% (9/30) at 50 ppm.

The percent of multiple liver tumors in male mice, either from the group that died during the test period or from the group that survived the 80-week period showed no dose related increase.

Liver Nodular hyperplasia

The percent incidences of liver nodular hyperplasia in male mice that both died during the experiment together with those that survived the 80-week period were 12% (6/50) at 0 ppm, 20.4% (10/49) at 4 ppm, 21.6% (11/51) at 8 ppm, 20% (9/45) at 16 ppm, and 14% (7/50) at 50 ppm.

The percent incidences of liver nodular hyperplasia in male mice that survived the test period and were then sacrificed at termination (maximum exposure) were 13% (5/38) at 0 ppm, 23% (9/39) at 4 ppm, 23.7% (9/38) at 8 ppm.

Female Mice

Of the seven routinely examined tissues in the 260 female mice, two pituitary adenoma were found in the 4 ppm dose level, two were found at the 8 ppm level and two more were found at 16

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ppm. One adrenal pheochromocytoma was found at 50 ppm. No pituitary adenoma or pheochromocytoma were found in control female mice. No tumors were found in thyroids and as in female mice, no tumors were found in spleen.

Liver tumors

The percent incidences of liver tumors, both animals with single and multiple liver tumors, in female mice that both died during the experimental test period and those that were sacrificed at termination were 16% (8/49) at 0 ppm, 28% (14/50) at 4 ppm, 19% (9/48) at 8 ppm, 23% (10/43) at 16 ppm and 16% (8/50) at 50 ppm.

The percent incidences of single liver tumors in female mice that were sacrificed at the end of test (maximum time exposure) were 4% (1/23) at 0 ppm, 9% (3/34) at 4 ppm, 15% (5/33) at 8 ppm, 13% (3/24) at 16 ppm and 9% (3/33) at 50 ppm.

Lymphosarcomas

The percent incidences of lymphosarcomas in female mice that died during the test period were 15% (4/26) at 0 ppm, 25% (4/16) at 4 ppm, 26.67% (4/15) at 8 ppm, 26.32% (5/19) at 16 ppm and 29% (5/17) at 50 ppm.

The percent incidences of lymphosarcomas in female mice that lived the entire test period and were sacrificed at termination (maximum time exposure) were 0% at 0 ppm, 0% at 4 ppm, 24% (4/17) at 8 ppm, 31% (4/13) at 16 ppm and 27% (6/22) at 50 ppm.

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The percent incidences of lymphosarcomas of both groups, those that died during test period together with those that were sacrificed at end of test were 8.16% (4/49) at 0 ppm, 8% (4/50) at 4 ppm, 17% (8/48) at 8 ppm, 21% (9/43) at 16 ppm, and 22% (11/50) at 50 ppm.

Tumors of the lymphoreticular tissue

The percent incidences (which include lymphosarcomas, myeloid leukaemia, reticulum cell sarcoma) in all female mice that survived the test period together with those that died during test were 14% (7/49) at 0 ppm, 22% (11/50) at 4 ppm, 21% (10/48) at 8 ppm, 35% (15/43) at 16 ppm and 26% (13/50) at 50 ppm.

It is questionable whether there can be validity in histopathology data in this oncogenicity study in those instances where tissues (other than those seven routinely examined from all animals) were selected for microscopic examination only on the basis of lesions visually observable throughout the control and treated groups. However, were it to be assumed that all tumors were visibly detectable, the following data become evident.

In the testes, one interstitial cell adenoma was found at 4 ppm, 4 were found at 8 ppm, two more were found at 16 ppm and two others were found at 50 ppm. One fibrosarcoma was found in the epididymis at 4 ppm. There were no tumors found in the testes of control mice.

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Lung tumors

The percent incidences of lung tumors (any grade) in the group of male mice that survived the whole test period and killed at test end were 29% (11/38) at 0 ppm, 41% (16/39) at 4%, (18/38) at 8 ppm, 47% (14/30) at 16 ppm and 23% (7/30) at 50 ppm.

The incidences of lung tumors (any grade) in the male mice that both survived the test period and those that died during test were 28% (14/50) at 0 ppm, 35% (17/49) at 4 ppm, 41% (12/51) at 8 ppm, 38% (17/45) at 16 ppm and 18% (9/50) at 50 ppm. There would appear to be no increase in lung tumors in female mice with increasing dose levels of test material.

Kidney adenomas in males were found, two at the 4 ppm dose level and one at the 16 ppm dose level. There were no kidney adenomas in the control male animals.

No kidney tumors were reported in female mice.

The remainder of the tumors are single tumors of various types in controls and in the treated groups, not unusual in this animal specie.

All Tumors - Female Mice

In the group of female mice that died during the test period, the tumor incidences (all tumors) were 42% (11/26) at the 0 ppm dose level, 56% (9/16) at the 4 ppm dose level, 60% (9/15) at the 8 ppm dose level, 68% (13/19) at the 16 ppm and 65% (11/17) at the

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50 ppm. There would appear to be a trend toward a % increase in tumors as the dose level increases with the exception at the highest dose level which seems to plateau.

All Tumors - Male Mice

The groups of male mice showing an increasing trend of % tumors with dose levels are those that survived the test period and were sacrificed at test end. The percent incidences in tumors were 63% (24/38) at 0 ppm, 74% (29/39) at 4 ppm, 79% (30/38) at 8 ppm, 80% (24/30) at 16 ppm and 60% (18/30) at 50 ppm. Again there appears to be a trend towards % increases in tumors with dose level with the exception at the highest dose level.

Summary

Male Mice

There is an overall increasing trend in single liver tumors in male mice at the two highest dose levels either when examining those mice that survived the whole test period or those that survived together with those that died during test period.

In examining liver nodular hyperplasia in male mice, there is a general increase in incidences in the first three dose levels whether examining the group that survived the 80 weeks or the group composed of those that survived together with those that suc-

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cumbed during test. At the highest dose level of test material incidences of nodular hyperplasia appear to return to control values.

Female Mice

Single and multiple liver tumors in female mice both those that succumbed during test together with those that survived showed increases at 4 ppm and 16 ppm with the 8 ppm and 50 ppm values close to control values.

Single liver tumor incidences of females that survived the test period generally appear to rise at 4, 8, and 16 ppm. Incidences decline at 50 ppm but still remain about twice control values.

Lymphosarcomas generally rise in all groups of female mice. Tumors of the lymphoreticular tissue rose with increasing dose levels appreciably except for the 50 ppm dose level. At 50 ppm, however, the percent incidence was still about twice control values.

There is no basis for the evaluation of tumor incidences in those tissues that were not routinely examined from all animals. With the exception of the seven tissues mentioned above, histopathology was performed only when visual inspection decreed the existence of a lesion. However, if there is reason to believe that

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all lesions that existed where lesions visually observable then there can be seen a trend towards increases in tumors (all types) in female mice with increasing dose levels with again the exception at 50 ppm where the tumor incidence tends to plateau but still remain well above control value. Again there is a continuing increase in tumors in male mice with increasing dose levels with the exception of the 50 ppm dose level. At 50 ppm, the tumor incidences decline to control values.

Lung tumors (any grade) again showed increasing incidences with increasing dose levels for male mice that survived the test period with the exception of those animals at the 50 ppm dose level.

Incidences of lung tumors for the group of male mice that survived together with those that succumbed the test period again showed increased values over controls with the exception at the highest dose level.

Comments

No statement was made that the diets were chemically analyzed for the actual concentration of DU 112307 during the 80-week period.

The report states: "At weekly intervals the intake of the test compound (mg/kg/day) was calculated from the food consumption and body weight data." The intake of test material needs to be

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calculated from the food consumption and the analytical concentrations of test material in the food to arrive at the actual dosage levels of DU 112307.

The tissues routinely examined were lymph nodes (cervical and mesenteric), liver, spleen, ovary, adrenals and pituitary along with other tissues which on macroscopic examination showed evidence of lesions.

In view of the uniqueness of DU 112307 both from the structural viewpoint as well as to its mechanism of action on chitin, it is recommended that as many tissues as possible be examined histologically. To the 25 tissues listed in the report as preserved (and not examined), I would add tendon, ligaments, synovial membranes, cartilages (articular), xiphoid process, larynx, bursa mucoas, vertebral discs, and eyes, for additional microscopic study.

Clarification is needed why only seven tissues were examined in histopathology routinely from all animals and about 25 tissues were excluded from examination in a study designed to detect oncogenicity.

It was stated that blood and bone marrow smears were taken but no data has been made available.

Clarification is needed in the basis for the choice of seven tissues chosen for histopathology examination.

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Clarification is needed why ovaries were chosen to be examined histologically routinely but not testis.

A compound which produces methemoglobinemia and sulfhemoglobinemia, which when administered to mice produces no clinical effects or splenic tissue effects raises more questions than the experiment is designed to answer. The question arises, as to whether this study was conducted at the maximum tolerated dose level. It would appear that the diet levels of test material are too low to warrant a definitive assessment of oncogenicity.

Validation: The data in the routinely, microscopically examined tissues is considered supplementary.

Recommendations: It is recommended that this study be repeated for two primary reasons.

(1) In an oncogenicity study, it is expected that the number of tissues examined histologically from each animal reflect the tumorigenicity potential of test material. In this experiment, the routine examination of seven tissues does not accomplish the intended purpose of the experiment. The microscopic examination of tissues as a basis for screening is not satisfactory.

(2) The dose levels used in this study were too low to establish definitively oncogenicity potential of DU 112307. It is already remarkable that tumors in number were observed over control

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values at a fraction of a mg/kg/day dose levels. It is expected that statistically significant values may be established for oncogenicity at higher dose levels, if the inferences in this study are real and not apparent. It is therefore recommended that this study be repeated at higher dose levels.

Validation: For the above stated reasons, this study is considered supplementary.

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STATISTICS

Summary

The following are tables derived from the 80-week oncogenicity test. The statistical methods employed are FISCHER'S EXACT METHOD and the CHI^2 test. These tests were employed considering the non-parametric nature of the data.

Statistical differences can be found in mice with lymphosarcomas at levels equal to or above 95% confidence limits in mice that survived the 80-week period and were sacrificed (maximum exposure) or in the group composed of both those mice that succumbed the test period together with those that survived. The mice with lymphosarcomas that died during test period, while not exceeding 76% confidence limits, nevertheless showed increasing trends for lymphosarcomas with dose.

Lymphoreticular tumors in female mice statistically were different at 16 ppm dose level to confidence limits of 98%.

Lung tumors in male mice rose to 92% confidence limits at the 8 ppm dose level.

All tumors (male mice) were statistically, significantly different at 8 and 16 ppm dose levels at about the 90% confidence limit while females (all tumors) were statistically significant from controls at about the 92% confidence limit at the 16 ppm dose level.



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SECTION 33 - VOLUME V OF VII

EFFECTS OF DU 112307 IN DIETARY ADMINISTRATION
TO RATS FOR 104 WEEKS

TEST COMPOUND: DU 112307 - Batch # P7227

TEST SPECIES: SPRAGUE-DAWLEY RATS, CD STRAIN

NUMBER OF RATS: Forty-Five Males and Forty-Five Females Per Test Group
In the Main Study for Tumorigenicity Study. Fifteen
Males and Fifteen Females Per Group--Satellite Study
For Toxicity Study.

ROUTE OF ADMINISTRATION: Dietary

DURATION OF STUDY: 104 Weeks

DIETARY LEVELS: 0 (CONTROLS)
10 ppm
20 ppm
40 ppm
160 ppm

TEST LABORATORY: Huntingdon Research Center,
Huntingdon, Cambridgeshire, England

SPONSOR: B. V. Philips-Duphar, Weesp, Holland

PETITIONER: Thompson Hayward Chemical Company

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At termination, all surviving rats were killed by CO₂ asphyxiation.

A "carcinogenicity screen" was performed by examining adrenals, thyroids, ovaries, liver, spleen, lymph nodes and pituitary glands plus all tissues which upon macroscopic examination show signs of lesions. Abnormalities found in blood smears were confirmed by examining bone marrow.

A toxicity screen was performed in examining microscopically these seven organs as listed above including kidney and all tissues which macroscopically showed lesions. The mean intake of DU 112307 was calculated from body weight and food consumption data. The dose levels were 10 ppm (Group 2), 20 ppm (Group 3), 40 ppm (Group 4) and 160 ppm (Group 5). The calculated intake for males was 0.35 mg/kg/day (Group 2), 0.70 mg/kg/day (Group 3), 1.43 mg/kg/day (Group 4) and 5.83 mg/kg/day (Group 5) respectively while for females the calculated intake was 0.43 mg/kg/day (Group 2), 0.88 mg/kg/day (Group 3), 1.73 mg/kg/day (Group 4) and 7.05 mg/kg/day (Group 5).

Results: The report states clinically there were no overt signs of reaction treatment. Mortalities and autopsy data appeared to have no relationship to treatment. Food intake changes or body weight gain and food efficiency could not be related to test material ingestion. No evidence was available that water intake was affected by DU 112307. Glucose in urine was found to be high in four control animals at 103 weeks but in only one animal at 160 ppm does level.

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Methodology: Six hundred Sprague-Dawley-CD rats were used in this study.

Four hundred and fifty were used in the oncogenicity test and the balance were used in a satellite study to determine toxicity. Five groups of animals were composed of 45 rats per sex per dose in the oncogenicity test and 15 rats per sex per dose were used in the toxicity test.

The dose levels were 0, 10 ppm, 20 ppm, 40 ppm, and 160 ppm. Randomization of rats took into consideration litter origin and body weight. Animals were identified by earmark. Rats were housed five to a suspended cage with wire mesh floors. Room temperature and relative humidity were $21 \pm 20^\circ$ C and $50 \pm 5\%$ respectively. Lighting was 12 hours light and 12 hours dark. Water was "ad lib." Food was Spratt's Laboratory Diet 2.

Test material was administered in the diet. Dietary administration continued for 104 weeks.

All rats were examined daily for ill-health, signs of toxicity and behavioral changes. Skin lesions, cataracts and palpable growths were recorded. All rats were examined macroscopically to determine cause of death. Body weight and food consumption were recorded weekly. All eyes were examined at 0, 13, 26, 52, 78, and 104 weeks from groups 1 (controls) and from group 5 (160 ppm). Urines were examined as frequently. Blood was also examined at these time intervals from selected number of animals.

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which predominated in both studies were pituitary adenomas and mammary fibro-adenomas well distributed throughout the four test groups and controls. A scattering of tumors, one of a type throughout the remaining tissues were observed.

Comment: A carcinogenicity study is designed to assess oncogenic potential of test material. A "carcinogenicity screen" performed only on the microscopic examination of seven tissues from each animal plus tissues decreed by visual inspection to have lesions does not satisfy the experimental design or intent.

In the toxicity satellite experiment composed of only 15 rats per sex per dose administered DU 112307 at 0, 10, 20, 40, 160 ppm, histology in seven tissues were performed on 11 rats in control, 12 rats at 10 ppm, 13 rats at 20 ppm, 10 rats at 40 ppm and 13 rats at 160 ppm while macroscopic examinations only were done on the remaining animals. No rationale could be found either in a statement of experimental design or in the inspection of the number and kinds of tissues examined, that a basis exists for the choice and number of tissues subjected to microscopic examination. Tissue chosen for microscopic examination on the basis of visibly observable lesions does not permit an analysis of lesions as to severity or frequency of lesions in any of the treated or control group nor between any treated groups and control group.

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Methaemoglobinemia began to increase at 13 weeks in both sexes as observed between group 1 (controls) and group 5 (160 ppm). At 26 weeks, methaemoglobinemia is checked not in group 1 and 5 but between group 1 and 4. Notwithstanding, there is again observed an increase over control values. At 52 weeks and 78 weeks, the increase is still obvious between controls and the 160 ppm dose levels. At 102 weeks, treated males at 160 ppm begin to approach control values while females continue to show an increased methaemoglobinemia.

Other blood parameters seem to be within normal limits considering this specie of animal and age.

Organ weight changes did not show consistent variations that could be attributed to the ingestion of test material.

Oncogenicity-female rats: The incidences of tumors observed in the females rats ranged from 90% to 95% in the four treated groups, on the basis of the ratio of number of rats with tumors to the number of rats examined. However, the meaning of so high a percentage of tumorigenicity is obfuscated by the 98% incidence of tumors in the control female rats. No conclusion, therefore, can be drawn as to the oncogenicity of DU 112307 in female rats when fed DU 112307 at 0, 10, 20, 40 and 160 ppm in the diet for two years.

Generally the same tumor incidences were observed in the 150 rats used to study toxicity in the satellite study. The tumors

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which predominated in both studies were pituitary adenomas and mammary fibro-adenomas well distributed throughout the four test groups and controls. A scattering of tumors, one of a type throughout the remaining tissues were observed.

Comment: A carcinogenicity study is designed to assess oncogenic potential of test material. A "carcinogenicity screen" performed only on the microscopic examination of seven tissues from each animal plus tissues decreed by visual inspection to have lesions does not satisfy the experimental design or intent.

In the toxicity satellite experiment composed of only 15 rats per sex per dose administered DU 112307 at 0, 10, 20, 40, 160 ppm, histology in seven tissues were performed on 11 rats in control, 12 rats at 10 ppm, 13 rats at 20 ppm, 10 rats at 40 ppm and 13 rats at 160 ppm while macroscopic examinations only were done on the remaining animals. No rationale could be found either in a statement of experimental design or in the inspection of the number and kinds of tissues examined, that a basis exists for the choice and number of tissues subjected to microscopic examination. Tissue chosen for microscopic examination on the basis of visibly observable lesions does not permit an analysis of lesions as to severity or frequency of lesions in any of the treated or control group nor between any treated groups and control group.

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The incidence of tumors both in female control animals and in the treated female animals at all dose levels are so high (about 90 percent) as to obfuscate the presence of oncogenicity which might be attributable to DU 112307 both in the main oncogenicity study as well as in the satellite toxicity study.

Conclusion: Due to the deficiencies enumerated under comments, no assessment of oncogenicity or long term toxicity can be made from the data submitted in this study.

Validation: This study is invalid unless clarification is forthcoming regarding comments.

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Section 34 - Vol. VII of VII

Effect of DU 112307 On Reproductive
Function of Multiple Generations
In the Rat

Report # PDR 173/7594

Test Compound: DU112307 (Batches P7227, 309181)

Test Animals: Charles River Specific Pathogen Free CD Strain

Number of Animals: Two hundred rats, one hundred males and 100 females

Route of Administration: Dietary

Doses: 0, 10, 20, 40 and 160 ppm, 40 animals per dose, 20 males
and 20 females.

Testing Laboratory: Huntingdon Research Center
Huntingdon, England

Sponsor: N. V. Philips-Duphar, Weesp, Holland

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Methodology: Animals in the F0 generation were placed on their respective treatment diets 60 days before mating. The animals were mated to produce a F1A generation. A second mating was permitted producing an F1B generation. Animals from the F1B were used as parents to produce an F2A generation. These were now used to produce the F3A generation.

Results: The animals in the F0 generation initially showed a low mating performance hence the F1A litters were sacrificed. The F0 generation was again mated to form the F1B group. In the 2d mating of the F0 generation there was an occurrence of more than 50% of total litter losses. Of the 17 litters lost in the 3 generations, 10 litters were lost at the 2nd mating of the F0 parents. There were no consistent dosage-related trends in regards to food consumption, or body weights when assessed over the three generations. Weight ranges of females during pregnancies and lactation showed no consistent dosage related trends. All other test parameters were not remarkable in respect to control values.

Comments: The study states "the occurrence of more than 50% of the total litter losses at the second mating of the F0 generation was considered to be associated with the consequences of the sialodacryoadenitis occurring at the first mating of the F0 generation..

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The assumption that an inflammatory process of the lacrimal and salivary glands was responsible for 50% litter loss is non-acceptable. The macroscopic examination of these tissues does not verify the existence of an inflammatory process of these tissues.

Validation: Core-Guidelines

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Mutagenic Study With TH 6040 In Albino Mice

IBT No. 622-05068
P.O. No. 9973

Test Compound: TH 6040

Test Specie: Charles River strain albino mice

Number of Mice: Thirty-six males, twelve per group

Route of Administration: Single intraperitoneal injection

Doses: 0 (Controls), 1,000 mg/kg (Group T-I). 2000mg/kg (Group T-II)

Testing Laboratory: Industrial Bio-Test Laboratories

Petitioner: Thompson Hayward Chemical Company

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Methodology: One group of 12 male mice were treated with a single intraperitoneal injection of TH 6040 at 1000 mg/kg and another group of 12 male mice were treated at 2000 mg/kg test material. Controls were given corn oil. Sequential mating for each male with 3 untreated females per week continued for six weeks. Mutagenic effects in this dominant lethal study were to be detected by examining implantation sites, resorption sites and embryos. Females were sacrificed at about one week after mating or about half way through gestation.

Results and Comments: While the report states that two dose levels were employed, TABLE 11 shows seven dose levels were examined. These dose levels were 4,000 mg/kg, 3000 mg/kg, 2,000 mg/kg, 1000 mg/kg, 300 mg/kg, 100 mg/kg and 30 mg/kg. It is reported that dose levels of 4,000 mg/kg, 3,000 mg/kg and 2,000 mg/kg produced excessive grooming, hypoactivity, and ruffled fur. These signs diminished with decreasing dose levels down to 2,000 mg/kg level. This report does not report data at the three ^{lower} dose levels. Hypoactivity at 4,000 mg/kg was deemed responsible for the lack of mating activity. Hypoactivity began 15 minutes after injection and lasted for about 3 days.

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Conclusion: Treating male mice with 1,000 mg/kg i.p. (12 males) or 2,000 mg/kg (12 males) with TH6040 did not result in an increase ~~in an increase~~ in dominant lethal mutations following sequential mating of each male with three untreated females per week for six consecutive weeks.

Validation: IBT Study. Results need to be validated by the sponsor.

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