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

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Date: August 2, 2004

MEMORANDUM

Subject: 1E6257
DP Barcode: D301436
Decision No.: 304365
PC Codes: 800435 (Decanamide, N,N-dimethyl-); 900435 (Octanamide, N,N-dimethyl-)

From: Byron T. Backus, Ph.D.
Technical Review Branch
Registration Division (7505C)

To: Kathryn Boyle/Dan Rosenblatt, RM05
Minor Use, Inerts & Emergency Response Branch
Registration Division (7505C)

Applicant: The C.P. Hall Company

ACTION REQUESTED:

The Data Package Instructions state the following:

> "Please complete secondary reviews on reviews from Oak Ridge (Assignment no. RD-2003-4) for inert chemical, Hallcomid, PC codes 900435 (Octanamide) and 800435 (Decanamide)."

BACKGROUND: In addition to the material in the package indicated above, TRB has accessed a review (dated 12 May 2003 from James O. Parker to Kathryn Boyle) of six acute studies for Hallcomid M-8-10 and has incorporated them into the attached document.

COMMENTS AND RECOMMENDATIONS:

The following is a summary of the available toxicity studies (acute, subacute, developmental toxicity, and mutagenicity) for Hallcomid M-8-10 incorporating secondary review revisions made by TRB:

Acute Mammalian Toxicity

The following is the acute toxicity profile of technical Hallcomid:

acute oral toxicity	III	Acceptable	MRID 45369714
acute dermal toxicity	II	Acceptable	MRID 45369716
acute inhalation toxicity	IV	Acceptable	MRID 45369717
primary eye irritation	I	Acceptable	MRID 45369721
primary skin irritation	II	Unacceptable[1]	MRID 45369723
dermal sensitization	No	Acceptable	MRID 45369724

[1] However, the study can be used for regulatory purposes

In an acceptable acute oral toxicity study (MRID 45369714) groups (5 males and 5 females at 5000 mg/kg; 2 males and 2 females at lower dose levels) of Sprague-Dawley rats were orally gavaged with undiluted Hallcomid M-8-10 at doses of 0.625, 1.25, 2.5 and 5.0 g/kg. Rats were observed for 14 days.

All rats dosed at 5 g/kg died within one day of dosage; as did 1/ 2 males and 2/2 females dosed at 2.5 g/kg (one male survived). There were no deaths at the two lower dose levels.

Signs of toxicity at 0.625 and 1.25 g/kg included depression, rapid breathing, shallow respiration and hypothermia. Additional signs at 1.25 g/kg included gasping, red stain around nostrils, fecal stains, saliva stains, reddish-brown discharge from the genitals and a clear greasy fluid around the anal area. Similar symptoms were observed at higher dose levels.

There were no remarkable necropsy findings at 0.625 g/kg. At 1.25 g/kg some rats showed discolored intestines.

The oral LD_{50} (males and females combined) was calculated as 1.77 g/kg with 95% confidence limits of 1.02 to 3.08 g/kg (EPA Toxicity Category III).

In an acceptable acute dermal toxicity study (MRID 45369716) groups (5 male and/or 5 female) of young adult Wistar rats received 24-hour occluded dermal exposure to doses of 50, 200, 400 (females only), 2000 and 5000 (males only) mg Hallcomid M-8-10/kg, with subsequent 14-day observation.

There were no deaths at doses of up to and including 400 mg/kg. At 2000 mg/kg 2/5

males and 5/5 females died, and at 5000 mg/kg 5/5 males died.

Signs of toxicity (starting 30 minutes after initiation of exposure) included piloerection, heavy breathing, decreased motility, decreased reactivity, spastic gait, temporary tremor, hypothermia, increased salivation and/or lacrimation, pallor, cyanosis, chromodacryorrhea and red urine. All survivors recovered from these symptoms within the 14-day observation period.

Dermal effects (reddening, dark color, scarring, partial hardening) were seen from Day 2 to, in some cases, the end of the study.

Necropsy findings from rats which died during the observation period included brownish-red urinary bladder with fluid contents and moderate discoloration of the liver. Rats which survived to terminal sacrifice had no unusual necropsy findings.

The female dermal LD_{50} = between 400 (0/5 died) and 2000 (5/5 died) mg/kg. The male dermal LD_{50} is probably >2000 mg/kg (2/5 died following dosage at this level). Based on the female dermal LD_{50} > 400 mg/kg (but less than 2000 mg/kg) Hallcomid M-8-10 is in EPA Toxicity Category II in terms of acute dermal toxicity.

In an acceptable acute inhalation study (MRID 45369717) groups (5 males and 5 females) of Wistar rats received 4-hour nose-only exposure to Hallcomid M-8-10 in either undiluted form (generation of higher concentrations) or as a mixture with polyethylene glycol 400 and ethanol (lower concentrations). Actual concentrations (measured gravimetrically and/or analytically) tested were 0.1185, 0.5864, 2.01 and 3.55 mg/L. The MMAD's ranged from 1.14 to 1.37 μm, while the GSD's were from 1.37 to 1.49.

One male died as a result of exposure to the highest concentration (3.55 mg/L). All other rats (including those exposed to 3.55 mg/L) survived.

Signs of toxicity included reddened noses, reduced motility, piloerection, blood-encrusted and swollen rhinarium, serous nasal discharge, decreased respiration, piloerection, wheezing, prostration and cyanosis.

Gross necropsy of the male which died showed distended, edematous and liver-like lung, hydrothorax, nose and rhinarium red and swollen, pale spleen, marbled kidneys, and slimy-yellow content of the duodenum. There were no remarkable findings in rats which survived to terminal sacrifice.

Based on the LC_{50} > 3.55 mg/L Hallcomid M-8-10 is in EPA Toxicity Category IV in terms of acute inhalation toxicity.

In an acceptable primary eye irritation study (MRID 45369721), 0.1 mL Hallcomid M-8-10 was instilled into the conjunctival sac of an eye of a single New Zealand White rabbit.

The eye subsequently showed a blistered appearance, corneal opacity, extreme swelling, a white mucous-like substance in the bottom lid of the eye, bleached appearance of the nictating membrane, a thickened appearance to the conjunctiva and corneal vascularization. Because of the evident corrosivity of the test material, the study was terminated after the Day 4 reading.

Due to the corrosive effects in the rabbit eye, Hallcomid M-8-10 is in EPA Toxicity Category I with respect to eye irritation potential.

In a dermal irritation study (MRID 45369723) 0.5 mL undiluted Hallcomid M-8-10 was applied to a site on each of 6 (3 male and 3 female) New Zealand White rabbits, with 4-hour occluded dermal exposure.

Based on the moderate to severe erythema observed at 48 hours (sites were not scored at 72 hours), Hallcomid M-8-10 is in EPA Toxicity Category II in terms of dermal irritation potential.

While the study was classified as unacceptable (no scoring at 72 hours), the Agency will accept this study for regulatory and labeling purposes because of the severe irritation at 48 hours; the Agency's position is that a new study would not provide any useful information justifying exposure of additional animals.

In an acceptable dermal sensitization study (MRID 45369724) a group of 20 Hartley albino guinea pigs received induction exposures to 2.5% Hallcomid M-8-10 in acetone. There was no indication of a subsequent response to a challenge application of the test material (previously exposed guinea pigs showed essentially the same response as their naive controls).

Based on the lack of a dermal sensitization response, Hallcomid M-8-10 is considered a nonsensitizer.

Subacute Mammalian Toxicity

In an acceptable 6-week oral toxicity dog study (MRID 45369727), Hallcomid-M-8-10 (100% purity, Batch No.002949) was administered daily by gavage in 0.5% Tylose solution (using a constant dose volume of 5 mL/kg) to groups of beagle dogs (2/sex/dose; ages at start of dosage: 21-30 weeks; males: 8.4-11.7 kg; females: 7.4-10.7 kg) at initial dose levels of 0, 20, 100, or 500 mg/kg/day. Due to lack of significant toxicity the high dose was increased from 500 to 1000 mg/kg/day after two weeks of treatment.

One male in the 500 mg/kg/day group died on day 7 from a gavage error. There were no treatment-related changes seen in clinical chemistry, hematology, urinalysis, organ weight and gross pathology examinations. Highest dose dogs showed reduced food consumption at 500 mg/kg/day in the period immediately after being given their daily

ration, and this continued after the dosage was raised; however, their overall food consumption was generally the same as dogs in other groups. Clinical signs seen, once the high dose was increased to 1000 mg/kg/day, included temporary lateral position in two dogs [day 36 in dog #329 (male)and days 26, 30, and 34 in dog #300 (female)], disturbed coordination in one dog (#300 at day 26), and prone position in one dog (#300 at day 30) one hour after administration of test formulation. Histopathology revealed vacuolation of the mucosal epithelial lining of jejunum (grade 3) in one male at 100 mg/kg/day, as well as the one surviving male and one of the two females at 500/1000 mg/kg/day; the affected female also had grade 1 vacuolation of the ileum. These changes were considered spontaneous by the investigators but the reviewer considers them to be treatment related; however, this vacuolation suggests increased lipid resorption, and is probably not an adverse effect. Both high-dose females had purulent pneumonia associated with intrabranchial foreign material, most likely from aspiration of the dosing solution during gavage. There were no toxicologically relevant changes in any parameter in the control and other dose groups.

The LOAEL for Hallcomid-M-8-10 in male and female dogs is 500/1000 mg/kg/day, based on clinical signs. The NOAEL is 100 mg/kg/day.

This 6-week pilot study in the dog is classified as **Acceptable/Non-guideline** as it was not intended to fulfill the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in the dog.

In an acceptable 90-day oral toxicity study (MRID 45369715) Hallcomid M-8-10 (98.17%, batch no. 002949) was administered to 10 Wistar [BOR:WISW (SPF-Cpb)] rats/sex/dose in the diet at concentrations of 0, 400, 2000 or 10,000 ppm (equivalent to 0, 27.4, 136.8 and 787.6 mg/kg bw/day, respectively, in males and 0, 35.2, 178.5 and 894.6 mg/kg/day, respectively, in females). These control and dosage groups were designated as the "main groups". Additional groups of 10 rats/sex/dose (designated as "recovery groups") also received dietary Hallcomid M-8-10 at dose levels of 0 or 10,000 ppm (equivalent to 0 and 726.7, respectively, in males and 0 and 907.7 mg/kg/day, respectively, in females) during a 90-day treatment period which was followed by a 28-day post-treatment observation period. Concentrations were selected based on a 4-week range finding study (MRID 45369713).

There were no significant compound-related effects on mortality, clinical signs, food consumption, hematology or gross pathology. The compound-related changes observed in main group animals dosed with 10,000 ppm Hallcomid M-8-10 were decreased body weight in males ($p \leq 0.01$, 11% decrease; however, only a 4% decrease (n.s.) in body weight was seen in recovery group animals during the treatment period), increased incidence/severity of renal basophilic cortical tubules and deposition of protein casts in the medullary tubules of kidneys in males as well as increased plasma cholesterol levels (males: week 4-5: +21% with $p \leq 0.05$; week 13: +25.3% with $p \leq 0.05$; females: week 5: +35.7%, $p \leq 0.01$; week 13: +36.3%, $p \leq 0.01$) and relative liver weights (males: +16%,

p≤0.01; females: +10%, p≤0.01). Plasma cholesterol was also elevated at 2000 ppm in females (week 5: +14.8%, p≤0.05; week 13: +19.1%, p≤0.05). However, there were no correlative histopathological changes to explain the increased cholesterol levels. Body weight gain was inhibited during the treatment period in recovery group females dosed with 10,000 ppm (-19%) Hallcomid M-8-10, but only a 1% decrease was seen in high dose main study females. This difference was due to mean weight gain differences between their respective controls during treatment (main group controls: 79 g; recovery group controls: 102 g). Therefore decreased body weights and body weight gains in 10,000 animals were not considered adverse due to the relatively small decreases and variability seen between main study and recovery group treated and control animals during the treatment period.

The LOAEL for Hallcomid M-8-10 is 10,000 ppm (787.6 mg/kg/day in main study males) based on an increased incidence/severity of renal basophilic cortical tubules and deposition of protein casts in medullary tubules of kidneys in males. A LOAEL was not identified for females. The NOAEL is 2000 ppm (136.8 mg/kg/day) in main study males and 10,000 ppm (894.6 mg/kg/day) in main study females.

This 90-day oral toxicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rat.

In an acceptable subacute inhalation toxicity study (MRID 45369720), groups of ten Wistar [Bor:WISW (SPF-Cpb) rats/sex/group were exposed to aerosolized Hallcomid M-8-10 (3.7% N, N-dimethyl hexane acid amide; 54.1% N, N-dimethyl octane acid amide; 38.5% N, N-dimethyl decane acid amide; 1.3% N, N-dimethyl dodecane acid amide; batch # 903069) head/nose only under dynamic conditions. The animals were exposed to nominal concentrations of 0, 100, 500 or 2500 mg/m^3 [analytical concentrations of 0, 24.6, 111.2 or 521.2 mg/m^3 (0, 0.025, 0.111, 0.521 mg/L)] for 6 hours/day for a total of 5 days and observed post-exposure for 2 weeks. Approximately 97% of the aerosol particles in the test atmosphere were respirable, i.e., relative mass ≤ 3 µm. Clinical signs, neurological reflexes, body weight, hematology, clinical chemistry and necropsy findings were evaluated. Five rats/sex/group were sacrificed on days 7 and 22 of the study.

All animals survived to the end of the study. No neurological effects were observed. All rats at 521.2 mg/m^3 exhibited one or more of the following clinical signs beginning on day 2: labored breathing, bradypnea, wheezing, reduced mobility, reddened nose and serous nasal discharge. Some of the signs lasted until the next exposure day and continued until day 7 of the study. Body temperature after exposure was statistically significantly decreased in male and female rats at 521.2 mg/m^3 on day 0 and in females at this concentration on day 7.

Males and females at 521.2 mg/m^3 had decreased body weights on days 4 (8-9% below controls) and 7 (4-8% below controls). All groups including controls lost weight during exposure; males at 521.2 mg/m^3 lost a mean of 22 g while their controls lost 1 g; females at 521.2 mg/m^3 lost a mean of 21 g while their controls lost 9 g. During the post-exposure period, males and females at 521.2 mg/m^3 gained more weight than controls.

The incidence of goblet cell hyperplasia in the nasal mucosa was increased in females at 521.2 mg/m^3 on days 7 and 22. An exposure-related increase in the incidence of subpleural round-cell infiltration of the lungs was observed in males on days 7 and 22. The incidence of marginal emphysema of the lungs was increased in males and females at 521.2 mg/m^3 on day 7.

The LOAEL for Hallcomid M-8-10 in male and female rats is 521.2 mg/m^3 (0.521 mg/L), based on clinical signs of toxicity, decreased body temperatures, decreased body weight and weight gain, and histopathological findings in the respiratory tract in both sexes. The NOAEL is 111.2 mg/m^3 (0.111 mg/L).

This subacute (5 days) inhalation toxicity study in the rat is **Acceptable/Non-guideline**; it was a range-finding study and was not intended to satisfy a guideline requirement.

Developmental Toxicity

In an acceptable developmental toxicity study (MRID 45369719), Hallcomid M-8-10 (3.45% N,N-dimethyl-hexanacidamide, 53.31% N,N-dimethyl-octanacidamide, 39.48% N,N-dimethyl-decanacidamide, 1.43% N,N-dimethyl-dodecanacidamide; batch # 903069) was administered to 25 female Wistar rats/dose by gavage at dose levels of 0, 50, 150, or 450 mg/kg bw/day on days 6 through 15 of gestation. Doses were selected on the basis of a range-finding study in the rat (MRID 45369718). On GD 21, all surviving dams were sacrificed and examined grossly. Each fetus was weighed and examined externally for abnormalities and for sex determination. Approximately one-half of the fetuses in each litter were examined viscerally. The remaining one-half of the fetuses in each litter were eviscerated and processed for skeletal examination.

All animals survived to scheduled termination. No treatment-related clinical signs of toxicity, or effects on body weight, body weight gain, or food consumption were observed in low- or mid-dose animals. All high-dose animals were observed to have ruffled fur, ventral recumbency, dyspnea, and apathy during GDs 8-14. In addition, a total of five high-dose animals were described as being in a comatose state on at least one day between GDs 10-12. Absolute body weight of the high-dose group was slightly less than that of the controls beginning about GD 15 with statistical significance ($p \leq 0.05$; 95% of controls) attained on GDs 18 and 19. However body weight gains by the high-dose group were significantly less than those of the controls throughout the dosing period. The most pronounced effect on body weight gain was during GDs 6-11 when the high-dose group gained only 47% of the control group level. Overall weight gain by

the high-dose group was 69% of the control group level during the dosing interval. Food consumption by the high-dose group was 76-82% ($p \leq 0.01$) of the control levels during the dosing interval.

Therefore, the maternal toxicity LOAEL for Hallcomid M-8-10 in rats is 450 mg/kg/day based on clinical signs, and decreased body weight gain and food consumption, and the maternal toxicity NOAEL is 150 mg/kg/day.

No treatment-related differences were noted between the treated and control groups for numbers of corpora lutea and implantations, gravid uterine weights, live fetuses per dam, resorptions, fetal sex ratios, and pre-implantation losses. Post-implantation loss by the high-dose group was slightly greater than that of the control group (9.4% vs 5.6% for the controls). Male and female fetal body weights from the high-dose group were significantly less ($p \leq 0.01$; 92% and 91% of controls, respectively) than those of the control. No female had complete litter resorption.

The number of fetuses (litters) examined for external malformations/variations in the control, low-, mid-, and high-dose groups was 287 (25), 287 (25), 260 (25), and 281 (25), respectively. The number of fetuses (litters) examined viscerally was 137 (25), 137 (25), 123 (25), and 134 (25), respectively, and the number of fetuses (litters) examined for skeletal malformations/variations was 150 (25), 150 (25), 137 (25), and 147 (25), respectively. No treatment-related external or visceral malformations/variations were observed. The number of fetuses(litters) in the control, low-, mid-, and high-dose groups with skeletal malformations was 5 (4), 4 (4), 3 (3), and 12 (9) ($p \leq 0.05$), respectively. Dumbell-shaped thoracic vertebral body was the most common malformation [8 (6) vs t (1) in the control group]. Also significantly increased (litter incidence, $p \leq 0.01$) in the high-dose group were the incidences of incomplete or non-ossification of the vertebrae [29 (16) vs 11 (7) controls] and sternebrae [65 (22) vs 28 (13) controls]. Reduced ossification combined with lower body weights indicates fetal growth retardation.

Therefore, the developmental toxicity LOAEL for Hallcomid M-8-10 in rats is 450 mg/kg/day based on increased post-implantation loss, decreased fetal body weight, and increased incidences of skeletal malformations/variations. The developmental toxicity NOAEL is 150 mg/kg/day.

This developmental toxicity study in the rat is classified **Acceptable/Guideline** and does satisfy the guideline requirements for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

In a second acceptable developmental toxicity study (MRID 45369726), Hallcomid M-8-10 (3.45% N,N-dimethyl-hexanacidamide, 53.31% N,N-dimethyl-octanacidamide, 39.48% N,N-dimethyl-decanacidamide, 1.43% N,N-dimethyl-dodecanacidamide, batch # 903069) was administered in bi-distilled water plus 0.5% cremophor to 16 naturally

mated female Chinchilla rabbits/dose by gavage at dose levels of 0, 100, 300, or 1000 mg/kg bw/day from days 6 through 18 of gestation. Doses were chosen on the basis of a range-finding study (MRID 45369725). On GD 28, all surviving does were sacrificed and examined grossly. Each uterus was weighed and the ovaries examined for number of corpora lutea. Each fetus was weighed and examined for external abnormalities. Fetuses were examined viscerally by fresh dissection, and the sex determined. After the skin was removed, the heads were examined for the degree of ossification. The heads were then fixed in trichloroacetic acid and formaldehyde and examined by serial sectioning. The remaining carcasses were processed for skeletal examination.

No treatment-related deaths or clinical signs of toxicity occurred in any animal and gross necropsy was unremarkable. No treatment-related effects on absolute body weight, weight change, or food consumption were observed in the low- or mid-dose groups during the study. Absolute body weight of the high-dose group was slightly less than that of the controls throughout the study (including prior to treatment initiation) with statistical significance attained only on GDs 21 and 22. Overall weight gain during the dosing interval by the high-dose group was 47% of the control level with the most pronounced effect on GDs 6-11 during which weight gain was only 11% of the control level. The high-dose group showed compensatory weight gain during the post-dosing interval. Food consumption by the high-dose group was 75-84% of the control group levels during the dosing interval. Although maternal effects were marginal, it is noted that the high dose was the limit dose for a developmental toxicity study.

Therefore, the maternal toxicity LOAEL for Hallcomid M-8-10 in Chinchilla rabbits is 1000 mg/kg/day based on **decreases in body weight gain and food consumption** and **the maternal toxicity NOAEL is 300 mg/kg/day.**

No statistically significant differences were noted between the treated and control groups for numbers of corpora lutea, implantations, live fetuses, or resorptions, gravid uterine weight, fetal sex ratios, and pre- or post-implantation losses. Fetal body weight was similar between the treated and control groups. The number of fetuses (litters) examined for external, visceral, and skeletal malformations/variations in the control, low-, mid-, and high-dose groups was 158 (16), 145 (14), 120 (12), and 147 (15), respectively. No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetus.

Therefore, the developmental toxicity NOAEL for Hallcomid M-8-10 in Chinchilla rabbits is ≥1000 mg/kg/day and the developmental toxicity LOAEL was not identified.

The developmental toxicity study in the rabbit is classified **Acceptable/Guideline** and does satisfy the guideline requirements for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits. This study was conducted prior to implementation of current guidelines although the high dose is the limit dose.

Mutagenicity Assays

In an acceptable reverse gene mutation assay in bacteria (MRID 45369728), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* were exposed to Hallcomid M-8-10 (Batch No. 002949, 98.08% of identified constituents) in ethanol in three independent assays. A standard plate assay procedure was used and all plating was in quadruplicate. In the first assay, bacteria were exposed at concentrations of 0, 8, 40, 200, 1000 or 5000 µg/plate without metabolic activation (S9-mix) and with 30% S9. In the second assay, bacteria were exposed at concentrations of 0, 25, 50, 100, 200, 400 or 800 µg/plate without S9-mix and with 10% S9. The third assay was conducted with activation only at the same test material concentrations as used in the second assay. The S9 concentration was 4%. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

Hallcomid M-8-10 was tested to upper concentrations limited by cytotoxicity. Excess cytotoxicity was seen at 1000 and 5000 µg/plate in the first assay; therefore, the dose range for the second and third assays was 25 to 800 µg/plate. Thinning of the background lawn and a reduction in the background number of revertants per plate were seen at 400 and 800 µg/plate in all strains with and without S9-mix. Bacteria titer determinations done in the presence of S9-mix additionally showed bacteriotoxic effects at 200 µg/plate with TA1535 and TA98. The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain in any assay. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline** and satisfies the intent of Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data. Four plates per dose were used and repeat assays were conducted, two without S9-mix and three with S9-mix. A different concentration of S9 was used in each of the assays with S9-mix although the 4% concentration is below the recommended range of 5% to 30%. There was no hint of a mutagenic effect seen in the study. The EPA Guideline recommends that five tester strains be used, and only four were used in this study. These four *S. typhimurium* strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidizing mutagens, crosslinking agents and hydrazines. Such substances may be detected by *Escherichia coli* WP2 strains or *S. typhimurium* TA102 which have an AT base pair at the primary reversion site. As the test material is not an oxidizer, shows no structural similarity to any known crosslinking agent, and does not include a hydrazine group, it is concluded that inclusion of *Escherichia coli* WP2(uvrA) would not have changed the conclusions.

In a mammalian cell gene mutation assay at the HGPRT locus (MRID 45369729), Chinese hamster V79 cells cultured in vitro were exposed for five hours to Hallcomid M-8-10, (98.26%, 98.08%, 98.17% a.i. depending on analysis date, batch # 002949) in ethanol at concentrations of 0, 25, 50, 100, 125, 150, 200 or 250 µg/mL in the presence

and absence of mammalian metabolic activation (S9-mix). Two independent assays were conducted and duplicate cultures were used at each test material concentration. The S9-fraction was obtained from Aroclor 1254 induced male Wistar rat liver.

Hallcomid M-8-10 was tested up to cytotoxic concentrations. In the absence of S9-mix, a dose-dependent decrease in survival relative to the solvent control values was seen in both assays. Relative survivals at 150 μg/mL were 42.4% and 72.9% in the first and second assays, respectively. Higher concentrations were excessively cytotoxic. Cytotoxicity was reduced in the presence of S9-mix with relative survivals exceeding 100% at all test material concentrations up to 200 μg/mL in the first assay. Essentially no viable cells were seen at 250 μg/mL. A dose-dependent reduction in relative survival was seen in the second assay with values of 69.2% at 200 μg/mL and 17.6% at 250 μg/mL. No biologically or statistically significant increase in the mutant frequency was seen at any test material concentration with or without S9-mix in either assay. The solvent control values were within the testing laboratory's historical solvent control ranges and positive controls (Ethyl methanesulfonate in the absence of S9-mix and Dimethylbenzanthracene with S9-mix) induced clear and statistically significant increases in the mutant frequencies. There was no evidence of induced mutant colonies over background in Hallcomid M-8-10 treated cells.

This study is classified as Acceptable/Guideline and satisfies the requirement for Test Guideline OPPTS 870.5300, OECD 476 for in vitro mutagenicity (mammalian forward gene mutation) data.

In an acceptable mammalian cell cytogenetics (chromosome aberrations) assay (MRID 45369730), Chinese hamster ovary CHO cell cultures were exposed to Hallcomid —8-10 (98.08% a.i. identified as 4.73% N,N-Dimethyl-hexanoic acid amide; 53.9% N,N-Dimethyl-octanoic acid amide; 38.9% N,N-Dimethyl-decanoic acid amide; 0.55% N,N-Dimethyl-dodecanoic acid amide; analyzed July 21, 1992), Batch # 002949) at concentrations of 0, 10, 40 or 160 μg/mL for 4 hours without metabolic activation (S9-mix) and to concentrations of 0, 7.2, 36 or 180 μg/mL with S9-mix. The vehicle was ethanol. The S9-fraction was obtained from Aroclor 1254 induced male F344 rat liver. Cells were harvested 20 hours post-exposure at all concentration with and without S9-mix and additionally at 4 hours and 26 hours post-exposure at 160 μg/mL without S9-mix and at 180 μg/mL with S9-mix. Cells were evaluated for structural aberrations.

Hallcomid M-8-10 was tested up to cytotoxic concentrations. Upper concentrations which caused about a 50% reduction in the relative mitotic index were chosen based on the results of a preliminary cytotoxicity test. There were no statistically or biologically significant increases in the percentage of cells with structural aberrations, including or excluding gaps, at any test material concentration or harvest time without S9-mix. A statistically significant increase in the percentage of aberrant cells was seen at the four-hour post-exposure sampling time in cells exposed at 180 μg/mL with S9-mix but at no other dose or sampling time with S9-mix. The actual value of 3.5% seen at 180 μg/mL was within the historical solvent control range and not considered biologically significant.

The four-hour post-exposure harvest time is too short to evaluate chromosomal aberration induction; however, the 20-hour post-exposure sampling time meets the guideline recommendation of sampling at 1.5 x the average generation time of the cells used (14 hours for the CHO cells in this study). The solvent and positive control (Mitomycin C without activation and Cyclophosphamide (Endoxan) with activation) values were appropriate. **There was no evidence of chromosome aberrations induced over background.**

This study is classified as **Acceptable/Guideline** and satisfies the Test Guideline *in vitro* mammalian cytogenetics (chromosomal aberrations) OPPTS 870.5375; OECD 473.

In an acceptable unscheduled DNA synthesis assay (MRID 45369731), primary rat hepatocyte cultures were exposed to Hallcomid M-8-10 (Batch No. 002949, 98.3% and 98.08% a.i.) in ethanol at concentrations of 0, 29.8, 39.6, 49.4, 59.5, 79.1, 98.8 or 118.6 µg/mL for 18 hours in the presence of 10 µCi/mL ^{3}H-thymidine. UDS was determined using the autoradiographic procedure. Hepatocytes were obtained from young adult male Sprague-Dawley rats.

Hallcomid M-8-10 was tested up to cytotoxic concentrations. It is reported that the highest dose was excessively cytotoxic (relative survival reduced to 52.1%) and no slides could be evaluated at this dose. Relative survival in the remaining dose groups ranged from 79.3% to 90.1% in a non dose-dependent manner. Mean grain counts were based on evaluation of 50 cells per slide, three slides per dose with the exception of the 79.1 µg/mL dose groups where cells on only two slides were evaluated. Duplicate cultures were used at each dose. The mean net nuclear grain count (-1.15 ± 0.30) and the average number of cells in repair (0.00%), defined as cells with five or more net nuclear grains, in the solvent control were within the historical control ranges. No statistically significant increase in the mean net nuclear grain count or the percentage of cells in repair over the solvent control values were seen at any test material concentration and no dose-dependency was apparent. The highest number of net nuclear grains seen in test material treated cells was 0.20 ± 0.56 at the lowest tested dose. The 2-acetylaminofluorene positive control values of 7.79 ± 1.22 net nuclear grains and 82.67% of cells in repair were appropriate. **There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5550; OECD 482 for other genotoxic mutagenicity data.

DATA EVALUATION RECORD

HALLCOMID-M-8-10

STUDY TYPE: SIX-WEEK ORAL TOXICITY GAVAGE-DOG

MRID 45369727

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. RD-2003-4

Primary Reviewer:
Sanjivani Diwan, M.S., Ph.D. Signature: _____
Date: _____

Secondary Reviewers:
Robert A. Young, Ph.D., DABT Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractors' signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract no. DE-AC05-00OR22725.

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Feeding-Dog [OPPTS 870.3150 (§82-1b)] (non-rodent) OECD 409.

TEST MATERIAL (PURITY): Hallcomid-M-8-10 (100% a.i.)

SYNONYMS: None

CITATION: Vliegen, M., and V. Geiss. (1996) Hallcomid-M-8-10: Subacute toxicity in dogs (6-week study by oral administration, gavage). Bayer AG, Department of Toxicology, Friedrich-Ebert-Str, 217-333, D-42096, Wuppertal, FRG. Report No. 25057, Study No. T8055297, September 5, 1996. MRID 45369727. Unpublished.

SPONSOR: Bayer AG, FRG. (Submitted by C.P.Hall Company, U.S.A.)

EXECUTIVE SUMMARY: In a 6-week oral toxicity study (MRID 45369727), Hallcomid-M-8-10 (100% purity, Batch No.002949) was administered daily by gavage in 0.5% Tylose solution (using a constant dose volume of 5 mL/kg) to groups of beagle dogs (2/sex/dose; ages at start of dosage: 21-30 weeks; males: 8.4-11.7 kg; females: 7.4-10.7 kg) at initial dose levels of 0, 20, 100, or 500 mg/kg/day. Due to lack of significant toxicity the high dose was increased from 500 to 1000 mg/kg/day after two weeks of treatment.

One male in the 500 mg/kg/day group died on day 7 from a gavage error. There were no treatment-related changes seen in clinical chemistry, hematology, urinalysis, organ weight and gross pathology examinations. Highest dose dogs showed reduced food consumption at 500 mg/kg/day in the period immediately after being given their daily ration, and this continued after the dosage was raised; however, their overall food consumption was generally the same as dogs in other groups. Clinical signs seen, once the high dose was increased to 1000 mg/kg/day, included temporary lateral position in two dogs [day 36 in dog #329 (male)and days 26, 30, and 34 in dog #300 (female)], disturbed coordination in one dog (#300 at day 26), and prone position in one dog (#300 at day 30) one hour after administration of test formulation. Histopathology revealed vacuolation of the mucosal epithelial lining of jejunum (grade 3) in one male at 100 mg/kg/day, as well as the one surviving male and one of the two females at 500/1000 mg/kg/day; the affected female also had grade 1 vacuolation of the ileum. These changes were considered spontaneous by the investigators but the reviewer considers them to be treatment related; however, this vacuolation suggests increased lipid resorption, and is probably not an adverse effect. Both high-dose females had purulent pneumonia associated with intrabranchial foreign material, most likely from aspiration of the dosing solution during gavage. There were no toxicologically relevant changes in any parameter in the control and other dose groups.

The LOAEL for Hallcomid-M-8-10 in male and female dogs is 500/1000 mg/kg/day, based on clinical signs. The NOAEL is 100 mg/kg/day.

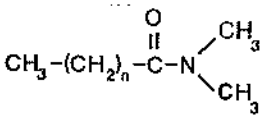
This 6-week pilot study in the dog is classified as **Acceptable/Non-guideline** and is not intended to fulfill the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in the dog.

COMPLIANCE: Signed Data Confidentiality statement was provided. A Quality Assurance statement was not included. The study differed from the requirements of 40 CFR Part 160 by complying with the GLP provisions of the "OECD Principles of Good Laboratory Practice".

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Hallcomid – 8-10

Description:	Clear, faintly yellow liquid
Lot/Batch #:	002949
Purity:	t00% a.i.
Compound Stability:	Stable at room temperature
CAS # if TGAI:	1118-92-9 (N,N-Dimethyloctanamide or N,N-Dimethyloctaneacidamine) and 14433-76-2 (N,N-Dimethyldecanamide or N,N-Dimethyldecaneacidamide)
Structure:	

2. Vehicle and/or positive control: 0.5% Tylose solution in water

3. Test animals:

Species:	Dog
Strain:	Beagle (Bor:Beag)
Age/weight at study initiation:	Males: 20-29 days, 7.7-11.3 kg; Females: 20-29 days, 6.9-10.1 kg
Source:	F. Winkelmann Breeders , Borchen.
Housing:	Individually in kennels with floor area of t. t0 x 1.15 m.
Diet:	Dog maintenance Sniff HH double-milled sole diet offered as daily rations of 350 g, during weeks 1-6.
Water:	Drinking water in dishes, *ad libitum*
Environmental conditions:	**Temperature:** 20-23 C **Humidity:** 30-50% **Air changes:** Not specified **Photoperiod:** 12 hrs day/12 hrs night
Acclimation period:	2 months

B. STUDY DESIGN:

1. **In life dates**: Start: March 28, 1994; End: May 9, 1994 (necropsy)

2. **Animal assignment**: Animals were assigned randomly to the test groups noted in Table 1.

TABLE 1: Study design			
Test group	Dose to animal (mg/kg/day)	# Male	# Female
Control	0	2	2
Low	20	2	2
Mid	100	2	2
High	500/1000[a]	2[b]	2

Data taken from page 17, study report MRID 45369727.
[a]The highest dose was increased to1000 mg/kg/day following two weeks of treatment.
[b]One of the two high-dose males died on day 7 from a dosing error, before the dose increase.

3. **Dose selection rationale:** No previous study was cited as the basis for dose selection. This dose-range finding pilot study was conducted to select doses for a subsequent subchronic 90-day guideline study.

4. **Diet preparation and analysis**: Test liquid was mixed in 0.5% Tylose once weekly using a magnetic mixer (IKA-Combining Ret). The concentrations in the test emulsions were monitored throughout entire study period. Prior to initiation of dosing the stability of the test substance in the emulsion was confirmed for a minimum of 8 days; the homogeneity analysis revealed that the test substance was homgeneously distributed in the emulsion.

Results:
Homogeneity/concentration analysis: Homogeneity analysis revealed concentration ranges for the upper, middle and lower portions of the low and high dose test samples, respectively, as follows: 95.3-104%, 94.9-96.3% and 94.9-101% and 100%, 97.1-98.9% and 96.5-101% of nominal on day 0 and 8, respectively.

Stability analysis: The results of homogeneity analysis indicate that the test substance was stable in Tylose solution for at least 8 days.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** Means and standard deviations were calculated for body weight, body weight gain, food consumption, hematology, clinical chemistry, electrocardiogram, blood-pressure, heart rates, urinalysis and organ weights. Because of small sample size no statistical analyses of data were performed.

C. METHODS:

1. Observations:

1a. **Cageside observations:** Animals were observed repeatedly daily for their appearance and behavior.

1b. **Clinical examinations:** Animals were tested for reflexes (pupillary, corneal, patellar, extensor, postural, and Flexor) before the start of the study (week -2), and in weeks 4 and 6. During the same time periods, body temperature was measured using a YSI telethermometer.

2. **Body weight:** Animals were weighed on study days -7 and 0, and at weekly intervals thereafter. Body weight change was calculated as the difference between the body weight on the day of weighing and the body weight on study day 0.

3. **Food consumption and compound intake:** All animals were provided with a daily ration of 350 g during weeks 1-6, and food consumption was monitored daily. However, data on actual amount consumed (g) for each animal were not provided. The reduced food consumption was presented in the form of grades.

4. **Ophthalmoscopic examination:** The eyes of all study animals were examined with a Zeiss ophthalmoscope before the start of the study (week -2), and in weeks 4 and 6.

5. **ECG and blood-pressure measurements:** Electrocardiograms were recorded before the start of the study (week -2), and in weeks 4 and 6. Each time the three tracings were plotted using a Schwarzer CK 12 in the lateral position from subcutaneous needle electrodes with paper feed at 50mm/sec. The heart rates at the femoral artery were determined before the start of the study (week -2), and in weeks 4 and 6. The heart rates determined only in the context of blood pressure measurements are referred to in this report. Pulse rates as well as systolic, diastolic and mean arterial pressures were calculated from at least five recorded blood pressure profiles.

6. **Hematology and clinical chemistry:** Blood was collected from all animals by puncturing the jugular vein. It was unclear whether the animals were fasted overnight. All hematology and clinical chemistry investigations were conducted before the start of the study (week -2), and in weeks 2, 4 and 6 of the study. The CHECKED (X) parameters were examined. In addition, N-demethylase, O-demethylase, and cytochrome P450 activities were also determined. Blood smear reticulocyte preparations were not evaluated.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	x	Reticulocyte count
	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.1350

b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium*		Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK, also APh)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase (CK)		
X	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT, also GPT)*		
X	Aspartate aminotransferase (AST, also GOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
X	Glutamate dehydrogenase (GLDH)		

* Recommended for subchronic non-rodent studies based on Guideline 870.1350

7. **Urinalysis:** Urine was collected overnight from fasted animals in metabolism cages before the start of the study (week -2), and in weeks 2, 4 and 6 of the study. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood / blood cells*
X	Sediment (microscopic)		Nitrate/nitrite
X	Protein*	X	Urobilinogen

* Recommended for subchronic non-rodent studies based on Guideline 870.1350

8. **Sacrifice and pathology** All animals were sacrificed under Narcoren® anaesthesia and subjected to gross pathological examination. The CHECKED (X) tissues were examined histologically. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroid*+
X	Rectum*	X	Urinary bladder*	XX	Thyroid*+
XX	Liver*+	XX	Testes*+		OTHER
X	Gall bladder*+	X	Epididymides*+	X	Bone (sternum and/or femur)
XX	Pancreas*	XX	Prostate*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries*+	X	Skin*
X	Trachea*	XX	Uterus*+	X	All gross lesions and masses*
XX	Lung*	X	Mammary gland*	X	Tonsils
	Nose*	X	Vagina		
	Pharynx*				
	Larynx*				

* Recommended for 90-day oral non-rodent studies based on Guideline 870.1350
\+ Organ weight required for non-rodent studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical observations/Signs of toxicity:** Monitoring of body temperature and heart rate revealed no changes among treated animals.

   All dogs from the mid and high dose groups showed defense reactions during test formulation administration.Clinical signs seen, once the high dose was increased to 1000 mg/kg/day, included temporary lateral position in two dogs [day 36 in dog #329 (male)and days 26, 30, and 34 in dog #300 (female)], disturbed coordination in one

dog (#300 at day 26), and prone position in one dog (#300 at day 30) one hour after administration of test formulation. Incidental clinical signs noted included salivation in dogs from all dose groups, nasal discharge in two dogs (male #321 and female #322) from the mid dose group and three dogs (male # 329 and two females #300 and 328, respectively) from the high dose group. Isolated incidences of vomiting and diarrhea were also noted.

2. **Mortality**: All animals survived to study termination with the exception of one high dose dog that died due to gavage error on day 7.

B. BODY WEIGHT AND WEIGHT GAIN:

No toxicologically significant treatment-related changes in body weight and body weight gain were seen in treated dogs from any dose groups. Although dogs treated with the high dose had body weight gains only 55% of control levels, the small number of animals (three in the high dose group and four controls by study end) precludes any determination of statistical significance. In addition, the two females in the high dose group had purulent pneumonia, presumably from aspiration of the test material, that may have had an effect on their final body weights.

TABLE 2. Mean body weight (and standard deviations) in dogs fed Hallcomid-M-8-10 for 6 weeks[a,b]

Parameter	Study week	Dose (mg/kg/day))			
		0	20	100	500/1000
Body weight (kg)	1 3 6	9.6±2.2 10.1±2.2 10.7_ 2.3	9.2±1.0 (96) 9.6±1.1 (95) 10.3_1.2(96)	9.3±1.0 (97) 9.9± 1.3 (98) 10.5± 1.3 (98)	9.3± 0.9 (97) 9.4± 1.1 (93) 9.9_ 1.2 (93)
Net weight gain (kg)	1 to 6	1.1	1.1	1.2	0.6 (54.5)
Number of animals/group		4	4	4	3

Data taken from p. 112, MRID 45369727.

[a] Net weight gain values were calculated by the Reviewer. No additional statistical analyses were conducted on these data.

[b] Numbers in parentheses represent the percent of the control value, as calculated by the Reviewer.

C. FOOD CONSUMPTION:

1. **Food consumption**: Reduced food consumption in the period immediately following offering of the food was repeatedly observed in the three surviving high dose dogs. However, their overall food consumption was apparently not different from other groups, although it is noted that the actual food consumption data (g) were not provided. There was no significant effect on food consumption in the remaining treated animals.

D. **OPHTHALMOSCOPIC EXAMINATION:** Ophthalmoscopic examination of the eyes of all study animals revealed no pathological findings.

E. **BLOOD ANALYSES:**

1. **Hematology:** The authors noted a marginal increase in leukocyte count in one control male, one mid dose female and two high dose females. The treated animals showed no changes compared with the control group in any of the other parameters measured.
2. **Clinical chemistry:** Alterations were noted in alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), N-demethylase and cytochrome P450 activities in treated animals. These changes consisted of marginal to distinct increases in ALT activity in one high dose female in week 2, LDH activity in one mid dose and one high dose female in week 6, CK activity in two mid dose females in week 6, N-demethylase activity in one low dose male and one male and one female from the high dose group as well as cytochrome P450 activity in one high dose male. However, these sporadic increases in a few treated animals were of low magnitude, and not dose-related. There were no differences in any other parameters noted between the control and treated animals.

F. **URINALYSIS**: No treatment-related changes were noted in urinary parameters.

G. **SACRIFICE AND PATHOLOGY:**

1. **Organ weight**: The study authors claim that increases in relative brain, lung, kidney, liver and pancreas weights seen in all dose groups were outside the range of the historical control data for the performing laboratory. However, similar increases were also seen in a few control animals.

2. **Gross pathology:** No treatment-related gross lesions were noted in any animal. At 500/1000 mg/kg/day, an unscheduled death of one dog revealed brownish discoloration in the lungs and the gastrointestinal tract. The trachea was filled with foam.

3. **Microscopic pathology**: Moderate vacuolation of the mucosal epithelial lining of the jejunum (grade 3) was observed in both sexes (male #329 and female #300) in the high dose group and in one male in the mid dose group. The affected high dose female also showed similar changes in the ileum (grade 1). These changes in high dose animals were not seen in controls. Incidental findings included inflammation of renal parenchyma and pelvis in nearly all animals of all dose groups as well as morphological changes such as differences in the number of goblet cells and presence of stratified squamous epithelium in the trachea of females of all dose groups.

III. **DISCUSSION AND CONCLUSIONS**:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that daily gavage administration of Hallcomid-M-8-10 to beagle dogs for 6 weeks failed to produce toxicologically relevant effects in both sexes. Due to lack of significant clinical findings at 500 mg/kg/day the dosing was increased to 1000 mg/kg/day following two weeks of treatment. One high dose male dog died due to gavage error on day 7. The dogs from the high dose groups repeatedly showed reduced food consumption in the period immediately after being offered food, but their overall food consumption was generally the same as was observed for other groups. Salivation was noted in dogs from all dose groups. Nasal discharge observed in two mid dose and three high dose dogs was possibly attributable to inadvertent passage of test formulation into the trachea. Following increase of the high dose to 1000 mg/kg/day, treatment-related transient signs consisting of lateral position, prone position and disturbed coordination were noted in two dogs from the high dose group. Occasional findings such as diarrhea/thin feces and pultaceous stools seen in dogs from all dose groups were not treatment-related. There were no significant differences between the control and treated groups in reflex tests, body temperature, heart rate, blood pressure and ECG measurements. Opthalmoscopic examinations, urinalyses, and hematology revealed no treatment-related findings.

The clinical chemistry analyses showed a marginal increase in ALT activity in high dose dogs at week 2, increased LDH activity in one dog each from the mid and high dose groups, and a marginal increase in CK in two mid dose dogs at week 6 compared to controls. These sporadic increases were considered unrelated to treatment because of lack of dose-response. Marginal increases in N-demethylase and cytochrome P450 activity and relative liver weight seen in only a few high dose animals were considered adaptive responses. Increases in relative brain, lung, kidney and pancreas weights were seen in both treated and control groups and were therefore considered insignificant. Gross and histopathology showed no test substance-related findings. Gross pathology revealed discoloration and surface changes in the lungs of high dose females possibly associated with purulent pneumonia caused by aspiration during gavage. Histopathology showed differences in the number of goblet cells and presence of stratified epithelium in trachea of dogs from all dose groups. These morphological differences were considered to be of spontaneous origin. There was vacuolation of the mucosal epithelial lining of jejunum (grade 3) in one male at 100 mg/kg/day, as well as in the one surviving male and one of the two females at 500/1000 mg/kg/day; the affected female also had grade 1 vacuolation of the ileum. These changes were considered spontaneous by the investigators but the reviewer considers them to be treatment related; however, this vacuolation suggests increased lipid resorption, and is probably not an adverse effect. The study authors concluded that 6-week treatment with Hallcomid-M-8-10 at 100 mg/kg/day produced no adverse effects in dogs (NOAEL). Increasing the high dose from 500 to 1000 mg/kg/day after 2 weeks of treatment caused transient clinical signs including lateral and prone position and disturbed coordination (LOAEL).

B. **REVIEWER COMMENTS:** Reduced food consumption in the high dose group in the period after food was offered was possibly from lack of appetite due to test

compound administration. The reviewer agrees with the study author's findings that the neurological clinical signs seen in high dose dogs (# 329 and #300) were treatment-related. The sporadic changes in clinical chemistry and hematological parameters seen in a few animals were not dose-related and therefore not considered toxicologically relevant. Changes in liver enzymes and relative liver weight seen in only a few high dose animals were considered adaptive responses. However, the reviewer considers the histopathological changes in the jejunum and/or ileum in two high dose dogs (# 329 and #300) to be treatment related.

The LOAEL for Hallcomid-M-8-10 in male and female dogs is 500/1000 mg/kg/day, based on clinical signs. The NOAEL is 100 mg/kg/day.

This 6-week pilot study in the dog is classified **Acceptable/Non-guideline** and is not intended to fulfill the guideline requirement for a 90-day subchronic oral toxicity study (OPPTS 870.3150; OECD 409) in the dog.

C. **STUDY DEFICIENCIES:** The group food consumption data were not provided. The most significant study deficiency was the small group size which makes it difficult to interpret the data.

DATA FOR ENTRY INTO ISIS

Subchronic Oral Study - non-rodents (870.3150)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range: mg/kg/day	Doses tested: mg/kg/day	NOAEL: mg/kg/day	LOAEL: mg/kg/day	Target organ(s)	Comments
xxxxxx	45369727	Subacute	dog	43 days	oral	gavage	20-1000	0, 20, 100, 500/ 1000 (_:and _)	100 (_:and _)	1000 (_:and _)	Clinical signs	Highest dose increased to 1000 mg/kg/day after 2 weeks of treatment.

REPEATED DOSE TOXICITY

TEST SUBSTANCE

_ **Identity**: Hallcomid-M-8-10

‡ **Remarks:** Clear, faintly yellow liquid with 100% purity; used as a solvent for agrochemical products. Test liquid was administered in 0.5% aqueous Tylose solution.

METHOD

· **Method/guideline followed:** Non-guideline range-finding study not intended to fulfill the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in dog.
_ **Test type**: Repeated dose 6-week pilot study
_ **GLP (Y/N)**: The study differed from the requirements of 40 CFR Part 160 by complying with the GLP provisions of the "OECD Principles of Good Laboratory Practice".
_ **Year (Study submitted):** September 5, 1996
_ **Species**: Dog
_ **Strain used**: Beagle (Bor:Beag)
_ **Route of administration:** Gavage
_ **Duration of test:** 6 weeks
_ **Doses/concentration levels:** 0, 20, 100, or 500 mg/kg/day. Due to lack of significant clinical findings at 500 mg/kg/day, after two weeks of treatment the high dose was increased to 1000 mg/kg/day.
· **Sex**: Male and female
_ **Exposure period:** 6 weeks
_ **Frequency of treatment:** Once daily
· **Control group and treatment:** Concurrent; 2/sex received 0.5% aqueous tylose solution
_ **Post exposure observation period**: None
_ **Statistical methods:** Mean and standard deviation for various parameters were calculated. The sample size was too small to conduct statistical analysis.
‡ **Remarks:**
This study was conducted to select doses for a subsequent 90-day subchronic toxicity guideline study.

RESULTS

_ **NOAEL**: 100 mg/kg/day
_ **LOAEL**: 1000 mg/kg/day
_ **Actual dose received by each sex:** 0, 20, 100, or 500 mg/kg/day for males and females. The highest dose was increased to 1000 mg/kg/day at week 3. In the following discussions, the high dose group will be called the the 1000 mg/kg/day group.
_ **Toxic response/effects by dose level:** At 1000 mg/kg/day, clinical signs were noted in one male and one female dog (#329 and 300, respectively) one hour following test compound administration. These were temporary lateral position in two dogs (day 36 in dog #329 and days 26, 30, and 34 in dog #300), disturbed coordination in one dog (#300 at day 26), and prone position in one dog (#300 at day 30) one hour after administration of test formulation. The dogs showed decreased food consumption which was possibly caused by the loss of appetite due to test compound administration. There was no decrease in body weight. Although dogs treated with the high dose had body weight gains only 55% of control levels, the small number of animals (three in the high dose group and four

controls by study end) precludes any determination of statistical significance. Among dogs from 100 and 1000 mg/kg/day dose groups, microscopic examination revealed moderate vacuolation of the mucosal epithelial lining of the jejunum in male #329 and female #300 at 1000 mg/kg/day and in one male (#321 at 100 mg/kg/day) and in the ileum of one high dose female. (#300). These changes were not seen at 20 mg/kg/day or in controls.

_ **Statistical results:** Because of the small number of animals statistical significance of the findings could not be determined.

‡ **Remarks:**

One male dog in the 500/1000 mg/kg/day dose group died due to gavage error on day 7 (before the dose was increased to 1000 mg/kg/day). No treatment-related ocular changes were detected in any animal. With the exception of the marginal increase in leukocyte count in one dog each from the control and 100 mg/kg/day dose groups and 2 dogs from the 1000 mg/kg/day dose group, no relevant changes in any of the hematological and urinalysis parameters were seen in treated dogs in comparison with the control group. No pathological findings were noted in the reflex tests. The marginal increase in ALAT activity level in one dog at 1000 mg/kg/day in week 2, increase in LDH activity in one dog each from the 100 and 1000 mg/kg/day dose groups, increased CK activity in two dogs from the 100 mg/kg/day dose group in week 6 were considered sporadic and unrelated to treatment. Increases in N-demethylase and cytochrome P450 activity and relative liver weight seen in only a few animals from the 1000 mg/kg/day dose group were considered adaptive responses. Increases in relative brain, lung, kidney and pancreas weights seen in treated dogs were not test substance-related since they were also seen in the control groups.

CONCLUSIONS

Hallcomid-M-8-10 administered daily by gavage at1000 mg/kg/day to beagle dogs for 6 weeks produced temporary clinical signs in one male and one female dog. In addition, vacuolation of the mucosal epithelial lining of jejunum (grade 3) was found in the same dogs from the 1000 mg/kg/day dose group and in only one male from 100 mg/kg/day dose group. The affected female had similar lesions (grade 1) in the ileum. This vacuolation suggests increased lipid resorption, and is probably not an adverse effect.

The LOAEL for Hallcomid-M-8-10 in male and female dogs is 500/1000 mg/kg/day, based clinical signs. The NOAEL is 100 mg/kg/day.

‡ **Remarks:**

The study authors claim that the vacuolation of the mucosal epithelial lining in the jejunum and ileum of dogs at 100 and 1000 mg/kg/day was not treatment related, since these findings have been observed in control animals of other species. The reviewer considers the lesions to be treatment related, but not necessarily indicative of an adverse effect These changes were not seen in concurrent controls or in the low dose group.. The highest dose was increased to 1000 mg/kg/day only after seeing the lack of toxicity for up to 2 weeks. Had this dose been administered to animals for 6 weeks, more significant toxicity may have been seen.

DATA QUALITY

_ **Reliability:** 1, reliable without restrictions.

REFERENCE

Vliegen, M., and V. Geiss. (1996) Hallcomid-M-8-10: Subacute toxicity in dogs (6-week study by oral administration, gavage). Bayer AG, Department of Toxicology, Friedrich-Ebert-Str, 217-333, D-42096, Wuppertal, FRG. Report No. 25057, Study No. T8055297, September 5, 1996. MRID 45369727. Unpublished.

FOUR-WEEK ORAL TOXICITY STUDY IN RATS

TEST SUBSTANCE

- **Identity**: Hallcomid M-8-10 (mixture of N, N-dimethylcaprylamide and N, N-dimethylcapramide)

‡ **Remarks: Description**: Light yellow liquid; purity: 98.30%; batch no. 002949; stable in laboratory diet for 14 days

METHOD

- **Method/guideline followed**: Not applicable
- **Test type**: Four-week oral toxicity (range-finding)
- **GLP (Y/N)**: No; the study was neither conducted according to GLP principles nor was the conduct of the study or the final report audited by a quality assurance unit.
- **Year (study performed)**: 1992
- **Species**: Rat
- **Strain**: Wistar [BOR:WISW (SPF-Cpb)]
- **Route of administration**: Oral (feed): Altromin®1321 laboratory diet *ad libitum*
- **Duration of test**: 4 weeks
- **Doses/concentration levels**: Dietary levels were 0, 1000, 3000 or 10,000 ppm (equivalent to 0, 82.9, 250.6 and 965.0 mg/kg/day, respectively, in males and 0, 93.7, 293.2, and 1075.7 mg/kg/day, respectively, in females).
- **Sex**: 5/sex/dose
- **Exposure period**: 4 weeks
- **Frequency of treatment**: Continuous (diet)
- **Control group and treatment**: 5/sex; maintained on laboratory diet with 1% peanut oil
- **Post exposure observation period**: None
- **Statistical methods**: Summarized data from the control and treatment groups for the parameters investigated in this study were compared using the Mann-Whitney U test (equivalent to the Wilcoxon rank sum test).

RESULTS

- **NOAEL/LOAEL**: Not applicable. This four-week study was a range finding study designed to set dose levels for a 90-day study.

- **Body weight**: Compared to controls, body weight gain was inhibited at 10,000 ppm in males (965.0 mg/kg/day) throughout the study (67 and 28 % during weeks 1 and 4, respectively) and in females at 10,000 ppm during weeks 1 (-79%) and 2 (-19%).
- **Food/water consumption**: No treatment-related effects
- **Clinical signs**: No treatment-related effects
- **Ophthalmologic findings**: Not performed
- **Hematologic findings**: No treatment-related effects
- **Clinical biochemical findings**: Plasma cholesterol levels were elevated at 10,000 ppm in males ($p<0.01$) and females ($p<0.05$).
- **Mortalities**: No treatment-related mortalities
- **Gross pathology**: No treatment-related changes
- **Organ weight changes**: Significant organ weight increases at 10,000 ppm included absolute and relative liver weights ($p<0.01$) and relative kidney weights ($p<0.05$) in females and relative liver ($p<0.01$) and kidney ($p<0.05$) weights in males; although increased absolute liver weights in females and relative liver weights in males were statistically significant ($p<0.05$) at 3,000 ppm, the increases were relatively small and no correlative changes were seen in the investigated parameters. Histopathologic changes in kidneys were seen in male rats dosed with 10,000 ppm Hallcomid

M-8-10 in a 90-day dietary study (MRID 45369715). There was an increased incidence/severity of basophilic cortical tubules and deposition of protein casts in medullary tubules, however, a concomitant increase in kidney weights was not observed in these animals.

- **Histopathology**: Not assessed

CONCLUSIONS

Dietary levels of Hallcomid M-8-10 up to and including 3,000 ppm did not cause significant treatment related effects on rats in this study.

There were no significant compound-related effects on mortality, clinical signs, food consumption, hematology or gross pathology at 10,000 ppm. The compound-related changes observed in animals dosed with 10,000 ppm Hallcomid M-8-10 were, in males, inhibited body weight gain, increased plasma cholesterol levels, and increased relative liver and kidney weights and in females, increased plasma cholesterol levels, increased absolute and relative liver weights as well as increased relative kidney weights.

Based on the results of this preliminary study, doses for the 13-week study were chosen as 0, 400, 2000, and 10,000 mg/kg/day.

DATA QUALITY

- **Reliability:** 2, reliable with restrictions

‡ **Remarks**:
Histopathology was not performed.

REFERENCE

Wirnitzer, U. (1993) Hallcomid M-8-10: Study for subacute toxicity on Wistar rats (Feeding study for range-finding over 4 weeks) Bayer AG, Institute of Toxicology, Friedrich-Ebert-Str. 217-333, 42096 Wuppertal 1. Report No. 22117. Study No. T9041022. March 11, 1993. MRID 4569713. Unpublished.

DATA EVALUATION RECORD

HALLCOMID M-8-10

STUDY TYPE: 90-DAY ORAL TOXICITY-RAT [OPPTS 870.3100 (§82-1a)]
MRID 45369715

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [feeding]- rat [OPPTS 870.3100 (§82-1a)](rodent) OECD 408.

PC CODE: NA

DP BARCODE: NA
SUBMISSION NO.: NA

TEST MATERIAL (PURITY): Hallcomid M-8-10 (98.17%)

SYNONYMS: none in this report

CITATION: Wirnitzer, U. and R_hl-Fehlert, C. (1993) Hallcomid M-8-10: Study on subacute toxicity in Wistar rats (Administration in feed over 13 weeks with 4-week post-treatment observation) Bayer AG, Institute of Toxicology, Friedrich-Ebert-Str. 217-333, 42096 Wuppertal 1. Report No. 22931. Study No. T4041117. March 11, 1993. MRID 45369715. Unpublished.

Wirnitzer, U. (1993) Hallcomid M-8-10: Study for subacute toxicity on Wistar rats (Feeding study for range-finding over 4 weeks) Bayer AG, Institute of Toxicology, Friedrich-Ebert-Str. 217-333, 42096 Wuppertal 1. Report No. 22117. Study No. T9041022. March 11, 1993. MRID 4569713. Unpublished.

SPONSOR: Bayer AG

EXECUTIVE SUMMARY: In a 90-day oral toxicity study (MRID 45369715) Hallcomid M-8-10 (98.17%, batch no. 002949) was administered to 10 Wistar [BOR:WISW (SPF-Cpb)] rats/sex/dose in the diet at concentrations of 0, 400, 2000 or 10,000 ppm (equivalent to 0, 27.4, 136.8 and 787.6 mg/kg bw/day, respectively, in males and 0, 35.2, 178.5 and 894.6 mg/kg/day, respectively, in females). These control and dosage groups were designated as the "main groups". Additional groups of 10 rats/sex/dose (designated as "recovery groups") also received dietary Hallcomid M-8-10 at dose levels of 0 or 10,000 ppm (equivalent to 0 and 726.7, respectively, in males and 0 and 907.7 mg/kg/day, respectively, in females) during a 90-day treatment period which was followed by a 28-day post-treatment observation period. Concentrations were selected based on a 4-week range finding study (MRID 4569713).

There were no significant compound-related effects on mortality, clinical signs, food consumption, hematology or gross pathology. The compound-related changes observed in main group animals dosed with 10,000 ppm Hallcomid M-8-10 were decreased body weight in males ($p \leq 0.01$, 11% decrease; however, only a 4% decrease (n.s.) in body weight was seen in recovery group animals during the treatment period), increased incidence/severity of renal basophilic cortical tubules and deposition of protein casts in the medullary tubules of kidneys in males as well as increased plasma cholesterol levels ($p \leq 0.01$ or 0.05; 21-36% increase) and relative liver weights in males ($p \leq 0.01$; 16%) and females ($p \leq 0.01$; 10%). Plasma cholesterol was also elevated at 2000 ppm in females ($p \leq 0.05$; 15-19%). However, there were no correlative histopathological changes to explain the increased cholesterol levels. Body weight gain was inhibited during the treatment period in recovery group females dosed with 10,000 ppm (-t9%) Hallcomid M-8-10, but only a 1% decrease was seen in high dose main study females.

The LOAEL for Hallcomid M-8-10 is 10,000 ppm (787.6 mg/kg/day in main study males) based on an increased incidence/severity of renal basophilic cortical tubules and deposition of protein casts in medullary tubules of kidneys in males. A LOAEL was not identified for females. The NOAEL is 2000 ppm (136.8 mg/kg/day) in main study males and 10,000 ppm (894.6 mg/kg/day) in main study females.

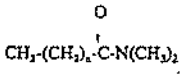
This 90-day oral toxicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rat.

COMPLIANCE: The submitter of the study (C.P. Hall Co.) stated that they do not know "whether it has been conducted in accordance with 40 CFR Part 160". The study director provided a statement which indicated that this study was performed according to the OECD Principles of Good Laboratory Practice and the Principles of Good Laboratory Practice as published in Annex 1 of the German Chemicals Act. Signed and dated Confidentiality and Quality Assurance statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Hallcomid M-8-10 (mixture of N,N-dimethylcaprylamide and N,N-dimethylcapramide)

Description:	Light yellow liquid
Lot/Batch #:	002949
Purity:	98.17 % a.i.
Compound Stability:	Stable in laboratory diet under test conditions for 14 days
CAS No. of TGAI:	1118-92-9 and 14433-76-2
Structure:	

2. **Vehicle:** 1% peanut oil

3. **Test animals:**

Species:	Rat
Strain:	Wistar [BOR:WISW (SPF-Cpb)]
Age/weight at study initiation:	6 - 7 weeks old/males: 116 - 145 g; females: 103 - 135 g
Source:	F. Winkelmann GmbH and Co., KG, Borchen
Housing:	Individual cages (Makrolon® type II)
Diet:	Altromin® 1324 during acclimation; Altromin® 1321 during the study, *ad libitum*
Water:	Tap water *ad libitum*
Environmental conditions:	**Temperature:** 22 ± 2° C **Humidity:** 55 ± 5% **Air changes:** 15 - 20/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	One week

B. STUDY DESIGN:

1. **In life dates:** Treatment period: start: May 15, 1992; End: main study: males - August 13; females and recovery animals - August 14, 1992. Recovery period: August 14 - September 11, 1992.

2. **Animal assignment**: Following stratification based on body weight, animals were randomly assigned to the test groups noted in Table 1.

TABLE 1: Study design

Test group	Conc. in diet (ppm)	Dose to animal (mg/kg/day)	No. Male	No. Female
	Main groups			
Control	0	0	10	10
Low	400	_(27.4) _(35.2)	10	10
Mid	2000	_(136.8) _(178.5)	10	10
High	10,000	_(787.6) _(894.6)	10	10
Recovery groups				
Control	0	0	10	10
High	10,000	_(726.7) _(907.7)	10	10

Data taken from p. 40, MRID 45360715.

3. **Dose selection rationale:** The dose levels were selected based on the results from a four-week study (MRID 45367913, Report no. 22117) with Hallcomid M-8-10 in Wistar rats which received 0, 1000, 3000 or 10,000 ppm in the diet. Inhibited body weight gain, increased cholesterol levels and elevated kidney and liver weights were seen at 10,000 ppm in both sexes.

4. **Diet preparation and analysis:** Diet was prepared by mixing appropriate amounts of test substance with Altromin® 1321 diet and 1% peanut oil; the frequency of test diet preparation and storage conditions for the test diet during the study were not reported. Test diets were analyzed three times during the study for active ingredient concentrations. Homogeneity and stability data were taken from another study (T 9041022) in which diet mixtures containing 100 or 20,000 ppm Hallcomid M-8-10 were analyzed. Stability was determined in diet mixtures that were stored 14 days "under conditions comparable to those in the actual feeding study".

Results:

Homogeneity analysis: Individual measurements of Hallcomid M-8-10 were 100 to 114 % and 96 to 108 % of nominal concentrations at 100 and 20,000 ppm, respectively.

Stability analysis: Following 14 days of storage, measured concentrations were 101 and 99 % of those determined at day 0 for the 100 and 20,000 ppm dietary mixtures of Hallcomid M-8-10, respectively.

Concentration analysis: Dietary concentrations of Hallcomid M-8-10 were 99-101%, 97-102% and 94-100% of nominal concentrations for the 400, 2000 and 10,000 dietary levels, respectively.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics: Summarized data from the control and treatment groups for the parameters investigated in this study were compared using the Mann-Whitney U test (equivalent to the Wilcoxon rank sum test).

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected twice daily on week days and once daily on weekends for signs of toxicity and mortality.

1b. Clinical examinations: Clinical examinations were conducted weekly.

1c. Neurological evaluations: A thorough neurological evaluation was not performed in this study. The following relevant assessments were performed weekly: posture, general behavior, breathing and excretions.

2. Body weight: Animals were weighed before treatment and weekly thereafter.

3. Food consumption and compound intake: Food consumption for each animal was determined weekly and mean daily diet consumption was calculated as g food/kg body weight/day as well as g food/animal/day. Cumulative food consumption values were also calculated. Food efficiency was not determined. Compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the food consumption (mg/kg bw/day) and active ingredient analytical data. Additional values for compound intake included g/animal/day and cumulative intake values.

4. Ophthalmoscopic examination: Eyes of animals in the main control and 10,000 ppm groups were examined before the treatment period and at the termination of the study.

5. Hematology and clinical chemistry: Blood was collected from all animals in the main groups during study weeks 4/5 and 13 and during week 17 for all animals in the recovery groups; animals were not fasted prior to blood collection. The times of collection were not reported. The CHECKED (X) parameters were examined.

a. Hematology:

x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)*
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)*
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)*
x	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
x	(Thromboplastin time)		
	(Clotting time)		

	(Prothrombin time)

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry:

	ELECTROLYTES		OTHER
x	Calcium	x	Albumin*
x	Chloride	x	Creatinine*
	Magnesium		Urea nitrogen*
x	Phosphorus	x	Total Cholesterol*
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	ENZYMES (more than 2 hepatic enzymes)*		Total bilirubin
x	Alkaline phosphatase (ALK)*	x	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Urea
x	Alanine aminotransferase (ALT/also SGPT)*		
x	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis**: Urine was collected from all animals in the main groups during study weeks 4 and 12 and during week 17 from the recovery groups. The report did not indicate if the animals were fasted before collection. Collection occurred during 16-hour overnight periods during which water was provided *ad libitum* and food was withheld. The CHECKED (X) parameters were examined.

	Appearance*	x	Glucose
x	Volume*	x	Ketones
x	Specific gravity/osmolality*	x	Bilirubin
x	pH*	x	Blood/blood cells*
x	Sediment (microscopic)		Nitrate
x	Protein*	x	Urobilinogen

[1] Optional for 90-day oral rodent studies
* Recommended for 90-day oral rodent studies

7. Sacrifice and pathology: All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. All checked tissues from the control and 10,000 ppm groups (main and recovery groups), liver and kidney from all 400 and 2000 ppm groups and all gross lesions were examined histologically. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
x	Tongue	x	Aorta*	xx	Brain*+
x	Salivary glands*	xx	Heart*+	x	Peripheral nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen*+	x	Eyes (optic nerve)*
x	Jejunum*	x	Thymus*+		GLANDULAR
x	Ileum*			xx	Adrenal gland*+
x	Cecum*		UROGENITAL	x	Lacrimal gland
x	Colon*	xx	Kidneys*+	x	Parathyroid*
x	Rectum*	x	Urinary bladder*	x	Thyroid*
xx	Liver*+	xx	Testes*+		OTHER
	Gall bladder (not rat)*	x	Epididymides*+	x	Bone (sternum and/or femur)
	Bile duct (rat)	x	Prostate*	x	Skeletal muscle
x	Pancreas*	x	Seminal vesicles*	x	Skin*
	RESPIRATORY	x	Ovaries*+	x	All gross lesions and masses*
x	Trachea*	x	Uterus*+	x	Zymbal gland
xx	Lung*	x	Mammary gland*	x	Ears
	Nose*	x	Ureter	x	Hardarian gland
	Pharynx*	x	Urethra	x	Head
x	Larynx*	x	Vagina	x	Tooth

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies.

II. RESULTS:

A. OBSERVATIONS:

1. Clinical signs of toxicity: The only finding reported was emaciation seen in 5/10 recovery group 10,000 ppm males. The study report did not provide specific information regarding the time of each observation or the severity of the findings.

2. Mortality: There were no treatment-related mortalities. There were three accidental deaths resulting from blood collection in the main groups: one control male, one control female and one 2000 ppm female.

3. Neurological evaluations: There were no indications of neurotoxicity from the limited assessment.

B. **BODY WEIGHT AND WEIGHT GAIN:** The final body weight of males in the main group receiving 10,000 ppm Hallcomid M-8-10 was significantly lower ($p \leq 0.01$; 11% decrease) than that of controls (Table 2), but 10,000 ppm recovery animals only showed a 4% decrease during the treatment period. Body weight gain in 10,000 ppm males was decreased in main study animals by 17% but only by 6% in recovery group animals (during the treatment period). Body weight gain was also depressed (23% for weeks 1- 4) in male rats (n=5) receiving 10,000 ppm Hallcomid M-8-10 in a previous 4-week dietary study (MRID 45369713). The body weight gain in the 2000 ppm main group males was depressed between weeks 1 and 9 but exceeded the control value thereafter. Both 10,000 ppm recovery group males and females gained body weight during the recovery period while respective control weights remained comparable to week 13 values.

TABLE 2. Mean body weight (BW)[a] and body weight gain (BWG) of rats fed diets containing Halcomid M-8-10

	Dietary concentration (ppm)					
	0	400	2000	10,000	0	10,000
	Main group				Recovery group	
MALES						
Initial BW	128 ± 8.4	131 ± 5.5	131 ± 4.6	129 ± 7.2	131 ± 7.1	130 ± 8.2
Final BW[b]	378 ± 26.6	364 ± 24.1 (-4)	351 ± 36.1 (-7)	337 ± 32.8** (-11)	364 ± 41.8	350 ± 31.7 (-4)
Final BW[c]					364 ± 43.6	366 ± 36.8 (+1)
BWG Wk 0-13[d]	250	233 (-7)[e]	220 (-12)	208 (-17)	233	220 (-6)
BWG Wk 0-17					233	236 (+1)
BWG Wk 0-1	36	44 (+22)	39 (+8)	33 (-8)	39	32 (-18)
BWG Wk 0-4	129	119 (-8)	108 (-16)	107 (-17)	132	122 (-8)
BWG Wk 4-9	97	92 (-5)	85 (-12)	80 (-18)	74	74 (0)
BWG Wk 9-13	24	22 (-8)	27 (+13)	21 (-13)	27	24 (-11)
BWG Wk 13-17					0	16
FEMALES						
Initial BW	117 ± 9.9	123 ± 7.0	117 ± 8.1	123 ± 7.5	121 ± 8.2	120 ± 5.3
Final BW[b]	196 ± 20.3	211 ± 17.6 (+8)	207 ± 20.8 (+6)	201 ± 17.7 (+3)	223 ± 16.4	203 ± 8.5 (-9)
Final BW[c]					222 ± 16.6	214 ± 19.6 (-4)
BWG Wk 0-13[d]	79	88 (+11)	90 (+14)	78 (-1)	102	83 (-19)
BWG Wk 0-17					101	94 (-7)
BWG Wk 0-1	16	20 (+25)	19 (+19)	15 (-6)	22	14 (-36)
BWG Wk 0-4	41	48 (+17)	49 (+20)	41 (0)	59	47 (-20)
BWG Wk 4-9	32	34 (+6)	34 (+6)	30 (-6)	31	25 (-19)
BWG Wk 9-13	6	6 (0)	7 (+17)	7 (+17)	12	11 (-8)
BWG Wk 13-17					-1	11

Data obtained from pages 121-124 of MRID 45369715

[a] Body weight data from the report expressed as group means (g) ± SD

[b] Body weight at the end of study week 13

[c] Body weight at the end of study week 17 (recovery period)

[d] Body weight gain (g) calculated by reviewer

[e] Percent difference from control in parenthesis calculated by reviewer

*p<0.05, **p<0.01

C. **FOOD CONSUMPTION AND COMPOUND INTAKE:**

1. **Food consumption**: Food consumption values were comparable between treated and control groups during both the treatment and recovery periods.

2. **Compound consumption**: Time-weighted averages are in Table 1.

3. **Food efficiency**: Food efficiency was not reported.

D. **OPHTHALMOSCOPIC EXAMINATION**: No treatment-related changes were observed.

E. **BLOOD ANALYSES:**

1. **Hematology**: No treatment-related changes were evident even though statistically significant changes were seen in some of the investigated parameters at the end of the treatment and recovery periods in treated males and females (Table 3). All of these changes were within the normal range of variation for respective values in Wistar rats and not all were dose-related. Additionally, decreased thromboplastin times are not toxicologically relevant. A decrease in mean corpuscular hemoglobin concentration and an increase in monocytes seen in 10,000 ppm males during weeks 4/5 were both statistically significant, however, both values were within respective ranges of normal variation of values in Wistar rats and were comparable to control values at the end of the treatment and recovery periods.

TABLE 3. Changes in selected hematological parameters of rats fed diets containing Hallcomid M-8-10[a]

	Dietary concentration (ppm)					
	0	400	2000	10,000	0	10,000
	Main group (Week 13)				Recovery group (Week 17)	
MALES						
RBC (10^{12}/l)	9.30 ± 0.36	9.06 ± 0.46	9.03 ± 0.24	8.83 ± 0.29**	9.39 ± 0.36	9.27 ± 0.24
HB (g/l)	154 ± 6.4	155 ± 5.1	152 ± 4.2	150 ± 4.9	156 ± 3.3	155 ± 2.5
HCT (l/l)	0.47 ± 0.021	0.46 ± 0.019	0.46 ± 0.011	0.45 ± 0.013*	0.50 ± 0.01	0.49 ± 0.01
TT (sec)	34.2 ± 2.61	31.7 ± 1.25*	32.9 ± 2.75	31.2 ± 2.32*	30.4 ± 2.07	30.6 ± 1.92
LYM (%)	88.1 ± 3.0	90.1± 3.7	87.9 ± 5.4	91.4 ± 5.1*	91.5 ± 2.0	88.2 ± 3.7*
FEMALES						
RBC (10^{12}/l)	8.46 ± 0.18	8.57 ± 0.17	8.20 ± 0.23*	8.66 ± 0.74	8.48 ± 0.31	8.50 ± 0.45
HB (g/l)	152 ± 3.4	151 ± 4.3	145 ± 4.7**	153 ± 11.6	146 ± 4.5	147 ± 4.6
HCT (l/l)	0.46 ± 0.014	0.46 ± 0.017	0.43 ± 0.014**	0.46 ± 0.038	0.47 ± 0.01	0.47 ± 0.02
TT (sec)	31.3 ± 1.16	30.4 ± 1.49	29.2 ± 2.53	29.9 ± 1.49	29.1 ± 2.97	29.5 ± 1.46
LYM (%)	88.1 ± 4.8	86.8 ± 4.5	89.6 ± 3.6	91.7 ± 3.0*	88.9 ± 3.1	90.4 ± 3.6

Data obtained from pages 143-150 of MRID 45369715

[a] Data are expressed as group means ± SD; *P<0.05; **P<0.01

2. **Clinical chemistry**: Sporadic increases during the treatment period in values for the following parameters in treated animals (Tables 4 and 5), although statistically significant, are not considered treatment-related, since the changes were transient, not dose-related or were small in magnitude resulting in values that were typically within the range of normal background values in Wistar rats for the respective parameters: ALAT in 2000 and 10,000 ppm females, total bilrubin in 400 ppm females, 2000 ppm males and 10,000 ppm males and females, sodium in 2000 ppm and 10,000 ppm males and females, chloride in 10,000 ppm males and phosphorous in 10,000 ppm males and females. Additionally, decreases in ALAT and/or ASAT seen in some treated animals are not toxicologically relevant. Significantly increased cholesterol values in 2000 ppm females ($p \leq 0.05$; 15% increase at week 5, 19% at week 13) and 10,000 ppm males ($p \leq 0.05$; 21% increase at week 5, 25% at week 13) and females ($p \leq 0.01$; 36% increase at weeks 5 and 13), although comparable to the range of historical control values in Wistar rats from studies at the test facility, demonstrated a dose-response relationship and were consistently higher than respective concurrent control values throughout the treatment period in this study. The increased values in 10,000 ppm males and females were consistent with those seen in 10,000 ppm males and females in a four-week range-finding study (MRID 45369713). Cholesterol levels were not elevated in treated animals at the end of the recovery period. The statistically significant increased levels of cholesterol seen in treated males and females in this study are considered to be treatment-related, however, there were no correlative changes observed in 2000 ppm females.

TABLE 4. Changes in selected clinical chemistry parameters of male rats fed Halicomid M-8-10[a]

Dietary conc. (ppm)	AST (u/l)	ALT (u/l)	Cholesterol (mmol/l)	Total bili. (µmol/l)	Na (mmol/l)	Cl (mmol/l)	P (mmol/l)
	WEEK 4/5						
0	36.6 ± 3.93	47.4 ± 3.39	2.15 ± 0.24	0.8 ± 0.11	141 ± 1.3	102 ± 0.9	2.13 ± 0.24
400	31.0 ± 2.25**	42.7 ± 5.71	2.30 ± 0.25	1.0 ± 0.12	141 ± 1.1	101 ± 1.6	2.29 ± 0.27
2000	34.4 ± 3.45	49.0 ± 4.64	2.24 ± 0.25	1.1 ± 0.12**	142 ± 1.0**	103 ± 0.9	2.29 ± 0.18
10,000	35.3 ± 3.33	45.4 ± 5.13	2.60 ± 0.45* (21%)[b]	1.0 ± 0.15*	143 ± 1.0**	103 ± 0.9	2.4 ± 0.26*
WEEK 13							
0	38.4 ± 3.88	50.5 ± 4.87	2.61 ± 0.19	1.5 ± 0.11	142 ± 0.6	101 ± 1.1	1.66 ± 0.15
400	33.0 ± 3.81*	46.2 ± 5.63	2.81 ± 0.47	1.5 ± 0.24	142 ± 0.9	102 ± 1.4	1.53 ± 0.14
2000	39.0 ± 12.35	53.0 ± 5.15	2.88 ± 0.47	1.7 ± 0.42	142 ± 1.0	102 ± 1.6	1.76 ± 0.23
10,000	34.5 ± 2.38*	49.2 ± 5.59	3.27 ± 0.63* (25%)	1.5 ± 0.30	142 ± 1.1	104 ± 1.2**	1.65 ± 0.14
WEEK 17 (Recovery groups)							
0	41.9 ± 9.48	51.1 ± 8.39	2.57 ± 0.46	1.6 ± 0.29	142 ± 1.6	99 ± 1.0	1.81 ± 0.45
10,000	38.7 ± 10.15	44.2 ± 5.71*	2.34 ± 0.29	1.6 ± 0.14	141 ± 1.4	102 ± 1.9**	1.46 ± 0.16

Data obtained from pages 151-160 of MRID 45369715

[a] Data expressed as group means ± SD; *p<0.05; **p<0.01

[b] Percent increase calculated by reviewer

TABLE 5. Changes in selected clinical chemistry parameters of female rats fed Halicomid M-8-10[a]

Dietary	ASAT	ALAT	Cholesterol	Total bili.	Na	Cl	P

conc. (ppm)	(u/l)	(u/l)	(mmol/l)	(µmol/l)	(mmol/l)	(mmol/l)	(mmol/l)
	WEEK 5						
0	38.0 ± 7.30	36.7 ± 3.95	1.82 ± 0.31	0.7 ± 0.16	139 ± 1.7	104 ± 3.0	1.52 ± 0.37
400	41.3 ± 12.10	40.4 ± 8.02	1.91 ± 0.20	0.9 ± 0.14*	139 ± 0.8	105 ± 1.8	1.72 ± 0.33
2000	36.0 ± 4.47	43.9 ± 5.17**	2.09 ± 0.26* (15%)[b]	0.9 ± 0.22	142 ± 1.0**	104 ± 2.5	1.65 ± 0.18
10,000	36.9 ± 3.23	47.4 ± 6.13**	2.47 ± 0.21** (36%)	1.0 ± 0.15*	142 ± 1.1**	103 ± 2.2	1.74 ± 0.30
	WEEK 13						
0	45.4 ± 5.93	51.6 ± 8.59	2.15 ± 0.38	1.3 ± 0.25	141 ± 1.2	104 ± 1.9	1.08 ± 0.32
400	42.3 ± 5.41	48.2 ± 10.99	2.38 ± 0.59	1.4 ± 0.16	142 ± 1.6	103 ± 2.6	1.01 ± 0.14
2000	46.6 ± 9.18	48.5 ± 9.14	2.56 ± 0.32* (19%)	1.5 ± 0.22	142 ± 1.2	104 ± 2.6	1.12 ± 0.31
10,000	39.2 ± 3.59*	58.2 ± 8.92	2.93 ± 0.24** (36%)	1.5 ± 0.12*	142 ± 0.7	103 ± 2.9	1.40 ± 0.27*
	WEEK 17 (Recovery groups)						
0	38.9 ± 6.71	45.5 ± 7.22	1.93 ± 0.24	1.2 ± 0.20	141 ± 1.1	103 ± 1.5	0.97 ± 0.22
10,000	40.0 ± 7.46	48.5 ± 10.56	2.03 ± 0.27	1.3 ± 0.20	140 ± 0.8	103 ± 1.4	1.18 ± 0.30

Data obtained from pages 151-160 of MRID 45369715
[a] Data expressed as group means ± SD
[b] Percent increase calculated by reviewer
*p<0.05; **p<0.01

F. URINALYSIS: There were no treatment-related effects observed. Slight but statistically significant increases in the levels of protein secretion in 2000 and 10,000 ppm males at twelve weeks were not dose-related and did not have histopathological correlates in kidney that were clearly associated with this change.

G. SACRIFICE AND PATHOLOGY

1. Organ weight: Sporadic statistically significant changes in brain, liver and spleen weights were seen in treated animals (Table 6), however, the increased absolute liver weights seen at the end of treatment in 10,000 ppm females ($p \leq 0.05$; 13% increase) and relative liver weights in both sexes receiving 10,000 ppm test material ($p \leq 0.01$; 16% increase for males and 10% for females) are the only changes that are considered treatment-related; relative liver weights in 10,000 ppm recovery group females were also significantly increased, however, this is evidently due to relatively low concurrent control values. Absolute liver weights were also increased in a four-week study in rats with Hallcomid M-8-10 (MRID 45369713) at 3,000 ppm and 10,000 ppm in females while relative weights were significantly increased at 3,000 ppm in males and at 10,000 ppm in both sexes. The decreased absolute brain weights in 10,000 ppm males at the end of the treatment period appear to be related to lower body weights in this group. Significantly elevated relative brain and spleen weights in 10,000 ppm recovery group females are apparently due to low respective control values based on mean weights of these organs in the control and treated animals in the main group.

TABLE 6. Changes in selected organ weights of rats fed diets containing Hallcomid M-8-10[a]

	Dietary concentration (ppm)					
	0	400	2000	10,000	0	10,000
	Main group (week 13)				Recovery group (week 17)	
MALES						
Absolute weight (mg)						
Brain	2013 ± 88.2	2015 ± 87.6	2014 ± 67.3	1911 ± 68.1*	2023 ± 82.7	1990 ± 89.9
Liver	12,629 ±1151	11,904 ± 1242	12,162 ± 1340	13,156 ± 1832	11,185 ± 2162	11,161 ± 1333
Spleen	668 ± 80.5	649 ± 75.4	680 ± 73.3	636 ± 109.5	584 ± 131.0	559 ± 88.4
Relative (to body) weight (mg/100g)						
Brain	534 ± 34.8	555 ± 36.1	579 ± 62.3	571 ± 60.4	565 ± 90.1	547 ± 55.3
Liver	3339 ± 130	3266 ± 180	3462 ± 184	3887 ± 242** (16%)[b]	3047 ± 287	3046 ± 172
Spleen	177 ± 21.0	179 ± 24.0	195 ± 25.2	188 ± 18.9	159 ± 28.1	152 ± 18.5
FEMALES						
Absolute weight (mg)						
Brain	1843 ± 94.0	1870 ± 119.0	1812 ± 67.8	1864 ± 207.7	1851 ± 78.1	1879 ± 75.6
Liver	7044 ± 1385	7186 ± 889	7189 ± 782	7936 ± 622* (13%)	6862 ± 572	7183 ± 912
Spleen	485 ± 88.6	502 ± 78.1	484 ± 46.5	445 ± 78.8	414 ± 59.7	469 ± 52.5
Relative weight						
Brain	950 ± 105.8	892 ± 74.7	883 ± 84.0	927 ± 57.4	836 ± 44.9	882 ± 50.3*
Liver	3583 ± 429	3413 ± 328	3476 ± 98	3951 ± 111** (10%)	3089 ± 142.1	3353 ± 200**
Spleen	247 ± 31.0	239 ± 32.4	235 ± 14.3	221 ± 29.6	186 ± 19.8	220 ± 22.7**

Data obtained from pages 167-176 of MRID 45369715
[a] Data expressed as group means ± SD
[b] Percent increase calculated by reviewer
*p<0.05; **p<0.01

2. **Gross pathology**: No treatment-related gross lesions were observed.

3. **Microscopic pathology**: The only significant histopathological findings were in the kidneys of males in the main group receiving 10,000 ppm test material (Table 7). There was an increase in the incidence and severity of basophilic tubules in the renal cortex. Protein casts in the medullary tubules were seen in some of these animals. The incidence of basophilic tubules was increased slightly in the 10,000 ppm recovery group males without an increase in severity; protein casts were not seen in the kidneys of these animals.

TABLE 7. Incidence of selected histopathologic kidney lesions in rats fed diets containing Halleomid M-8-10[a]

	Dietary concentration (ppm)					
	0	400	2000	10,000	0	10,000
	Main Group (Week 13)				Recovery Group (Week 17)	
MALES						
Basophilic tubules	3/1.0[a]	5/1.0	5/1.2	9/2.4	7/1.1	9/1.1
Protein casts	0	0	0	6	2	0
FEMALES						
Basophilic tubules	0	1/1.0	2/1.0	2/1.5	1/1.0	1/1.0
Protein casts	0	0	0	1	0	0

Data from pages 262-271 of MRID 45369715

[a] Values expressed as incidence/average severity where 1=minimal, 2=slight, 3=moderate and 4=marked

n=10 for all groups

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The investigator concluded that the administration of Hallcomid M-8-10 in the diet of male and female rats at levels up to and including 10,000 ppm had no effect evident in assessments of mortality, general behavior, feed and water consumption, ophthalmology, hematology, urinalysis and gross pathology.

Daily observations revealed that 5/10 males in the 10,000 ppm groups showed signs of emaciation starting at week 11.

Body weight gain was inhibited in both sexes at 10,000 ppm but adjusted to that of control animals during the recovery period.

A significant increase in the plasma cholesterol levels was seen in 2000 ppm females and in both sexes at 10,000 ppm. The significant increase in cholesterol in 2000 ppm females is not considered toxicologically relevant due to the relatively small increase and the absence of increased cholesterol in female rats receiving 3,000 ppm dietary Hallcomid M-8-10 in a four-week study (Report No. 22117; MRID 45369713). Cholesterol levels were comparable between treated and control animals at the end of the recovery period. The increased plasma cholesterol levels in 10,000 ppm males and females is considered evidence of hepatic fat metabolism impairment. Since there were no histological findings in the liver, it is not possible to conclude whether the increased liver weights seen at the end of the treatment period in 10,000 ppm males and females are the result of impaired fat metabolism or a non-specific adaptation due to higher metabolic demand. Regarded in isolation, these liver weight changes have no toxicological significance".

Slightly increased urinary protein excretion was seen in 10,000 ppm males at the end of the study. The increased incidence of basophilic regenerated tubules in the renal cortex could possibly be a correlative histopathological change in these animals. Urinary protein excretion was comparable between treated and

control animals after the recovery period. There were no other significant histopathological changes in treated animals.

Dietary levels of Hallcomid M-8-10 up to and including 2000 ppm did not cause adverse effects in male and female rats under the conditions described for this study.

B. **REVIEWER COMMENTS:** The reviewer agrees with the investigator that dietary levels of Hallcomid M-8-10 up to and including 2000 ppm did not cause adverse effects on rats in this study.

The reviewer does not agree with the investigators speculation that the slightly increased level of urinary protein in main group 10,000 ppm males may be correlated with the increased incidence of basophilic cortical tubules in the renal cortex of kidneys in these animals. Urinary protein was actually higher in 2000 ppm males than 10,000 ppm males at the end of the treatment period even though the incidence and severity of basophilic tubules were greater in the 10,000 ppm group.

The decreased body weights and body weight gains in 10,000 animals were not considered adverse due to the relatively small decreases and variability seen between main study and recovery group (during the treatment period) treated and control animals.

The increased plasma cholesterol and relative liver weights seen in males and females maintained on a diet containing 10,000 ppm Hallcomid M-8-10 in this study are consistent with data generated in a four-week dietary study in rats with this test material (MRID 45369713). However, neither of these effects had histopathological correlates and their toxicological significance is unknown.

The LOAEL for Hallcomid M-8-10 is 10,000 ppm (787.6 mg/kg/day in main study males) based on an increased incidence/severity of renal basophilic cortical tubules and deposition of protein casts in medullary tubules of kidneys in males. A LOAEL was not identified for females. The NOAEL is 2000 ppm (136.8 mg/kg/day) in main study males and 10,000 ppm (894.6 mg/kg/day) in main study females.

C. **STUDY /REPORT DEFICIENCIES:**

Study deficiencies: None that would compromise the acceptability of the study. The following assessments, guideline recommendations for a 90-day study in rodents, were not performed: histopathology of nose and pharynx, weight for uterus, ovary and epididymides, and appearance of urine. Blood samples used for clinical examinations were collected from non-fasted animals.

Report deficiency: Body weight gain, the frequency of test diet preparation and storage conditions for the test diet preparation were not reported.

DATA FOR ENTRY INTO ISIS

Subchronic (90 day) Oral Study - rodents (870.3100)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
NA	45369715	subchronic	rat	90-days	oral	dietary	27.4-907.7	Main groups: males: 0, 27.4, 136.8, 787.6; females: 35.2, 178.5, 894.6, Recovery groups: males: 0, 726.7; females: 0, 907.7	males: 136.8; females: 894.6	males: 787.6; females: Not identified	kidney	Toxicity

SUBCHRONIC ORAL TOXICITY STUDY IN RATS

TEST SUBSTANCE

· **Identity:**
Hallcomid M-8-10 (mixture of N, N-dimethylcaprylamide and N, N-dimethylcapramide)

‡ **Remarks:**
Description: light yellow liquid
Purity: 98.17%; batch no. 002949
Stable in laboratory diet for 14 days

METHOD

· **Guideline**: OPPTS 870.3100/OECD 408
· **Test type**: Subchronic oral toxicity
· **GLP**: Yes, according to the OECD Principles of Good Laboratory Practice and the Principles of Good Laboratory Practice as Published in Annex 1 of the German Chemicals Act.
· **Year study performed**: 1992
· **Species**: Rat
· **Strain**: Wistar [BOR:WISW (SPF-Cpb)]
· **Route of administration**: Oral (feed): Altromin®1321 laboratory diet *ad libitum*
· **Duration of test**: 90-day treatment period for all animals; 28-day recovery period for recovery group animals
· **Doses/concentration levels**: Main groups- 0, 400, 2,000 or 10,000 ppm (equivalent to 0, 27.4, 136.8 and 787.6 mg/kg/day, respectively, in males and 0, 35.2, 178.5, and 894.6 mg/kg/day, respectively, in females). Recovery groups- 0 and 10,000 ppm (equivalent to 0 and 726.7, respectively, in males and 0 and 907.7, respectively in females).
· **Sex**: 10/sex/dose
· **Exposure period**: 90 days
· **Frequency of treatment**: Continuous (diet)
· **Control group and treatment**: 10/sex in main and recovery groups; maintained on laboratory diet with 1% peanut oil
· **Post exposure observation period**: 28 days for recovery group animals only
· **Statistical methods**: Summarized data from the control and treatment groups for the parameters investigated in this study were compared using the Mann-Whitney U test (equivalent to the Wilcoxon rank sum test).

‡ **Remarks:**
Study design: The following assessments, guideline recommendations for a 90-day study in rodents, were not performed: histopathology of nose and pharynx, weights for uterus, ovary and epididymides,and appearance of urine. Blood samples used for clinical examinations were collected from non-fasted animals.

Satellite groups (designated as recovery groups) were added to assess the reversibility of possible compound-related effects.

RESULTS

· **NOAEL**: 2000 ppm males (136.8 mg/kg/day); 10,000 ppm females (894.6 mg/kg/day)

- **LOAEL**: 10,000 ppm males (787.6 mg/kg/day); females- not identified
- Toxic response/effects by dose level: 10,000 ppm (main groups)- **males** (787.6 mg/kg/day): increased incidence and severity of basophilic cortical tubules and deposition of protein casts in medullary tubules of the kidneys; **females** - none identified
- **Statistical results**: Several parameters were statistically different from controls, but none of these were considered adverse. The kidney changes in males were not analyzed statistically.
- **Body weight**: No treatment related effects
- **Food/water consumption**: No treatment-related effects
- **Clinical signs**: No treatment-related effects
- **Ophthalmologic findings**: No treatment-related effects
- **Hematologic findings**: No treatment-related effects
- **Clinical biochemical findings**: Plasma cholesterol levels were elevated in main group 10,000 ppm males at weeks 4/5 and 13 (21% and 25%, respectively; $p<0.05$ at both intervals) and in females at 2000 ppm (15% and 19%; $p<0.05$) and 10,000 ppm females (36% and 36%; $p<0.01$) at weeks 5 and 13, respectively. Although the increases were not large, they were dose-related, statistically significant and consistent with the increases observed at 10,000 ppm in a four-week dietary range-finding study with Hallcomid M-8-10 in rats (MRID 45369713). Plasma cholesterol levels were comparable between control and treated animals at the end of the recovery period.
- **Mortalities**: No treatment-related mortalities
- **Gross pathology**: No treatment-related changes
- **Organ weight changes**: There were significant increases in absolute liver weight at 10,000 ppm in females (13%; $p<0.05$) and relative liver weights at 10,000 ppm in males and females (16% and 10%, respectively; $p<0.01$)..
- **Histopathology**: there was an increase in the incidence and severity of basophilic tubules in the renal cortex of kidneys in males dosed with 10,000 ppm Hallcomid M-8-10; compared to controls, the incidence increased from 3/10 to 9/10 and the average severity from 1.0 to 2.4 (scale: 1- 4); deposition of protein casts was also found in the medullary tubules of kidneys in these animals. There were no changes seen in the other parameters assessed in this study that were clearly associated with these histopathologic effects.

CONCLUSIONS

Dietary levels of Hallcomid M-8-10 up to and including 2000 ppm in males and 10,000 in females did not cause adverse effects on rats in this study.

There were no significant compound-related effects on mortality, clinical signs, food consumption, hematology or gross pathology. The adverse treatment related effects observed in main group animals dosed with 10,000 ppm Hallcomid M-8-10 were an increased incidence/severity of basophilic cortical tubules in the kidneys of males.

The LOAEL for Hallcomid M-8-10 is 10,000 ppm (787.6 mg/kg/day in main study males) based on an increased incidence/severity of renal basophilic cortical tubules and deposition of protein casts in medullary tubules of kidneys in males. A LOAEL was not identified for females. The NOAEL is 2000 ppm (136.8 mg/kg/day) in main study males and 10,000 ppm (894.6 mg/kg/day) in main study females.

DATA QUALITY

Reliability: 1, reliable without restrictions

REFERENCE

Wirnitzer, U. and R_hl-Fehlert, C. (1993) Hallcomid M-8-10: Study on subacute toxicity in Wistar rats (Administration in feed over 13 weeks with 4-week post-treatment observation) Bayer AG, Institute of Toxicology, Friedrich-Ebert-Str. 217-333, 42096 Wuppertal 1. Report No. 22931. Study No. T4041117. March 11, 1993. MRID 45369715. Unpublished.

APPENDIX

(MRID 45369713)

STUDY TYPE: Four-week oral toxicity (range finding) in Wistar rats

PC CODE: NA

DP BARCODE: NA
SUBMISSION NO.: NA

TEST MATERIAL (PURITY): Mixture of N,N-dimethylcaprylamide and N,N-dimethylcapramide (98.30%)

SYNONYMS: Hallcomid M-8-10

CITATION: Wimitzer, U. (1993) Study for subacute toxicity on Wistar rats (Feeding study for range-finding over 4 weeks). Bayer GA, Institute of Toxicology, Friedrich-Ebert-Str. 217-333, 5600 Wuppertal 1. Report No. 22117. MRID 45369713. Unpublished.

SPONSOR: Bayer AG; submitted by C.P. Hall Co.

EXECUTIVE SUMMARY: In a preliminary oral toxicity feeding study (MRID 45369713), Hallcomid M-8-10 (98.30%, batch # 002949) was administered for 4 weeks in the diet to five Wistar [BOR:WISW (SPF-Cpb)] rats/sex/dose at concentrations of 0, 1000, 3000, or 10,000 ppm (equivalent to 0, 82.9, 250.6 and 965.0 mg/kg/day, respectively, in males and 0, 93.7, 293.2, and 1075.7 mg/kg/day, respectively, in females).

No treatment-related deaths or clinical signs of toxicity were observed, and there were no treatment-related effects on food or water consumption, opthalmologic findings, hematological findings, or gross pathology. Body weight gain was decreased at 10,000 ppm in males throughout the study (67% and 23 % during weeks 1 and 4, respectively) and in females at 10,000 ppm during weeks 1 (-79%) and 2 (-19%). Plasma cholesterol levels were increased in 10,000 ppm in males (34%; $p \leq 0.01$) and females (51%; $p \leq 0.05$). Absolute liver weight was increased (21%; $p \leq 0.01$) in 10,000 ppm males, and relative liver weight was increased in 10,000 ppm males and females (24% for males and 22% for females; $p \leq 0.01$). Relative kidney weights were also increased in 10,000 ppm males and females (27% for males and 13% for females; $p \leq 0.05$). Although increased absolute liver weights in females and relative liver weights in males were statistically significant ($p \leq 0.05$) at 3,000 ppm, the increases were relatively small (≤10%) and no correlative changes were seen in the investigated parameters.

Therefore, based on the results of this preliminary study, doses for the 13-week study were chosen as 0, 400, 2000, and 10,000 mg/kg/day.

DATA EVALUATION RECORD

HALLCOMID M-8-10/PC Code

STUDY TYPE: SUBACUTE INHALATION STUDY
MRID 45369720

Prepared for

Registration Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. RD-2003-4

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Quality Assurance:
Lee Ann Wilson, M.A.

Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

TXR#:

DATA EVALUATION RECORD

STUDY TYPE: Subacute Inhalation Toxicity - rat

TEST MATERIAL (PURITY): Hallcomid M-8-10

SYNONYMS: None

CITATION: Pauluhn J (1992) Orientation Study for Subacute Inhalation Toxicity in the Rat (Expos: 5x6h). Bayer AG, Department of Toxicology, Wuppertal, Germany. Study no. T7039960, September 17, 1992. MRID 45369720. Unpublished.

SPONSOR: Bayer AG

EXECUTIVE SUMMARY: In a subacute inhalation toxicity study (MRID 45369720), groups of ten Wistar [Bor:WISW (SPF-Cpb) rats/sex/group were exposed to aerosolized Hallcomid M-8-10 (3.7% N, N-dimethyl hexane acid amide; 54.1% N, N-dimethyl octane acid amide; 38.5% N, N-dimethyl decane acid amide; 1.3% N, N-dimethyl dodecane acid amide; batch # 903069) head/nose only under dynamic conditions. The animals were exposed to nominal concentrations of 0, 100, 500 or 2500 mg/m^3 [analytical concentrations of 0, 24.6, 111.2 or 521.2 mg/m^3 (0, 0.025, 0.111, 0.521 mg/L)] for 6 hours/day for a total of 5 days and observed post-exposure for 2 weeks. Approximately 97% of the aerosol particles in the test atmosphere were respirable, i.e., relative mass ≤ 3 µm. Clinical signs, neurological reflexes, body weight, hematology, clinical chemistry and necropsy findings were evaluated. Five rats/sex/group were sacrificed on days 7 and 22 of the study.

All animals survived to the end of the study. No neurological effects were observed. All rats at 521.2 mg/m^3 exhibited one or more of the following clinical signs beginning on day 2: labored breathing, bradypnea, wheezing, reduced mobility, reddened nose and serous nasal discharge. Some of the signs lasted until the next exposure day and continued until day 7 of the study. Body temperature after exposure was statistically significantly decreased in male and female rats at 521.2 mg/m^3 on day 0 and in females at this concentration on day 7.

Males and females at 521.2 mg/m^3 had decreased body weights on days 4 (8-9% below controls) and 7 (4-8% below controls). All groups including controls lost weight during exposure; males at 521.2 mg/m^3 lost a mean of 22 g while their controls lost 1 g; females at 521.2 mg/m^3 lost a mean of 21 g while their controls lost 9 g. During the post-exposure period, males and females at 521.2 mg/m^3 gained more weight than controls.

The incidence of goblet cell hyperplasia in the nasal mucosa was increased in females at 521.2 mg/m^3 on days 7 and 22. An exposure-related increase in the incidence of subpleural round-cell infiltration of the lungs was observed in males on days 7 and 22. The incidence of marginal emphysema of the lungs was increased in males and females at 521.2 mg/m^3 on day 7.

The LOAEL for Hallcomid M-8-10 in male and female rats is 521.2 mg/m^3 (0.521 mg/L), based on clinical signs of toxicity, decreased body temperatures, decreased body weight and weight gain, and histopathological findings in the respiratory tract in both sexes. The NOAEL is 111.2 mg/m^3 (0.111 mg/L).

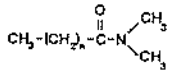
This subacute (5 days) inhalation toxicity study in the rat is **Acceptable/Non-guideline**; it was a range-finding study and was not intended to satisfy a guideline requirement.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Hallcomid M-8-10

Description:	Clear, light yellow liquid
Batch #:	903069
Purity:	3.7% N, N-dimethyl hexane acid amide; 54.1% N, N-dimethyl octane acid hexane amide; 38.5% N, N-dimethyl decane acid amide; 1.3% N, N-dimethyl dodecane acid amide
Compound Stability:	The study report states that the stability is guaranteed for the study period.
CAS # of TGAI:	1118-92-9 and 14433-76-2 (N, N-dimethyl octane acid hexane amide and N, N-dimethyl decane acid amide)
Structure:	

n=(4), 6, 8 or (10); ( )= minor constituents

2. Vehicle: 1:1 mixture of polyethylene glycol 400 and ethanol.

3. Test animals:

Species:	Rat
Strain:	Wistar (Bor:WISW (SPF-Cpb))
Age/weight at study initiation:	2-3 months/180-200 g
Source:	Winkelmann, Borchen, District of Paderborn, Germany
Housing:	Makrolon® cages type III, 5 animals per cage, during acclimation and test periods
Diet:	Altromin® 1324 Diet for Rats and Mice, ad *libitum* (except during exposure)
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 22 ± 2°C **Humidity:** Approximately 50% **Air changes:** Approximately 10/hr

Photoperiod 12 hrs dark/12 hrs light
:

Acclimation period: At least one week

B. STUDY DESIGN:

1. **In life dates:** Start:April 15, 1991; End: May 7, 1991

2. **Animal assignment:** Animals were assigned using a computer software program to the test groups noted in Table 1.

TABLE 1: Study design

Test group	Nominal conc. (mg/m^3)	Analytical conc.[a] (mg/m^3)	MMAD[a] µm	GSD[a]	Rats/sex
Control	0	0	1.36	1.45	10
Low (LCT)	100	24.6	1.46	1.48	10
Mid (MCT)	500	111.2	1.43	1.47	10
High (HCT)	2500	521.2	1.47	1.49	10

[a] Data obtained from pages 60 and 69-70 in the study report, MRID 45369720

3. **Dose selection rationale:** The study report states that exposure levels were selected based on the results from an acute inhalation study where the LC_{50} in rats exposed to Hallcomid M-8-10 for four hours was >3551 mg/m^3. Rats exposed to a concentration of 119 mg/m^3 air had no signs of toxicity. Rats exposed to 586 mg/m^3 had transient redness of the nose and reduced motility. At 3551 mg/m^3 (undiluted test material), bradypnea, serous nasal discharge, dyspnea, wheezing and hypothermia were observed; one male died at this concentration.

4. **Generation of the test atmosphere / chamber description:** The test material in the vehicle (mixture of polyethylene glycol and ethanol, 1:1) was prepared daily and was nebulized using a Braun infusion pump with a 50 mL glass syringe. The aerosol was sprayed under dynamic conditions into a commercially available (TSE Labortechnik Co.) cylindrical chamber with a baffle and volume of 20 L. Compressed air (15 L air/min) was produced with two in-parallel Boge compressors. The animals were exposed head/nose only while confined in plexiglass tubes with tails outside the tubes to avoid hyperthermia. The aerosol generation process allowed for approximately 45 air exchanges/hour. The nominal concentration of the test material was calculated as follows:

$$\text{Nominal concentration} = \frac{V1 \times Ci}{100 \times V2} \ mg/m^3 \text{ air}$$

V1 = nebulized vehicle volume per exposure day (54000 µl)
V2 = total inlet air per exposure day (5.4 m^3)
Ci = concentration of spray solution (g/v): 0, 1.0, 5.0 or 25.0% (nominal)

Time to equilibrium occurred within 4 minutes of operation. The study report states that temperature and humidity were measured every 10 minutes during the exposure. The temperature inside the chamber was 24-26°C (within the OECD guideline), and the relative humidity was 18-30% (occasionally slightly lower than that required by the guideline).

Test atmosphere concentration around the breathing zone was determined at the following times: start of test (after reaching steady state concentration), half-way though the test and towards the end of the test according to the study report. Concentration analyses were performed by gas chromatography. Results are in table 1 above.
Particle size determination in a vicinity of the breathing zone was determined using an aerodynamic particle sizer with laser velocimeter. Particle size was characterized based on the following parameters: MMAD (Mass Median Aerodynamic Diameter) and GSD (geometric standard deviation). Analyses showed that approximately 97% of the particles were respirable, i.e., relative mass ≤ 3 µm. Results are in table 1 above.

5. **Statistics**: Body weights, along with hematology and clinical chemistry parameters were evaluated with the Rank Test (U Test). Data were then subjected to a Box Text for homogeneity and an Analysis of Variance (ANOVA). Organ weights were subjected to a one-way ANOVA. The histopathological findings were evaluated using the pairwise Fisher's Test with a preceding RxC Chi square test.

C. **METHODS**:

1. **Observations**:

1a. **Cageside observations**: Animals were inspected twice daily for signs of toxicity and mortality during exposure-free periods. The rats were assessed during the exposure periods if there were obvious clinical signs. At the end of the exposure period, the animals were evaluated with regard to the following signs: gross appearance of the mucous membranes of the eyes and respiratory tract; general state of the muzzle skin and pinna, state of fur and grooming activity; respiration; cardiovascular parameters; somato-motor system and behavioral pattern; and central and autonomic nervous system signs. Rectal temperatures were measured on days 0, 4 and 7 in 5 rats/sex/group directly after the exposure.

1b. **Clinical examinations**: Clinical examinations were not specified in the study report.

1c. **Neurological evaluations**: The following evaluations (measurements) were performed on days 0, 4 and 7: visual placing response and grip strength; tonus of abdominal muscles; pupil reflex; cornea reflex; pinna reflex; righting reflex; tail-pinch response; startle reflex (noises); and changes in behavior induced through touching.

2. **Body weight:** Animals were weighed before the first exposure (day 0) and on days 4, 7, 14, and 21.

3. **Food consumption:** Food consumption was not measured.

4. **Hematology and clinical chemistry:** Blood was collected by heart puncture at the interim necropsy (day 7) and final necropsy (day 22) for hematology and clinical chemistry analysis from all surviving animals.

a. **Hematology:** The following parameters were examined: hematocrit (HCT), hemoglobin (HGB), leucocyte count (WBC), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), platelet count and thromboplastin time.

b. **Clinical chemistry**: The following parameters were examined: aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH) and alkaline phosphatase (ALK).

5. **Sacrifice and pathology**: Fifty percent of the rats per group and sex were sacrificed on days 7 and 22 and subjected to gross pathological examination. The following organs were weighed: brain, heart, liver, lung and kidney. The following organs in all rats were prepared for histological examination: eyes, heart, head (nasopharynx, oropharynx and nasal and paranasal cavities), larynx, liver, lung (with main bronchi), lymph nodes (mediastinal and lung associated lymph nodes), kidneys, trachea, and organs showing gross changes. All organs and tissues collected were examined microscopically.

II. RESULTS:

A. OBSERVATIONS :

1. **Clinical signs of toxicity:** All rats in the 521.2 mg/m^3 group exhibited one or more of the following clinical signs beginning on day 2: labored breathing, bradypnea, wheezing, reduced mobility, reddened nose and serous nasal discharge. Some of the signs lasted until the next exposure day and continued until day 7 of the study. The incidence and/or severity of the clinical signs increased with exposure time. No clinical signs were observed in controls or the 24.6 or 111.2 mg/m^3 groups.

Body temperatures were statistically significantly decreased in male and female rats in the 521.2 mg/m^3 group on day 0 (first day of exposure) and in females at this concentration on day 7 (Table 2).

TABLE 2: Mean rectal temperatures[a]		
Analytical concentration	Males	Females

(mg/m^3)	Day 0	Day 4	Day 7	Day 0	Day 4	Day 7
0	37.8	38.1	38.3	37.2	38.1	38.8
24.6	38.3	38.2	38.4	37.1	38.1	38.6
111.2	37.9	37.8	38.6	37.8	38.0	38.7
521.2	35.3*	37.4	37.9	33.8**	35.6	38.0**

[a] Data obtained from page 43 in the study report, MRID 45369720.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

2. **Mortality:** All animals survived until scheduled sacrifice.

3. **Neurological evaluations:** There were no neurological effects in the control or treated groups.

B. **BODY WEIGHT AND WEIGHT GAIN:** Males and females in the 521.2 mg/m^3 group had statistically significantly decreased body weights relative to controls on days 4 (8 -9%) and day 7 (4-8%) (Table 3). All groups including controls lost weight during exposure; males at 521.2 mg/m^3 lost a mean of 22 g while their controls lost 1 g; females at 521.2 mg/m^3 lost a mean of 21 g while their controls lost 9 g. Males and females at 24.6 and 111.2 mg/m^3 also lost more weight than controls, and males showed an exposure-related weight loss but not the females at these concentrations; however, mean weight losses for these groups were not significantly different from those of controls. All groups gained weight post-exposure, with the males and females in the 521.2 mg/m^3 group gaining considerably more weight than controls and the other groups gaining about the same amount or only slightly less than controls. There was no clear exposure-related and statistically significant effect observed at 24.6 or 111.2 mg/m^3.

TABLE 3. Average body weights and body weight gains [a]

Analytical concentration (mg/m^3)	Body weights (g±SD)				Weight gain (g)[b]	
	Day 0 (n=10)	Day 4 (n=10)	Day 7 (n=10)	Day 21 (n=5)	Days 0-4	Days 4-21
Male						
0	197.1 ± 5.5	196.0 ± 5.8	211.6 ± 7.8	283.8 ± 11.3	-1.1	87.8
24.6	202.1 ± 6.8	198.2 ± 10.5	214.4 ± 12.7	284.6 ± 12.3	-3.9	86.4
111.2	197.7 ± 8.2	192.0 ± 9.5	205.8 ± 12.1	264.2 ± 20.5	-5.7	72.2
521.2	201.5 ± 8.6	178.7 ± 7.9** (91)[c]	194.9 ± 9.7** (92)[c]	283.0 ± 9.7	-22.8	104.3
Female						

0	184.2 ± 5.9	175.1 ± 6.1	179.0 ± 4.8	194.2 ± 6.6	-9.1	19.1
24.6	183.2 ± 7.6	175.9 ± 7.4	177.8 ± 8.7	188.8 ± 11.8	-7.3	12.9
111.2	184.0 ± 10.6	175.6 ± 7.0	178.2 ± 8.0	189.2 ± 9.4	-8.4	13.6
521.2	182.6 ± 10.1	161.6 ± 9.5** (92)[c]	171.0 ± 10.6 (96)[c]	187.2 ± 13.9	-21.0	25.6

[a] Data obtained from pages 40 and 121- 137 in the study report, MRID 45369720.
[b] Calculated by the reviewer.
[c] Percent of control value calculated by the reviewer.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

C. BLOOD ANALYSES:

1. **Hematology:** Mean WBC counts in females of the 521.2 mg/m^3 group were statistically significantly increased on day 7. However, the mean WBC count value in the control group females was considerably lower on day 7 than on day 22. No laboratory reference values were provided. The MCV was significantly lower and the MCHC was significantly higher in females of the 521.2 mg/m^3 group; however, there were no changes in other RBC parameters. The hematology findings were not considered treatment-related.

2. **Clinical chemistry:** Females in the 521.2 mg/m^3 group had a significant increase (+49%) in ALT activity and a non-significant increase (+20%) in AST activity on day 7 (Table 4). Males in the 521.2 mg/m^3 had a non-significant increase (+22%) in ALT activity.

TABLE 4: Selected clinical chemistry findings on day 7 of treatment[a]

Analytical concentration (mg/m^3)	Males		Females	
	AST (µL)	ALT (µL)	AST (µL)	ALT (µL)
0	49.1 ± 4.5	42.6 ± 4.9	47.6 ± 8.1	35.9 ± 3.8
24.6	52.1 ± 3.4	50.0 ± 9.8	44.2 ± 3.3	34.6 ± 3.6
111.2	53.2 ± 6.9	47.8 ± 4.7	44.4 ± 5.1	37.8 ± 10.5
521.2	51.2 ± 6.7	52.0 ± 6.2	57.0 ± 8.0	53.5 ± 5.2**

[a] Data obtained from pages 148 and 150 in the study report, MRID 45369720.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

D. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** The absolute brain weight was not statistically significantly increased, but the relative brain weight was statistically significantly increased (+22%) in males of the 521.2 mg/m^3

group at the interim (day 7) necropsy; the increase was due in part to the decreased body weight. Also at the day 7 necropsy, absolute and relative liver weights were non-significantly increased in females (16-18% above controls) of the 521.2 mg/m^3 group. There were no histological changes in the liver; therefore, the toxicological significance of the increase is questionable.

2. **Gross pathology**: There were no treatment-related effects.

3. **Microscopic pathology**: The incidence of goblet cell hyperplasia in the nasal mucosa was increased in females of the 521.2 mg/m^3 group at days 7 and 22 (Table 5). An exposure-related increase in the incidence of subpleural round-cell infiltration of the lungs in males was observed on days 7 and 22. The incidence of marginal emphysema of the lungs was increased in males and females at 521.2 mg/m^3 on day 7.

TABLE 5: Incidence of selected histopathology findings[a]								
Lesion/ Necropsy Day	Analytical Concentration (mg/m^3)							
	Males (n=5)				Females (n=5)			
	0	24.6	111.2	521.2	0	24.6	111.2	521.2
Nasal and Paranasal Cavities - goblet cell hyperplasia								
Day 7	0	0	0	0	1	2	2	5*
Day 22	0	0	0	1	1	1	1	4
Lungs - round-cell infiltration - subpleural								
Day 7	1	0	2	3	0	1	1	0
Day 22	0	1	2	3	2	1	0	1
Lungs - marginal emphysema								
Day 7	1	1	0	3	1	1	1	3
Day 22	3	0	1	2	1	1	0	1

[a] Data obtained from page48 in the study report, MRID 45369720.
* Statistically different ($p < 0.05$) from the control.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that 111.2 mg/m^3 (nominal 500 mg/m^3) Hallcomid M-8-10 was tolerated without any effects on the parameters measured. At 521.2 mg/m^3 (nominal 2500 mg/m^3), rats showed signs of a transient influence on body weight, body temperature and clinical signs related to an irritant potential in the respiratory tract (hyperplasia of the mucosa cells in the nasal septum). The signs became more severe with increasing exposure time.

B. REVIEWER COMMENTS: With the exception of decreased body weight, the effects from 5-day inhalation exposure to Hallcomid M-8-10 are confined to the respiratory tract. All rats at 521.2 mg/m^3 group exhibited one or more of the following clinical signs with increasing incidence beginning on day 2 : labored breathing, bradypnea, wheezing, reduced mobility, reddened nose and serous nasal discharge. Body temperature after exposure was statistically significantly decreased in male and female rats at 521.2 mg/m^3 group on day 0 and in females at this concentration on day 7.

Males and females at 521.2 mg/m^3 group had significantly decreased body weights at day 4 and 7. Although all groups lost weight during the exposure period, the 521.2 mg/m^3 groups lost

considerably more weight than controls. Compensatory weight gain was observed in the 521.2 mg/m^3 groups during the post-exposure period. Males in the 24.6 and 111.2 mg/m^3 groups gained less weight than controls during the exposure period; however, the lack of effect on absolute body weight compared with that of controls and the small absolute loss of weight compared with controls suggest that the decrease in weight gain is unlikely to be biologically significant. The decreased body weight and weight gain at 521.2 mg/m^3 could have been a result of restraining the animals during exposure (all groups) and the irritant effect of the test substance (521.2 mg/m^3). Measurement of food consumption would have helped to establish this effect. Females at 521.2 mg/m^3 had a significant increase in ALT activity and a non-significant increase in AST activity on day 7, and males at 521.2 mg/m^3 had a non-significant increase in ALT activity. Absolute and relative liver weights were non-significantly increased in females at 521.2 mg/m^3 at the day 7 necropsy, and no histological changes in the liver were observed; therefore, the toxicological significance of the enzyme and liver findings is questionable.

The incidence of goblet cell hyperplasia in the nasal mucosa was increased in females at 521.2 mg/m^3 group on the day 7 and 22. There was also an exposure-related increase in the incidence of subpleural round-cell infiltration of the lungs in males on days 7 and 22. In addition, the incidence of marginal emphysema of the lungs was increased in males and females at 521.2 mg/m^3 on day 7. Because of the irritant nature of the test substance, the increased incidences of the findings in the lungs are considered related to exposure to the test substance.

The LOAEL for Hallcomid M-8-10 in male and female rats is 521.2 mg/m^3 (0.521 mg/L), based on clinical signs of toxicity, decreased body temperatures, decreased body weight and weight gain, and histopathological findings in the respiratory tract in both sexes. The NOAEL is 111.2 mg/m^3 (0.111 mg/L).

C. **STUDY DEFICIENCIES:** None

DATA FOR ENTRY INTO ISIS

Subchronic Inhalation Study - rodents (870.3200)

PC code	MRID	Study	Species	Duration	Route	Admin	Conc. range mg/m^3	Conc. mg/L	NOAEL mg/L	LOAEL mg/L	Target organ	Comments
	45369720	subacute	rat	5 days	inhalation	inhalation	0.025-0.521	0, 0.025, 0.111, 0.52	0.111	0.521	body weight, respiratory tract	Toxicity

DATA EVALUATION RECORD

HALLCOMID M-8-10

Study Type: DEVELOPMENTAL TOXICITY– RAT [870.3700 (83-3A)[
MRID 45369719 (main study), 45369718 (range-finding study)

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. RD-2003-4

Primary Reviewer: Carol S. Wood, Ph.D., D.A.B.T.	Signature: Date:	_____ _____
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Robert H. Ross, M.S., Group Leader	Signature: Date:	_____ _____
Quality Assurance: Susan Chang, M.S.	Signature: Date:	_____ _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat [OPPTS 870.3700a (§83-3a)] OECD 414.

TEST MATERIAL (PURITY): N,N-dimethyl-hexanacidamide - 3.45%
N,N-dimethyl-octanacidamide - 53.31%
N,N-dimethyl-decanacidamide - 39.48%
N,N-dimethyl-dodecanacidamide - 1.43%

SYNONYMS: Hallcomid M-8-10

CITATION: Becker, H. and K. Biedermann (1991) Embryotoxicity study (including teratogenicity) with Hallcomid M-8-10 in the rat. RCC, Research and Consulting Company AG and RCC Umweltchemie AG, PO Box CH 4452 Itingen, Switzerland. Project no. 274983. October 21, 1991. MRID 45369719. Unpublished.

Becker, H. and K. Biedermann (1991) Dose range-finding embryotoxicity study (including teratogenicity) with Hallcomid M-8-10 in the rat. RCC, Research and Consulting Company AG and RCC Umweltchemie AG, PO Box CH 4452 Itingen, Switzerland. Project no. 274972. January 28, 1991. MRID 45369718. Unpublished.

SPONSOR: Bayer AG; submitted by C.P. Hall Co.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45369719), Hallcomid M-8-10 (3.45% N,N-dimethyl-hexanacidamide, 53.31% N,N-dimethyl-octanacidamide, 39.48% N,N-dimethyl-decanacidamide, 1.43% N,N-dimethyl-dodecanacidamide; batch # 903069) was administered to 25 female Wistar rats/dose by gavage at dose levels of 0, 50, 150, or 450 mg/kg bw/day on days 6 through 15 of gestation. Doses were selected on the basis of a range-finding study in the rat (MRID 45369718). On GD 21, all surviving dams were sacrificed and examined grossly. Each fetus was weighed and examined externally for abnormalities and for sex determination. Approximately one-half of the fetuses in each litter were examined viscerally. The remaining one-half of the fetuses in each litter were eviscerated and processed for skeletal examination.

All animals survived to scheduled termination. No treatment-related clinical signs of toxicity, or effects on body weight, body weight gain, or food consumption were observed in low- or mid-dose animals. All high-dose animals were observed to have ruffled fur, ventral recumbency, dyspnea, and apathy during GDs 8-14. In addition, a total of five high-dose animals were described as being in a comatose state on at least one day between GDs 10-12. Absolute body weight of the high-dose group was slightly less than that of the controls beginning about GD 15 with statistical significance ($p \leq 0.05$; 95% of controls) attained on GDs 18 and 19. However body weight gains by the high-dose group were significantly less than those of the controls throughout the dosing period. The most pronounced effect on body weight gain was during GDs 6-11 when the high-dose group gained only 47% of the control group level. Overall weight gain by the high-dose group was 69% of the control group level during the dosing interval. Food consumption by the high-dose group was 76-82% ($p \leq 0.01$) of the control levels during the dosing interval.

Therefore, the maternal toxicity LOAEL for Hallcomid M-8-10 in rats is 450 mg/kg/day based on clinical signs, and decreased body weight gain and food consumption, and the maternal toxicity NOAEL is 150 mg/kg/day.

No treatment-related differences were noted between the treated and control groups for numbers of corpora lutea and implantations, gravid uterine weights, live fetuses per dam, resorptions, fetal sex ratios, and pre-implantation losses. Post-implantation loss by the high-dose group was slightly greater than that of the control group (9.4% vs 5.6% for the controls). Male and female fetal body weights from the high-dose group were significantly less ($p \leq 0.01$; 92% and 91% of controls, respectively) than those of the control. No female had complete litter resorption.

The number of fetuses (litters) examined for external malformations/variations in the control, low-, mid-, and high-dose groups was 287 (25), 287 (25), 260 (25), and 281 (25), respectively. The number of fetuses (litters) examined viscerally was 137 (25), 137 (25), 123 (25), and 134 (25), respectively, and the number of fetuses (litters) examined for skeletal malformations/variations was 150 (25), 150 (25), 137 (25), and 147 (25), respectively. No treatment-related external or visceral malformations/variations were observed. The number of fetuses(litters) in the control, low-, mid-, and high-dose groups with skeletal malformations was 5 (4), 4 (4), 3 (3), and 12 (9) ($p \leq 0.05$), respectively. Dumbell-shaped thoracic vertebral body was the most common malformation [8 (6) vs 1 (1) in the control group]. Also significantly increased (litter incidence, $p \leq 0.01$) in the high-dose group were the incidences of incomplete or non-ossification of the vertebrae [29 (16) vs 11 (7) controls] and sternebrae [65 (22) vs 28 (13) controls]. Reduced ossification combined with lower body weights indicates fetal growth retardation.

Therefore, the developmental toxicity LOAEL for Hallcomid M-8-10 in rats is 450 mg/kg/day based on increased post-implantation loss, decreased fetal body weight, and increased incidences of skeletal malformations/variations. The developmental toxicity NOAEL is 150 mg/kg/day.

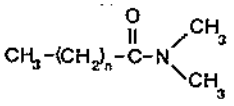
This developmental toxicity study in the rat is classified **Acceptable/Guideline** and does satisfy the guideline requirements for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A Flagging statement was not included.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Hallcomid M-8-10

Description:	Clear, yellow liquid
Lot #:	903069
Purity:	N,N-dimethyl-hexanacidamide - 3.45% N,N-dimethyl-octanacidamide - 53.31% N,N-dimethyl-decanacidamide - 39.48% N,N-dimethyl-dodecanacidamide - 1.43%
Compound Stability:	stable until November 17, 1990
CAS #of TGAI:	Not given
Structure:	

2. **Vehicle and/or positive control:** Bi-distilled water with 0.5% Cremophor (BASF) was used as the vehicle and negative control. No positive control was used in this study.

3. **Test animals:**

Species:	Rat
Strain:	Wistar Hanlbm: WIST (SPF)
Age/weight at study initiation:	at least 11 weeks; 179-226 g
Source:	BRL, Biological Research Laboratories Ltd., Wölferstrasse 4, CH 4414 Füllinsdorf, Switzerland
Housing:	Animals were individually housed in Makrolon cages with wire mesh tops.
Diet:	Pelleted standard Kliba 343 rat/mouse maintenance diet was available *ad libitum*.
Water:	Tap water was available *ad libitum*.
Environmental conditions:	Temperature: 22±3°C Humidity: 40-70% Air changes: 10-15/hour Photoperiod: 12 hrs dark/12 hrs light
Acclimation period:	10 days

B. PROCEDURES AND STUDY DESIGN:

1. In life dates: Start: August 6, 1990; End: August 31, 1990

2. **Mating:** Females were placed with male rats (same strain and source) overnight at a ratio of 1:1. Females were considered to have mated when either a vaginal plug and/or sperm in a vaginal smear was observed. The day evidence of mating was observed was considered gestation day (GD) 0.

3. **Animal assignment:** Mated females were allocated to the treatment groups indicated in Table 1 using a computer-generated random algorithm.

TABLE 1. Animal assignment

Dose (mg/kg bw/day)	0	50 (LDT)	150 (MDT)	450 (HDT)
# Females	25	25	25	25

4. **Dose selection rationale:** Doses were selected on the basis of a range-finding study conducted by the testing facility (see Appendix). Briefly, groups of 5 mated females/dose were administered 0, 100, 300, or 1000 mg/kg/day on GD 6-15. At the high dose, severe clinical signs of toxicity and marked reductions in food consumption and body weight were observed, and one animal died. Clinical signs of toxicity were observed in the low- and mid-dose groups during the first few days of dosing. Post-implantation loss for the control, low-, mid-, and high-dose groups was 7.1, 9.4, 12.9, and 100%, respectively. No fetal effects were noted in the low- or mid-dose groups. Therefore, doses chosen for the main study were 0, 50, 150, and 450 mg/kg/day.

5. **Dosage preparation and analysis**: Dosing solutions were prepared daily by weighing the appropriate amount of test article and adding vehicle. The dosing solutions were mixed using a homogenizer. Solutions were constantly stirred with a magnetic stirrer during use. Concentration, homogeneity, and stability were determined previously as part of the preliminary study in rats (MRID 45369718) and confirmed once during the current study.

Results

Homogeneity analysis: Samples from the top, middle, and bottom of the dose solutions differed from each other in concentration by <9%.

Stability analysis: After 2 hours at room temperature, concentrations of the dosing solutions were 98-108% of their initial measured concentrations.

Concentration analysis: Absence of test article was confirmed in the vehicle. Concentrations of the low-, mid-, and high-dose suspensions were within ±4% of nominal.

Analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration:** All doses were administered once daily by gavage, on GD 6 through 15, in a volume of 10 mL/kg of body weight/day.

C. OBSERVATIONS:

1. **Maternal observations and evaluations**: The animals were checked twice daily for mortality and clinical signs of toxicity. Each animal was weighed daily and food consumption was recorded on GDs 0-6, 6-11,

11-16, and 16-21. Dams were sacrificed on GD 21 by carbon dioxide asphyxiation and subjected to gross examination. The number of corpora lutea on each ovary was counted. Gravid uteri were removed, weighed, and examined for the number and position of fetuses. Implantation sites were classified as empty (early resorption), with an embryonic or fetal resoprtion, or with a live or dead fetus.

2. **Fetal evaluations**: Live fetuses were weighed and examined externally for abnormalities and for sex determination. Approximately one-half of the fetuses in each litter were examined viscerally by the Wilson technique. The remaining one-half of the fetuses in each litter were processed for skeletal examination.

D. DATA ANALYSIS:

1. **Statistical analyses:** Maternal body weight and food consumption, reproduction, and skeletal examination data were analyzed by a one-way analysis of variance (ANOVA). The ANOVA was followed by the Dunnett-test if the variables could be assumed to follow a normal distribution or the Steel-test if the data could not be assumed to follow a normal distribution. Fisher's exact test was applied if the variables could be dichotomized without loss of information.

2. **Indices:** The following indices were calculated:

   Pre-implantation loss (%) = [(No. corpora lutea - No. implant.)/No. corpora lutea] × 100

   Post-implantation loss (%) = [(No. implant. - No. live fetuses)/No. implants.] × 100

3. **Historical control data:** Historical control data for cesarean section parameters and fetal malformations and variations were included from 28 studies conducted from February, 1987 through January, 1990.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** All animals survived to scheduled termination. No treatment-related clinical signs of toxicity were observed in low- or mid-dose animals. All high-dose animals were observed to have ruffled fur, ventral recumbency, dyspnea, and apathy during GDs 8-14. In addition, a total of five animals were considered to be in a comatose state on at least one day between GDs 10-12. Individual animal data for clinical signs were not given in the study report, but these findings were described in the text.

2. **Body weight**: Selected body weight and body weight change data are summarized in Table 2. Body weight and body weight change values for the low-and mid-dose groups were similar to the control group throughout the study. Absolute body weight of the high-dose group was slightly less than that of the controls beginning about GD 15 with statistical significance ($p \leq 0.05$; 95% of controls) attained on GDs 18 and 19. However, body weight gains by the high-dose group were significantly less than those of the controls throughout the dosing period. The most pronounced effect on body weight gain was during GDs 6-11 when the high-dose group gained only 47% of the control group level. Overall weight gain by the high-

dose group was 69% of the control group level during the dosing interval. During the post-dosing interval weight gain was similar between the treated and control groups.

3. **Food consumption**: Food consumption (g/animal/day) by the low- and mid-dose groups was similar to that of the control group throughout the study. Food consumption by the high-dose group was 76-82% ($p \leq 0.01$) of the control levels during the dosing interval (Table 2).

TABLE 2. Maternal body weight (g), body weight change (g), and food consumption (g/animal/day) data

Gestation Day/Interval	0 mg/kg/day	50 mg/kg/day	150 mg/kg/day	450 mg/kg/day
Body weight GD 0	198 ± 9.7	201 ± 8.3	200 ± 11.6	201 ± 9.8
Body weight GD 6	215 ± 11.7	217 ± 9.5	215 ± 11.5	219 ± 11.1
Body weight GD 11	230 ± 13.3	232 ± 12.2	227 ± 12.2	226 ± 15.2
Body weight GD 16	254 ± 14.5	255 ± 15.2	249 ± 14.5	246 ± 18.7
Body weight GD 21	305 ± 20.6	308 ± 20.3	299 ± 22.2	296 ± 29.1
Wt. change GD 0-6	17	16	15	18
Wt. change GD 6-11	15	15	12	7 (47)[a]
Wt. change GD 11-16	24	23	22	20 (83)
Wt. change GD 16-21	51	53	50	50
Wt change GD 6-16 (dosing interval)	39	38	34	27 (69)
Food consumption GD 0-6	17.5 ± 2.1	17.7 ± 2.1	17.8 ± 1.7	18.1 ± 1.9
Food consumption GD 6-11	19.5 ± 2.4	18.8 ± 2.2	18.2 ± 2.2	14.8** ± 3.0 (76)
Food consumption GD 11-16	23.3 ± 2.1	23.1 ± 2.5	21.9 ± 2.3	19.1** ± 3.4 (82)
Food consumption GD 16-21	22.5 ± 2.1	22.6 ± 2.5	22.4 ± 2.2	21.8 ± 2.9

Data taken from tables on pp. 25, 30-31, and 40, MRID 45369719; weight change data not analyzed statistically and standard deviations not included on the report summary table.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: **$p \leq 0.01$.

4. **Gross pathology**: No treatment-related gross lesions were observed in any animal at necropsy. Blood in the uterus was found in one control and two mid-dose animals.

5. Cesarean section data: Cesarean section data are given in Table 3. No treatment-related differences were noted between the treated and control groups for numbers of corpora lutea and implantations, gravid uterine weights, live fetuses per dam, resorptions, fetal sex ratios, and pre-implantation losses. Post-implantation loss by the high-dose group was slightly greater than that of the control group (9.4% vs 5.6% for the controls). Male and female fetal body weights from the high-dose group were significantly less ($p \leq 0.01$; 92% and 91% of controls, respectively) than those of the control. No female had complete litter resorption.

TABLE 3. Cesarean section observations

Observation	0 mg/kg/day	50 mg/kg/day	150 mg/kg/day	450 mg/kg/day
# Animals assigned (mated)	25	25	25	25
# Animals pregnant	25	25	25	25
Pregnancy rate (%)	100	100	100	100
# Nonpregnant	0	0	0	0
Maternal wastage				
# Died	0	0	0	0
# Aborted	0	0	0	0
Gravid uterine wt. (g)	73.5 ± 16.9	73.6 ± 13.9	68.1 ± 21.0	66.0 ± 18.3
Mean corpora lutea/Dam	13.6 ± 2.0	13.4 ± 2.0	13.0 ± 2.4	13.0 ± 1.8
Mean implantations/Dam	12.2 ± 2.7	12.1 ± 2.5	11.2 ± 3.9	12.4 ± 2.4
Mean live fetuses/Dam	11.5 ± 2.9	11.5 ± 2.3	10.4 ± 3.6	11.2 ± 3.0
Total # dead fetuses	0	0	0	0
Resorptions/Dam				
Early	0.6 ± 1.0	0.6 ± 0.8	0.8 ± 1.9	1.2 ± 1.7
Late	0.0 ± 0.2	0.0	0.0	0.0
Litters with total resorptions	0	0	0	0
Mean fetal weight (g)				
Males	4.8 ± 0.2	4.9 ± 0.3	5.0 ± 0.3	4.4** ± 0.5
Females	4.5 ± 0.2	4.6 ± 0.3	4.8* ± 0.4	4.1** ± 0.5
Sex ratio (% male)	46.3	49.5	50.8	55.9
Pre-implantation loss (%)	10.9	9.9	13.3	4.6
Post-implantation loss (%)	5.6	5.0	7.5	9.4

Data taken from tables on pp. 36-39 and 41-42, MRID 45369719.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

B. **DEVELOPMENTAL TOXICITY**: The number of fetuses (litters) examined for external malformations/variations in the control, low-, mid-, and high-dose groups was 287 (25), 287 (25), 260 (25), and 281 (25), respectively. The number of fetuses (litters) examined viscerally was 137 (25), 137 (25), 123 (25), and 134 (25), respectively, and the number of fetuses (litters) examined for skeletal malformations/variations was 150 (25), 150 (25), 137 (25), and 147 (25), respectively.

1. **External examination**: No treatment-related external malformations/variations were observed (Table 4a). One fetus in each of the low- and mid-dose groups had malpositioned hind limbs. A runt was found in one mid- and one high-dose litter.

2. **Visceral examination**: No treatment-related visceral malformations/variation were observed (Table 4a). One control fetus and one high-dose fetus had dilatation of the renal pelvis.
3. **Skeletal examination**: The high-dose group had a significantly ($p \leq 0.05$) greater number of litters containing fetuses with skeletal malformations and variations compared with the control group (Table 4b). Dumbell-shaped thoracic vertebral body was the most common malformation observed in the high-dose group. The most prevalent variation in the high-dose group included significantly increased incidences of incomplete or non-ossification of the vertebrae and sternebrae.

TABLE 4a. External and visceral examinations

Observations	0 mg/kg/day	50 mg/kg/day	150 mg/kg/day	450 mg/kg/day
#Fetuses (litters) examined externally	287 (25)	287 (25)	260 (25)	281 (25)
#Fetuses (litters) with external malformations	0 (0)	1 (1)	2 (2)	1 (1)
#Fetuses (litters) examined viscerally	(37 (25)	137 (25)	123 (25)	134 (25)
#Fetuses (litters) with visceral malformations	1 (1)	0 (0)	0 (0)	1 (1)

Data taken from tables on p. 47, MRID 45369719.

TABLE 4b. Skeletal Examinations

Observations	0 mg/kg/day	50 mg/kg/day	150 mg/kg/day	450 mg/kg/day
#Fetuses (litters) examined	150 (25)	(50 (25)	137 (25)	147 (25)
#Fetuses (litters) with skeletal malformations	5 (4)	4 (4)	3 (3)	12 (9*)
#Fetuses (litters) with dumbell-shaped thoracic vertebral body	1 (1)	0 (0)	2 (2)	8 (6)
#Fetuses (litters) with wavy ribs	3 (2)	4 (4)	1 (1)	3 (3)

#Fetuses (litters) with non-ossified cervical vertebra 3	11 (7)	5 (4)	16 (10)	29 (16**)
#Fetuses (litters) with incompletely ossified sternebra 2	28 (13)	20 (9)	16 (13)	65 (22**)

Data taken from tables on pp. 48-53, MRID 45369719.
Litter incidence significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study authors concluded that administration of the test article produced maternal toxicity at 150 and 450 mg/kg/day. At 450 mg/kg/day, clinical signs of toxicity and reduced food consumption and body weight gain were observed. Reduced food consumption was also observed in dams at 150 mg/kg/day. In addition, 450 mg/kg/day resulted in a slight increase in post-implantation loss, reduced mean fetal body weight, and increased incidences of skeletal malformations and variations. The authors considered the skeletal effects a result of reduced fetal body weight and not a direct teratogenic potential of the test article. Thus, the authors identified NOAELs for maternal rats and fetuses of 50 and 150 mg/kg/day, respectively.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** Maternal toxicity was evident in the high-dose group as clinical signs of toxicity, decreases in body weight gain, and reduced food consumption during the dosing interval. The magnitude of lower weight gain in the high-dose group resulted in statistically decreased absolute body weight on GDs 18 and 19. The reviewer disagrees with the study authors about the significance of slightly lower food consumption by the mid-dose group. In the absence of any other maternal or fetal effects at the mid-dose, the magnitude of reduced food consumption during the dosing interval (-6.7% GD 6-11; -6.0% GD 11-16) was neither biologically or toxicologically significant.

   Therefore, the maternal toxicity LOAEL for Hallcomid M-8-10 in rats is 450 mg/kg/day based on clinical signs, and decreased body weight gain and food consumption, and the maternal toxicity NOAEL is 150 mg/kg/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** Post-implantation loss was slightly higher for the high-dose group compared with the controls. Although not severe enough to affect the number of live fetuses/litter, complete litter loss was found in the range-finding study at higher doses; therefore, the post-implantation loss at the high-dose is considered to be an adverse effect of treatment.

   b. **Altered growth:** Fetal body weights for high-dose males and females were significantly less than those of the controls. In addition, ossification rates of several bones were decreased and the incidence rates of several common skeletal malformations were increased.

c. **Developmental variations:** Litters from the high-dose group had an increased incidence of fetuses with skeletal variations, mainly reduced ossification, which corresponded to reduced growth. Taken together these effects suggest growth retardation.

d. **Malformations:** Fetuses from high-dose litters had increases in skeletal malformations. The most common finding was a dumbell-shaped thoracic vertebral body.

Therefore, the developmental toxicity LOAEL for Hallcomid M-8-10 in rats is 450 mg/kg/day based on increased post-implantation loss, decreased fetal body weight, and increased incidences of skeletal malformations/variations. The developmental toxicity NOAEL is 150 mg/kg/day.

C. **STUDY DEFICIENCIES:** No major deficiencies were identified in the conduct of this study.

APPENDIX

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700a [§83-3a]; OECD 414.

TEST MATERIAL (PURITY): N,N-dimethyl-hexanacidamide - 3.45%
N,N-dimethyl-octanacidamide - 53.31%
N,N-dimethyl-decanacidamide - 39.48%
N,N-dimethyl-dodecanacidamide - 1.43%

SYNONYMS: Hallcomid M-8-10

CITATION: Becker, H. and K. Biedermann (1991) Dose range-finding embryotoxicity study (including teratogenicity) with Hallcomid M-8-10 in the rat. RCC, Research and Consulting Company AG and RCC Umweltchemie AG, PO Box CH 4452 Itingen, Switzerland. Project no. 274972. January 28, 1991. MRID 45369718. Unpublished.

SPONSOR: Bayer AG; submitted by C.P. Hall Co.

EXECUTIVE SUMMARY: In a preliminary developmental toxicity study (MRID 45369718), Hallcomid M-8-10 (3.45% N,N-dimethyl-hexanacidamide, 53.31% N,N-dimethyl-octanacidamide, 39.48% N,N-dimethyl-decanacidamide, 1.43% N,N-dimethyl-dodecanacidamide; batch # 903069) was administered in bi-distilled water with 0.5% cremophor to 5 female Wistar rats/dose by gavage at dose levels of 0, 100, 300, or 1000 mg/kg bw/day from days 6 through 15 of gestation. On GD 21, all surviving dams were sacrificed and examined grossly. Each uterus was weighed and the ovaries examined for number of corpora lutea. Each fetus was weighed and examined externally for abnormalities and for sex determination.

Clinical signs of toxicity were observed in all treated animals. In the low- and mid-dose group, the animals consumed the bedding material after dosing on the first two days and had ruffled fur on the first three days of dosing. High-dose animals were observed with ventral recumbency, apathy, ruffled fur, cold to the touch, comatose state, dyspnea, and watery discharge from the eyes throughout the dosing interval. One high-dose animal died on GD 13.

Body weight, body weight gain, and food consumption were similar between the low-, mid-dose, and the control group. High-dose animals had significantly ($p \leq 0.05$ or 0.01) lower absolute body weight compared with that of the controls beginning on GD 9. Reduced body weight was due to marked weight loss by the high-dose group during the treatment interval. Food consumption by the high-dose animals was 55% of the control level during treatment.

Post-implantation loss for the control, low-, mid-, and high-dose groups was 7.1, 9.4, 12.9, and 100%, respectively. No effects on fetal body weight or abnormal external findings were noted in the low- or mid-dose groups. **Therefore, doses of 0, 50, 150, and 450 mg/kg/day were chosen for the main study.**

DATA FOR ENTRY INTO ISIS

Developmental Study - rats (870.3700a)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NDAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
NA	45369719	developmental	rats	10 days	oral	gavage	50-450	0, 5, 150, 450	150	450	clinical signs; decr. body weight gain and food consumption	Maternal
NA	45369719	developmental	rats	10 days	oral	gavage	50-450	0, 50, 150, 450	150	450	incr. post-implantation loss; decr. fetal body weight; skeletal malformations and variations	Developmental

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

- **Identity:** N,N-dimethyl-hexanacidamide - 3.45%
  N,N-dimethyl-octanacidamide - 53.31%
  N,N-dimethyl-decanacidamide - 39.48%
  N,N-dimethyl-dodecanacidamide - 1.43%

‡ **Remarks:** no further comments

METHOD

- **Method/guideline followed:** OPPTS 870.3700a; OECD 414
- **GLP (Y/N):** yes
- **Year (study performed):** 1991
- **Species:** rat
- **Strain:** Wistar
- **Route of administration:** oral (gavage)
- **Doses/concentration levels:** 0, 50, 150, or 450 mg/kg/day
- **Sex:** female
- **Exposure period:** 10 days
- **Frequency of treatment:** daily on gestation days 6-15
- **Control group and treatment:** bi-distilled water plus 0.5% cremophor
- **Duration of test:** gestation days 0-21
- **Statistical methods:** Maternal body weight and food consumption, reproduction, and skeletal examination data were analyzed by a one-way analysis of variance (ANOVA). The ANOVA was followed by the Dunnett-test if the variables could be assumed to follow a normal distribution or the Steel-test if the data could not be assumed to follow a normal distribution. Fisher's exact test was applied if the variables could be dichotomized without loss of information.

‡ **Remarks:** Hallcomid M-8-10 was administered in bi-distilled water plus 0.5% cremophor to 25 presumed pregnant Wistar rats/dose by gavage at dose levels of 0, 50, 150, or 450 mg/kg bw/day from days 6 through 15 of gestation. The animals were checked twice daily for mortality and clinical signs of toxicity. Body weights were recorded daily. Food consumption was recorded on GDs 0-6, 6-11, 11-16, and 16-21. Dams were sacrificed on GD 21 carbon dioxide asphyxiation and subjected to gross examination. The number of corpora lutea on each ovary was counted. Gravid uteri were removed, weighed, and examined for the number and position of fetuses. Implantation sites were classified as empty (early resorption), embryonic, or fetal resoprtions. Live fetuses were weighed and examined externally for abnormalities and for sex determination. Approximately one-half of the fetuses in each litter were examined viscerally by the Wilson technique. The remaining one-half of the fetuses in each litter were processed for skeletal examination.

RESULTS

- **NOAEL (NOEL) and LOAEL (LOEL) maternal toxicity:** NOAEL = 150 mg/kg/day; LOAEL = 450 mg/kg/day
- **NOAEL (NOEL) and LOAEL (LOEL) developmental toxicity:** NOAEL = 150 mg/kg/day; LOAEL = 450 mg/kg/day
- **Actual dose received by dose level by sex if available:** 0, 50, 150 or 450 mg/kg/day
- **Maternal data with dose level (with NOAEL value):** All animals survived to scheduled termination. No treatment-related clinical signs of toxicity, or effects on body weight, body weight gain, or food consumption were observed in low- or mid-dose animals. All high-dose animals were observed with ruffled fur, ventral recumbency, dyspnea, and apathy during GDs 8-14. In addition, a total of five animals were described as being in a comatose state on at least one day between GDs 10-12. Absolute body weight of the high-dose group was slightly less than that of the controls beginning about GD 15 with statistical significance ($p \leq 0.05$; 95% of controls) attained on GDs 18 and 19. However body weight gains by the high-dose group were significantly less than those of the controls throughout the dosing period. The most pronounced effect on body weight gain was during GDs 6-11 when the high-dose group gained only 47% of the control group level. Overall weight gain by the high-dose group was 69% of the control group level during the dosing interval. Food consumption by the high-dose group was 76-82% ($p \leq 0.01$) of the control levels during the dosing interval.

Maternal body weight (g), body weight change (g), and food consumption (g/animal/day) data				
Gestation Day/Interval	0 mg/kg/day	50 mg/kg/day	150 mg/kg/day	450 mg/kg/day
Body weight GD 0	198 ± 9.7	201 ± 8.3	200 ± 11.6	201 ± 9.8
Body weight GD 6	215 ± 11.7	217 ± 9.5	215 ± 11.5	219 ± 11.1
Body weight GD 11	230 ± 13.3	232 ± 12.2	227 ± 12.2	226 ± 15.2
Body weight GD 16	254 ± 14.5	255 ± 15.2	249 ± 14.5	246 ± 18.7
Body weight GD 21	305 ± 20.6	308 ± 20.3	299 ± 22.2	296 ± 29.1
Wt. change GD 0-6	17	16	15	18
Wt. change GD 6-11	15	15	12	7
Wt. change GD 11-16	24	23	22	20
Wt. change GD 16-21	51	53	50	50
Wt change GD 6-16 (dosing interval)	39	38	34	27
Food consumption GD 0-6	17.5 ± 2.1	17.7 ± 2.1	17.8 ± 1.7	18.1 ± 1.9
Food consumption GD 6-11	19.5 ± 2.4	18.8 ± 2.2	18.2 ± 2.2	14.8** ± 3.0
Food consumption GD 11-16	23.3 ± 2.1	23.1 ± 2.5	21.9 ± 2.3	19.1** ± 3.4
Food consumption GD 16-21	22.5 ± 2.1	22.6 ± 2.5	22.4 ± 2.2	21.8 ± 2.9

Significantly different from control: **$p \leq 0.01$.

- **Fetal data with dose level (with NOAEL value):** No treatment-related differences were noted between the treated and control groups for numbers of corpora lutea and implantations, gravid uterine weights, live fetuses per dam, resorptions, fetal sex ratios, and pre-implantation losses. Post-implantation loss by the high-dose group was slightly greater than that of the control group (9.4% vs 5.6% for the controls). Male and female fetal body weights from the high-dose group were significantly less ($p \leq 0.01$; 92% and 91% of controls, respectively) than those of the control. No female had complete litter resorption.

Cesarean section observations				
Observation	0 mg/kg/day	50 mg/kg/day	150 mg/kg/day	450 mg/kg/day
# Animals assigned (mated)	25	25	25	25
# Animals pregnant	25	25	25	25
Pregnancy rate (%)	100	100	100	100
# Nonpregnant	0	0	0	0
Maternal wastage				
# Died	0	0	0	0
# Aborted	0	0	0	0
Gravid uterine wt. (g)	73.5 ± 16.9	73.6 ± 13.9	68.1 ± 21.0	66.0 ± 18.3
Mean corpora lutea/Dam	13.6 ± 2.0	13.4 ± 2.0	13.0 ± 2.4	13.0 ± 1.8
Mean implantations/Dam	12.2 ± 2.7	12.1 ± 2.5	11.2 ± 3.9	12.4 ± 2.4
Mean live fetuses/Dam	11.5 ± 2.9	11.5 ± 2.3	10.4 ± 3.6	11.2 ± 3.0
Total # dead fetuses	0	0	0	0
Resorptions/Dam				
Early	0.6 ± 1.0	0.6 ± 0.8	0.8 ± 1.9	1.2 ± 1.7
Late	0.0 ± 0.2	0.0	0.0	0.0
Litters with total resorptions	0	0	0	0
Mean fetal weight (g)				
Males	4.8 ± 0.2	4.9 ± 0.3	5.0 ± 0.3	4.4** ± 0.5
Females	4.5 ± 0.2	4.6 ± 0.3	4.8* ± 0.4	4.1** ± 0.5
Sex ratio (% male)	46.3	49.5	50.8	55.9
Pre-implantation loss (%)	10.9	9.9	13.3	4.6
Post-implantation loss (%)	5.6	5.0	7.5	9.4

Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

The number of fetuses (litters) examined for external malformations/variations in the control, low-, mid-, and high-dose groups was 287 (25), 287 (25), 260 (25), and 281 (25), respectively. The number of fetuses (litters) examined viscerally was 137 (25), 137 (25), 123 (25), and 134 (25), respectively, and the number of fetuses (litters) examined for skeletal malformations/variations was 150 (25), 150 (25), 137 (25), and 147 (25), respectively. No treatment-related external or visceral

malformations/variations were observed. The number of fetuses(litters) in the control, low-, mid-, and high-dose groups with skeletal malformations was 5 (4), 4 (4), 3 (3), and 12 (9) ($p \leq 0.05$), respectively. Dumbell-shaped thoracic vertebral body was the most common malformation. Also increased in the high-dose group were the incidences of incomplete or non-ossification of the vertebrae and sternebrae.

- **Statistical results, as appropriate:** as described in results
‡ **Remarks:** no further comments

CONCLUSIONS

‡ Maternal and developmental toxicity were evident at 450 mg/kg/day as clinical signs in the dams, reduced maternal body weight gain and feed consumption, increased post-implantation loss, decreased fetal body weight, and fetal skeletal malformations/variations. No effects were noted at doses less than or equal to 150 mg/kg/day.

DATA QUALITY

- Reliability: (1) reliable without restriction

‡ **Remarks:** Flag, key study for endpoint

REFERENCES

Becker, H. and K. Biedermann (1991) Embryotoxicity study (including teratogenicity) with Halicomid M-8-10 in the rat. RCC, Research and Consulting Company AG and RCC Umweltchemie AG, PO Box CH 4452 Itingen, Switzerland. Project no. 274983. October 21, 1991. MRID 45369719. Unpublished.

DATA EVALUATION RECORD

HALLCOMID M-8-10

Study Type: DEVELOPMENTAL TOXICITY– RABBIT [870.3700 (83-3B)]
MRIDs 45369726 (main study) and 45369725 (range-finding study)

Prepared for

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U.S. Environmental Protection Agency
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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbit; OPPTS 870.3700b [§83-3b]; OECD 414.

TEST MATERIAL (PURITY): N,N-dimethyl-hexanacidamide - 3.45%
N,N-dimethyl-octanacidamide - 53.31%
N,N-dimethyl-decanacidamide - 39.48%
N,N-dimethyl-dodecanacidamide - 1.43%

SYNONYMS: Hallcomid M-8-10

CITATION: Becker, H. and K. Biedermann (1991) Embryotoxicity study (including teratogenicity) with Hallcomid M-8-10 in the rabbit. RCC, Research and Consulting Company AG and RCC Umweltchemie AG, PO Box CH 4452 Itingen, Switzerland. Project no. 275005. August 27, 1991. MRID 45369726. Unpublished.

Becker, H. and K. Biedermann (1991) Dose range-finding embryotoxicity study (including teratogenicity) with Hallcomid M-8-10 in the rabbit. RCC, Research and Consulting Company AG and RCC Umweltchemie AG, PO Box CH 4452 Itingen, Switzerland. Project no. 274994. January 28, 1991. MRID 45369725. Unpublished.

SPONSOR: Bayer AG; submitted by C.P. Hall Co.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45369726), Hallcomid M-8-10 (3.45% N,N-dimethyl-hexanacidamide, 53.31% N,N-dimethyl-octanacidamide, 39.48% N,N-dimethyl-decanacidamide, 1.43% N,N-dimethyl-dodecanacidamide, batch # 903069) was administered in bi-distilled water plus 0.5% cremophor to 16 naturally mated female Chinchilla rabbits/dose by gavage at dose levels of 0, 100, 300, or 1000 mg/kg bw/day from days 6 through 18 of gestation. Doses were chosen on the basis of a range-finding study (MRID 45369725). On GD 28, all surviving does were sacrificed and examined grossly. Each uterus was weighed and the ovaries examined for number of corpora lutea. Each fetus was weighed and examined for external abnormalities. Fetuses were examined viscerally by fresh dissection, and the sex determined. After the skin was removed, the heads were examined for the degree of ossification. The heads were then fixed in trichloroacetic acid and formaldehyde and examined by serial sectioning. The remaining carcasses were processed for skeletal examination.

No treatment-related deaths or clinical signs of toxicity occurred in any animal and gross necropsy was unremarkable. No treatment-related effects on absolute body weight, weight change, or food consumption were observed in the low- or mid-dose groups during the study. Absolute body weight of the high-dose group was slightly less than that of the controls throughout the study (including prior to treatment initiation) with statistical significance attained only on GDs 21 and 22. Overall weight gain during the dosing interval by the high-dose group was 47% of the control level with the most pronounced effect on GDs 6-11 during which weight gain was only 11% of the control level. The high-dose group showed compensatory weight gain

during the post-dosing interval. Food consumption by the high-dose group was 75-84% of the control group levels during the dosing interval. Although maternal effects were marginal, it is noted that the high dose was the limit dose for a developmental toxicity study.

Therefore, the maternal toxicity LOAEL for Hallcomid M-8-10 in Chinchilla rabbits is 1000 mg/kg/day based on decreases in body weight gain and food consumption and the maternal toxicity NOAEL is 300 mg/kg/day.

No statistically significant differences were noted between the treated and control groups for numbers of corpora lutea, implantations, live fetuses, or resorptions, gravid uterine weight, fetal sex ratios, and pre- or post-implantation losses. Fetal body weight was similar between the treated and control groups. The number of fetuses (litters) examined for external, visceral, and skeletal malformations/variations in the control, low-, mid-, and high-dose groups was 158 (16), 145 (14), 120 (12), and 147 (15), respectively. No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetus.

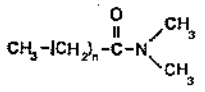
Therefore, the developmental toxicity NOAEL for Hallcomid M-8-10 in Chinchilla rabbits is ≥1000 mg/kg/day and the developmental toxicity LOAEL was not identified.

The developmental toxicity study in the rabbit is classified **Acceptable/Guideline** and does satisfy the guideline requirements for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits. This study was conducted prior to implementation of current guidelines although the high dose is the limit dose.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1.	**Test material:**	Hallcomid —8-10
	Description:	Clear, yellow fluid
	Lot #:	903069
	Purity:	N,N-dimethyl-hexanacidamide - 3.45% N,N-dimethyl-octanacidamide - 53.31% N,N-dimethyl-decanacidamide - 39.48% N,N-dimethyl-dodecanacidamide - 1.43%
	Compound Stability:	Until November 17, 1990
	CAS #of TGAI:	Not given, but CAS # 1 1 18-92-9 and #14433-76-2 for the two major constituents
	Structure:	

n=(4), 6, 8, (10); ( ) = minor constituents

2. **Vehicle and/or positive control:** Bi-distilled water with 0.5% Cremophor (BASF) was used as the vehicle and negative control. No positive control was used in this study.

3. **Test animals:**

Species:	Rabbit
Strain:	Chinchilla (Chbb: Ch, hybrids, SPF quality)
Age/weight at study initiation:	4-6 months; 2810-4825 g
Source:	Dr. Karl Thomae GmbH, Birkendorferstrasse 65, D-W-7950 Biberach / Riss
Housing:	Animals were individually housed in stainless steel cages with an automatic cleaning system.
Diet:	Pelleted Kilba 341 rabbit maintenance diet was available *ad libitum*.
Water:	Tap water was available *ad libitum*.
Environmental conditions:	**Temperature:** 20±3°C **Humidity:** 40-70% **Air changes:** 10-15/hour **Photoperiod:** 12 hrs light/12 hrs dark
Acclimation period:	Minimum of 7 days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: August 7, 1990; End: September 20, 1990

2. **Mating procedure:** Females were caged 1:1 with sexually mature males of the same strain and source until copulation was observed. The day of mating was designated as gestation day (GD) 0.

3. **Animal assignment:** Animals were assigned to the treatment groups indicated in Table 1 so that a similar number of females were allocated to each group on each day of mating and ensuring an acceptable distribution of males with which the females were mated.

TABLE 1: Animal assignment

Dose (mg/kg bw/day)	0	100 (LDT)	300 (MDT)	1000 (HDT)
# Females	16	16	16	16

4. **Dose selection rationale**: Doses were selected on the basis of a range-finding study conducted by the testing facility (see Appendix). Briefly, groups of 5 mated females/dose were administered 0, 100, 300, or 1000 mg/kg/day on GD 6-18. No treatment-related deaths or clinical signs of toxicity were observed. In high-dose does, food consumption was reduced and body weight loss was recorded during the first few days after the initiation of treatment. One doe in each of the mid- and high-dose groups had complete litter resorption. No effects were found on any fetal parameter. Therefore, doses for the main study were chosen as 0, 100, 300, and 1000 mg/kg/day.

5. **Dosage preparation and analysis**: Dosing solutions were prepared daily by weighing the appropriate amount of test article and adding vehicle. The dosing solutions were mixed using a homogenizer. Solutions were constantly stirred with a magnetic stirrer during use. Concentration, homogeneity, and stability were determined previously as part of the preliminary study in rabbits (MRID 45369725) and confirmed once during the current study.

Results:
Homogeneity analysis: Samples from the top, middle, and bottom of the dosing solutions differed from each other in concentration by <2% in the range-finding study and by <8% in the main study.

Stability analysis: After two hours at room temperature, concentrations of the dosing solutions were 96-101% of their initial measured concentrations in both the range-finding and main studies.

Concentration analysis: Absence of test article was confirmed in the vehicle. Mean concentrations of the low-, mid-, and high-dose suspensions were ±7% of nominal.

Analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration**: All doses were administered once daily by gavage, on GD 6 through 18, in a volume of 4 mL/kg of body weight/day. Dose volume was adjusted daily for the animal's body weight for that day.

C. **OBSERVATIONS**:

1. **Maternal observations and evaluations**: The animals were checked twice daily for mortality and clinical signs of toxicity. Body weights were recorded daily. Food consumption was recorded on GDs 0-6, 6-11, 11-15, 15-19, 19-24, and 24-28. Does were sacrificed on GD 28 by cervical dislocation and subjected to gross examination. The number of corpora lutea on each ovary was counted. Gravid uteri were removed, weighed, and the contents were examined. For animals that

appeared non-pregnant, the uteri were stained with an aqueous ammonium sulfide solution.

2. **Fetal evaluations**: Each fetus was weighed and examined for external abnormalities. Fetuses were examined viscerally by fresh dissection, and the sex determined. After the skin was removed, the heads were examined for the degree of ossification. The heads were then fixed in trichloroacetic acid and formaldehyde and examined by serial sectioning. The remaining carcasses were processed for skeletal examination.

D. **DATA ANALYSIS**:

1. **Statistical analyses**: Maternal body weight and food consumption, reproduction, and skeletal examination data were analyzed by a one-way analysis of variance (ANOVA). The ANOVA was followed by the Dunnett-test if the variables could be assumed to follow a normal distribution or the Steel-test if the data could not be assumed to follow a normal distribution. Fisher's exact test was applied if the variables could be dichotomized without loss of information.

2. **Indices**: Pre-and post-implantation losses were given in the summary tables but the formulas used for calculation were not provided.

3. **Historical control data**: Historical control data for cesarean section parameters and fetal malformations and variations were included from 6-7 studies/year conducted from 1987-1989.

II. **RESULTS**:

A. **MATERNAL TOXICITY**:

1. **Mortality and clinical observations**: No treatment-related clinical signs of toxicity were observed in any animal. Two low-dose animals died on GDs 7 and 15, respectively, due to gavage error. In addition, one animal in each of the low-, mid-, and high-dose groups was found dead (GDs 12, 28, and 12, respectively) with no prior signs or apparent cause of death. All remaining animals survived to scheduled sacrifice.

2. **Body weight**: Selected body weight and body weight change data are summarized in Table 2. Body weight and body weight change values for the low-and mid-dose groups were similar to the control group throughout the study. Absolute body weight of the high-dose group was slightly less than that of the controls throughout the study (including prior to treatment initiation) with statistical significance attained only on GDs 21 and 22. However, mean weight change for the high-dose group was consistently less than that of the controls during dosing. Overall weight gain during the dosing interval by the high-dose group was 47% of the control level with the most pronounced effect on GDs 6-11 during which weight gain was only 11% of the

control level. The high-dose group showed compensatory weight gain during the post-dosing interval, with 2.6 times the weight gain of controls.

TABLE 2: Mean (±SD) maternal body weight (g) and mean body weight change (g)

Gestation day/interval	0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
GD 0	3628 ± 621	3567 ± 473	3445 ± 472	3433 ± 411
GD 6	3851 ± 594	3764 ± 419	3695 ± 403	3644 ± 326
GD 11	3935 ± 567	3858 ± 382	3781 ± 391	3653 ± 282
GD 15	3989 ± 549	3914 ± 348	3859 ± 357	3692 ± 273
GD 19	4069 ± 558	3981 ± 363	3934 ± 341	3747 ± 246
GD 28	4145 ± 452	4150 ± 336	4049 ± 326	3945 ± 261
Wt. change GD 0-6	223	197	250	211
Wt. change GD 6-11	84	94	86	9 (11)[a]
Wt. change GD 11-15	54	56	78	39 (72)
Wt change GD 15-19	80	67	75	55 (69)
Wt. change GD 19-28	76	169	115	198 (261)
Wt. change GD 6-19	218	217	239	103 (47)

Data taken from tables on pp. 31-32 and 42, MRID 45369726; weight change data not analyzed statistically and standard deviations not included on the report summary table.
[a]Number in parentheses is percent of control; calculated by reviewer.

3. **Food consumption**: Food consumption by the high-dose group was 75-84% of the control group levels during the dosing interval. No differences in food consumption were observed between the low- and mid-dose groups and the control group during the study.

4. **Gross pathology**: No treatment-related gross lesions were observed in any animal at necropsy.

5. **Cesarean section data**: Cesarean section data are given in Table 3. No treatment-related differences were noted between the treated and control groups for numbers of corpora lutea, implantations, gravid uterine weights, live fetuses, or resorptions, fetal sex ratios, and pre- or post-implantation losses. Fetal body weights were similar between the treated and control groups.

TABLE 3: Cesarean section observations

Observation	0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
# Animals assigned (mated)	16	17[a]	16	16

# Animals pregnant	16	17	15	16
Pregnancy rate (%)	100	100	94	100
# Nonpregnant	0	0	1	0
Maternal wastage				
# Died/killed	0	3	1	1
# Aborted	0	0	0	0
Gravid uterine wt. (g)	519.1 ± 105.2	535.2 ± 65.6	498.7 ± 129.6	485.2 ± 84.7
Corpora lutea/doe	11.2 ± 2.3	11.2 ± 1.8	11.3 ± 2.5	10.4 ± 1.4
Implantations/doe	11.0 ± 2.8	10.8 ± 1.7	10.8 ± 3.1	10.3 ± 1.5
Live fetuses/doe	9.9 ± 2.9	10.4 ± 2.2	10.0 ± 3.2	9.8 ± 1.8
Resorptions/doe				
Early	0.1 ± 0.3	0.4 ± 1.1	0.3 ± 0.9	0.1 ± 0.4
Late	1.1 ± 1.4	0.0	0.6 ± 1.1	0.3 ± 0.6
Litters with total resorptions	0	0	2	0
Mean fetal weight (g)				
Males	32.2 ± 4.8	34.1 ± 4.8	33.2 ± 6.4	32.3 ± 3.4
Females	32.0 ± 2.5	33.4 ± 5.8	33.1 ± 5.1	30.9 ± 2.7
Sex ratio (% male)	49.4	55.2	47.5	57.1
Preimplantation loss (mean%)	1.7	3.8	3.7	1.3
Postimplantation loss (mean%)	10.2	4.0	7.7	4.5

Data taken from tables on pp. 20, 38-41, 43, and 44, MRID 45369726.
[a]One female died on GD 7 due to a gavage error and was replaced.

B. DEVELOPMENTAL TOXICITY: The number of fetuses (litters) examined for external, visceral, and skeletal malformations/variations in the control, low-, mid-, and high-dose groups was 158 (16), 145 (14), 120 (12), and 147 (15), respectively.

1. **External examination**: No treatment-related external malformations/variations were observed (Table 4). A runt was found in each of two control litters and two runts were found in one mid-dose litter.

2. **Visceral examination**: No treatment-related visceral malformations/variations were observed (Table 4). Malformations of the aorta, diaphragm, or kidneys were observed at single incidences in each treated group.

3. **Skeletal examination**: No treatment-related skeletal malformations/variations were observed (Table 4). Major malformations of the thoracic vertebral bodies, sternebrae, or ribs occurred in one to three fetuses from different litters of all groups. Common variations in fetuses from all groups included incomplete ossification of the sternebrae and digits.

TABLE 4. External, visceral, and skeletal examinations				
Observations	**0 mg/kg/day**	**100 mg/kg/day**	**300 mg/kg/day**	**1000 mg/kg/day**
#Fetuses (litters) examined	158 (16)	145 (14)	120 (12)	147 (15)
#Fetuses (litters) with external findings	2 (2)	0 (**0**)	2 (1)	0 (**0**)
#Fetuses (litters) with visceral findings	0 (**0**)	1 (1)	2 (2)	1 (1)
#Fetuses (litters) with skeletal malformations	1 (1)	2 (2)	3 (3)	1 (1)

Data taken from tables on pp. 49 and 50, MRID 45369726.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that administration of the test article produced maternal toxicity in the high-dose group as evidenced by decreased weight gain and food consumption. Fetal parameters were not affected at any dose and no teratogenic potential was observed. The maternal NOAEL was 300 mg/kg/day and the developmental NOAEL was 1000 mg/kg/day.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** Slight maternal toxicity was evident in this study as decreases in body weight gain and food consumption by the high-dose group during the dosing interval. The most pronounced effect on weight gain was for GDs 6-11, immediately after the initiation of dosing. However, reduced weight gain by the high-dose group was not severe enough to cause biologically significant depression of absolute body weight although statistical significance was attained on two occasions. Compensatory weight gain was apparent during the post-dosing interval. Although maternal effects were marginal, it is noted that the high dose was the limit dose for a developmental toxicity study.

   Therefore, the maternal toxicity LOAEL for Hallcomid M-8-10 in Chinchilla rabbits is 1000 mg/kg/day based on decreases in body weight gain and food consumption and the maternal toxicity NOAEL is 300 mg/kg/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** Maternal treatment with the test article did not result in an increase in resorptions or fetal deaths. No doe aborted prior to scheduled sacrifice.

b. **Altered growth:** Fetal body weights were not affected by treatment with the test article. Ossification rates were similar between the treated and control groups.

c. **Developmental variations:** No increase in fetal variations occurred at any maternal dose.

d. **Malformations:** Maternal treatment with the test article did not result in an increase of any specific fetal malformation.

Therefore, the developmental toxicity NOAEL for Hallcomid M-8-10 in Chinchilla rabbits is ≥1000 mg/kg/day and the developmental toxicity LOAEL was not identified.

C. **STUDY DEFICIENCIES:** No major deficiencies were identified in the conduct of this study. It is noted that the number of litters available for evaluation in all groups was less than the required number of 20. However, this study was conducted prior to implementation of current guidelines. A minor deficiency was that litter incidences were not summarized for fetal findings and had to be calculated from the individual animal data.

APPENDIX

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbit; OPPTS 870.3700b [§83-3b]; OECD 414.

TEST MATERIAL (PURITY): N,N-dimethyl-hexanacidamide - 3.45%
N,N-dimethyl-octanacidamide - 53.31%
N,N-dimethyl-decanacidamide - 39.48%
N,N-dimethyl-dodecanacidamide - 1.43%

SYNONYMS: Hallcomid M-8-10

CITATION: Becker, H. and K. Biedermann (1991) Dose range-finding embryotoxicity study (including teratogenicity) with Hallcomid M-8-10 in the rabbit. RCC, Research and Consulting Company AG and RCC Umweltchemie AG, PO Box CH 4452 Itingen, Switzerland. Project no. 274994. January 28, 1991. MRID 45369725. Unpublished.

SPONSOR: Bayer AG; submitted by C.P. Hall Co.

EXECUTIVE SUMMARY: In a preliminary developmental toxicity study (MRID 45369725), Hallcomid M-8-10 (3.45% N,N-dimethyl-hexanacidamide, 53.31% N,N-dimethyl-octanacidamide, 39.48% N,N-dimethyl-decanacidamide, 1.43% N,N-dimethyl-dodecanacidamide, batch # 903069) was administered in bi-distilled water plus 0.5% cremophor to 5 naturally mated female Chinchilla rabbits/dose by gavage at dose levels of 0, 100, 300, or 1000 mg/kg bw/day from days 6 through 18 of gestation. On GD 28, all surviving does were sacrificed and examined grossly. Each uterus was weighed and the ovaries examined for number of corpora lutea. Each fetus was weighed and examined externally and viscerally for abnormalities and for sex determination.

No treatment-related deaths or clinical signs of toxicity were observed in any doe. One high-dose animal died on GD 17 from an injury in the cage. Body weight, body weight gain, and food consumption by the low- and mid-dose groups were similar to those of the control group throughout the study. The high-dose group had transient weight loss on GDs 6-8 corresponding to food consumption 85% of the control levels during GDs 6-11. Maternal necropsy was unremarkable.

The numbers of corpora lutea and implantations were similar between the treated and control groups. One doe in each of the mid- and high-dose groups had complete litter resorption. Fetal body weight was slightly reduced in the high-dose group compared with the controls. No treatment-related external or visceral abnormalities were observed in any fetus.

Therefore, based on the results of this preliminary study, doses for the main study were chosen as 0, 100, 300, and 1000 mg/kg/day.

DATA FOR ENTRY INTO ISIS

Developmental Study - rabbits (870.3700b)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
NA	45369726	developmental	rabbits	13 days	oral	gavage	100-1000	0, 100, 300, 1000	300	1000	body wt gain; food consumption	Maternal
NA	45369726	developmental	rabbits	13 days	oral	gavage	100-1000	0, 100, 300, 1000	1000	not identified	none	Developmental

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

- **Identity**: N,N-dimethyl-hexanacidamide - 3.45%
  N,N-dimethyl-octanacidamide - 53.31%
  N,N-dimethyl-decanacidamide - 39.48%
  N,N-dimethyl-dodecanacidamide - 1.43%

‡ **Remarks:** no further comments

METHOD

- **Method/guideline followed:** OPPTS 870.3700b; OECD 414
- **GLP (Y/N):** yes
- **Year (study performed):** 1991
- **Species:** Rabbit
- **Strain:** Chinchilla
- **Route of administration:** oral (gavage)
- **Doses/concentration levels:** 0, 100, 300, or 1000 mg/kg/day
- **Sex:** female
- **Exposure period:** gestation days 6-18
- **Frequency of treatment:** daily
- **Control group and treatment:** bi-distilled water plus 0.5% cremophor
- **Duration of test:** gestation days 0-28
- **Statistical methods:** Maternal body weight and food consumption, reproduction, and skeletal examination data were analyzed by a one-way analysis of variance (ANOVA). The ANOVA was followed by the Dunnett-test if the variables could be assumed to follow a normal distribution or the Steel-test if the data could not be assumed to follow a normal distribution. Fisher's exact test was applied if the variables could be dichotomized without loss of information.

‡ **Remarks:**

Hallcomid M-8-10 was administered in bi-distilled water plus 0.5% cremophor to 16 naturally mated female Chinchilla rabbits/dose by gavage at dose levels of 0, 100, 300, or 1000 mg/kg bw/day from days 6 through 18 of gestation. The animals were checked twice daily for mortality and clinical signs of toxicity. Body weights were recorded daily. Food consumption was recorded on GDs 0-6, 6-11, 11-15, 15-19, 19-24, and 24-28. Does were sacrificed on GD 28 by cervical dislocation and subjected to gross examination. The number of corpora lutea on each ovary was counted. Gravid uteri were removed, weighed, and the contents were examined. For animals that appeared non-pregnant, the uteri were stained with an aqueous ammonium sulfide solution. Each fetus was weighed and examined for external abnormalities. Fetuses were examined viscerally by fresh dissection, and the sex determined. After the skin was removed, the heads were examined for the degree of ossification. The heads were then fixed in trichloroacetic acid and formaldehyde and examined by serial sectioning. The remaining carcasses were processed for skeletal examination.

RESULTS

- **NOAEL (NOEL) and LOAEL (LOEL) maternal toxicity:** NOAEL = 300 mg/kg/day; LOAEL = 1000 mg/kg/day
- **NOAEL (NOEL) and LOAEL (LOEL) developmental toxicity:** NOAEL equal to or greater than 1000 mg/kg/day; LOAEL not identified
- **Actual dose received by dose level by sex if available:** 0, 100, 300, or 1000 mg/kg/day
- **Maternal data with dose level (with NOAEL value):** No treatment-related clinical signs of toxicity were observed in any animal. Two low-dose animals died on GDs 7 and 15, respectively, due to gavage error. In addition, one animal in each of the low-, mid-, and high-dose groups was found dead (GDs 12, 28, and 12, respectively) with no prior signs or apparent cause of death. All remaining animals survived to scheduled sacrifice. No treatment-related gross lesions were observed in any animal at necropsy. Body weight, body weight change, and food consumption values for the low-and mid-dose groups were similar to the control group throughout the study. Absolute body weight of the high-dose group was slightly less than that of the controls throughout the study (including prior to treatment initiation) with statistical significance attained only on GDs 21 and 22 (see table). However, mean weight change was for the high-dose group was consistently less than that of the controls during dosing. Overall weight gain during the dosing interval by the high-dose group was 47% of the control level with the most pronounced effect on GDs 6-11 during which weight gain was only 11% of the control level. The high-dose group showed compensatory weight gain during the post-dosing interval. Food consumption by the high-dose group was 75-84% of the control group levels during the dosing interval.

Mean (±SD) maternal body weight (g) and mean body weight change (g)				
Gestation Day/Interval	0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
GD 0	3628 ± 621	3567 ± 473	3445 ± 472	3433 ± 411
GD 6	3851 ± 594	3764 ± 419	3695 ± 403	3644 ± 326
GD 11	3935 ± 567	3858 ± 382	3781 ± 391	3653 ± 282
GD 15	3989 ± 549	3914 ± 348	3859 ± 357	3692 ± 273
GD 19	4069 ± 558	3981 ± 363	3934 ± 341	3747 ± 246
GD 28	4145 ± 452	4150 ± 336	4049 ± 326	3945 ± 261
Wt. change GD 0-6	223	197	250	211
Wt. change GD 6-11	84	94	86	9 (11)[a]
Wt. change GD 11-15	54	56	78	39 (72)
Wt change GD 15-19	80	67	75	55 (69)
Wt. change GD 19-28	76	169	115	198 (261)
Wt. change GD 6-19	218	217	239	103 (47)

[a]Number in parentheses is percent of control.

- **Fetal data with dose level (with NOAEL value):** No treatment-related differences were noted between the treated and control groups for numbers of corpora lutea, implantations, gravid uterine weights, live fetuses, or resorptions, fetal sex ratios, and pre- or post-implantation losses. Fetal body weights were similar between the treated and control groups.

Cesarean section observations				
Observation	0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	t000 mg/kg/day
# Animals assigned	16	17[a]	16	16
# Animals pregnant	16	17	15	16
Pregnancy rate (%)	100	100	94	100
# Nonpregnant	0	0	1	0
Maternal wastage				
# Died/killed	0	3	1	1
# Aborted	0	0	0	0
Gravid uterine wt. (g)	519.1 ± 105.2	535.2 ± 65.6	498.7 ± 129.6	485.2 ± 84.7
Corpora lutea/doe	11.2 ± 2.3	11.2 ± 1.8	11.3 ± 2.5	10.4 ± 1.4
Implantations/doe	11.0 ± 2.8	10.8 ± 1.7	10.8 ± 3.1	10.3 ± 1.5
Live fetuses/doe	9.9 ± 2.9	10.4 ± 2.2	10.0 ± 3.2	9.8 ± 1.8
Resorptions/doe				
Early	0.1 ± 0.3	0.4 ± 1.1	0.3 ± 0.9	0.1 ± 0.4
Late	1.1 ± 1.4	0.0	0.6 ± 1.1	0.3 ± 0.6
Litters with total resorptions	0	0	2	0
Mean fetal weight (g)				
Males	32.2 ± 4.8	34.1 ± 4.8	33.2 ± 6.4	32.3 ± 3.4
Females	32.0 ± 2.5	33.4 ± 5.8	33.1 ± 5.1	30.9 ± 2.7
Sex ratio (% male)	49.4	55.2	47.5	57.1
Preimplant. loss (%)	1.7	3.8	3.7	1.3
Postimplant. loss (%)	10.2	4.0	7.7	4.5

[a]One female died on GD 7 due to a gavage error and was replaced.

The number of fetuses (litters) examined for external, visceral, and skeletal malformations/ variations in the control, low-, mid-, and high-dose groups was 158 (16), 145 (14), 120 (12), and 147 (15), respectively. No treatment-related external, visceral, or skeletal malformations/ variations were observed (Table 4). A runt was found in each of two control litters and two runts were found in one mid-dose litter. Malformations of the aorta, diaphragm, or kidneys were observed at single incidences in each treated group. Major malformations of the thoracic vertebral bodies, sternebrae, or ribs occurred in one to three fetuses from different litters of all groups. Common skeletal variations in fetuses from all groups included incomplete ossification of the sternebrae and digits.

- **Statistical results, as appropriate:** as described in results

RESULTS

‡ **Remarks:** It is noted that the number of litters available for evaluation in all groups was less than the required number of 20. However, this study was conducted prior to implementation of current guidelines.

CONCLUSIONS

Maternal toxicity occurred at the highest dose, 1000 mg/kg/day, which is the limit dose for developmental toxicity studies. No evidence of developmental toxicity was observed.

DATA QUALITY

- **Reliability:** (2) reliable with restriction
- ‡ **Remarks:** Fewer than the required number of litters per group were available for evaluation.

REFERENCES

Becker, H. and K. Biedermann (1991) Embryotoxicity study (including teratogenicity) with Hallcomid M-8-10 in the rabbit. RCC, Research and Consulting Company AG and RCC Umweltchemie AG, PO Box CH 4452 Itingen, Switzerland. Project no. 275005. August 27, 1991. MRID 45369726. Unpublished.

DATA EVALUATION RECORD

HALLCOMID M-8-10

***SALMONELLA*/MAMMALIAN ACTIVATION GENE MUTATION ASSAY; OPPTS 870.5100 [§84-2]**
MRID 45369728

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. RD-2003-4

Primary Reviewer:
B.L. Whitfield, Ph.D. Signature: _____
Date: _____

Secondary Reviewers:
Cheryl B. Bast, Ph.D., D.A.B.T. Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Susan Chang, M.S. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: ***In vitro* Bacterial Gene Mutation** *(Bacterial system, Salmonella typhimurium)*/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: N/A

DP BARCODE: N/A
SUBMISSION NO.: N/A

TEST MATERIAL (PURITY): Hallcomid M-8-10 (98.08% a.i. identified as 4.73% N,N-Dimethyl-hexanoic acid amide; 53.9% N,N-Dimethyl-octanoic acid amide; 38.9% N,N-Dimethyl-decanoic acid amide; 0.55% N,N-Dimethyl-dodecanoic acid amide; analyzed July 21, 1992))

SYNONYMS: No others provided

CITATION: Herbold, B.A.. (1992) Hallcomid M-8-10 *Salmonella*/microsome test. Bayer AG, Fachbereich Toxicology, Friedrich-Ebert-Straße 217-333, D-5600 Wuppertal 1, F.R.G.. Laboratory Report No.: 21849, Study No.: T 3039100, November 17, 1992. MRID 45369728. Unpublished

SPONSOR: Bayer AG. (Submitted by The C.P. Hall Company, 311 South Wacker Drive, Suite 4700, Chicago, Illinois, 60606)

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 45369728), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* were exposed to Hallcomid M-8-10 (Batch No. 002949, 98.08% of identified constituents) in ethanol in three independent assays. A standard plate assay procedure was used and all plating was in quadruplicate. In the first assay, bacteria were exposed at concentrations of 0, 8, 40, 200, 1000 or 5000 µg/plate without metabolic activation (S9-mix) and with 30% S9. In the second assay, bacteria were exposed at concentrations of 0, 25, 50, 100, 200, 400 or 800 µg/plate without S9-mix and with 10% S9. The third assay was conducted with activation only at the same test material concentrations as used in the second assay. The S9 concentration was 4%. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

Hallcomid M-8-10 was tested to upper concentrations limited by cytotoxicity. Excess cytotoxicity was seen at 1000 and 5000 µg/plate in the first assay; therefore, the dose range for the second and third assays was 25 to 800 µg/plate. Thinning of the background lawn and a reduction in the background number of revertants per plate were seen at 400 and 800 µg/plate in all strains with and without S9-mix. Bacteria titer determinations done in the presence of S9-mix additionally showed bacteriotoxic effects at 200 µg/plate with TA1535 and TA98. The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain in any assay. The solvent and positive controls induced the appropriate responses

in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

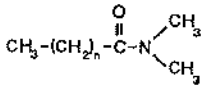
This study is classified as **Acceptable/Guideline** and satisfies the intent of Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data. Four plates per dose were used and repeat assays were conducted, two without S9-mix and three with S9-mix. A different concentration of S9 was used in each of the assays with S9-mix although the 4% concentration is below the recommended range of 5% to 30%. There was no hint of a mutagenic effect seen in the study. The EPA Guideline recommends that five tester strains be used, and only four were used in this study. These four *S. typhimurium* strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidizing mutagens, crosslinking agents and hydrazines. Such substances may be detected by *Escherichia coli* WP2 strains or *S. typhimurium* TA102 which have an AT base pair at the primary reversion site. As the test material is not an oxidizer, shows no structural similarity to any known crosslinking agent, and does not include a hydrazine group, it is concluded that inclusion of *Escherichia coli* WP2(uvrA) would not have changed the conclusions.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Hallcomid M-8-10

Description:	Clear, yellowish liquid
Lot/Batch #:	002949
Purity:	98.08% a.i. (identified as 4.73% N,N-Dimethyl-hexanoic acid amide; 53.9% N,N-Dimethyl-octanoic acid amide; 38.9% N,N-Dimethyl-decanoic acid amide; 0.55% N,N-Dimethyl-dodecanoic acid amide; analyzed July 21, 1992
CAS # of TGAI:	1118-92-9 (N,N-Dimethyloctananmide) and 14433-76-2 (N,N-Dimethyldecanamide)
Structure:	

n = (4) ,6, 8 or (10); ( ) = minor constituents

Solvent Used: Ethanol

2. Control materials:

Negative:	None
Solvent (final conc'n):	Ethanol / 0.1 mL/plate
	Nonactivation:

Positive:

Sodium azide 10 µg/plate TA1535
2-Nitrofluorene ___ µg/plate
9-Aminoacridine ___ µg/plate
Other (list):
Nitrofurantoin 0.2 µg/plate TA100
4-Nitro-1,2-phenylene diamine 10 µg/plate TA1537
4-Nitro-1,2-phenylene diamine 0.5 µg/plate TA98

Activation:
2-Aminoanthracene (2-anthramine) 3 µg/plate All strains

3. **Activation: S9 derived from:**

x	Induced	x	Aroclor 1254	x	Rat	x	Liver
	Non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other (name)
			Other:		Other (name)		

S9-fraction was obtained from male Sprague-Dawley rats.

Describe S9 mix composition:

S9-fraction 4%, 10% or 30% (volume difference at 4% and 10% replaced with 0.15 M KCl)
Cofactor solution (per mL):

$MgCl_2 \cdot 6\ H_2O$	2.32 mg
KCl	3.51 mg
Glucose-6-phosphate, disodium salt	2.56 mg
NADP, disodium salt	4.50 mg
Phosphate buffer	100 mM

4. **Test organisms: *S. typhimurium* strains**

	TA97	x	TA98	x	TA100		TA102		TA104
x	TA1535	x	TA1537		TA1538		*E.coli* WP2(uvrA)		

Properly maintained? [x] Yes [ ] No
Checked for appropriate genetic markers (*rfa* mutation, R factor)? [x] Yes [ ] No

5. **Test compound concentrations used:**

Mutation assays: (all strains, quadruplicate plating)

First assay:
Nonactivated conditions: 8, 40, 200, 1000, 5000 µg/plate
Activated conditions (30% S9): 8, 40, 200, 1000, 5000 µg/plate

Second assay:

Nonactivated conditions: 25, 50, 100, 200, 400, 800 µg/plate
Activated conditions (10% S9): 25, 50, 100, 200, 400, 800 µg/plate

Third assay:
Activated conditions (4% S9): 25, 50, 100, 200, 400, 800 µg/plate

B. TEST PERFORMANCE:

1. **Type of *Salmonella* assay:**
   _x_ standard plate test
   __ pre-incubation (___ minutes)
   __ "Prival" modification *(i.e. azo-reduction method)*
   __ spot test
   __ other

2. **Protocol**: A standard plate test was conducted by adding 0.1 mL of a bacterial tester strain culture, 0.1 mL of the desired concentration of test material or solvent and 0.5 mL of either S9-mix or sodium phosphate buffer (pH 7.4) to a small test tube followed by the addition of 2.0 mL of molten selective top agar containing 0.5 mM histidine (this value was not provided in the study but is the value reported in other *Salmonella*/microsome studies from the same testing laboratory). The contents were mixed and then poured onto minimal agar plates. After the top agar had solidified, the plates were incubated at 37°C for 48 hours and held in the cold, if necessary, until the revertant count was made. Revertant colonies were counted using an automatic colony counter. Cytotoxicity was evidenced by a thinning of the background lawn of bacteria, by a reduction in the background number of revertants per plate and by a determination of the bacterial titer.

3. **Statistical analysis**: No statistical analysis was performed.

4. **Evaluation criteria**: Criteria for an acceptable assay were solvent and positive control values within the ranges seen in published literature and the laboratory's historical ranges. Also, bacterial titer determinations had to demonstrate sufficient bacterial density for the assay. Criteria for a positive response were a reproducible, dose-related increase in the number of revertants per plate in test material treated bacteria over the concurrent solvent control value in at least one strain. A two-fold or greater increase was required for TA98, TA100 and TA1535 and a three-fold or greater increase for TA1537.

II. **REPORTED RESULTS:** Hallcomid M-8-10 concentrations of 0.05 and 100 mg/mL in ethanol were analyzed (procedure not provided) and found to be 95.7% and 101.0% of the nominal values, respectively, after 0 hours storage and 99.6% and 95.0% of the nominal values, respectively, after 24 hours of storage at room temperature.

A. PRELIMINARY CYTOTOXICITY ASSAY: No preliminary cytotoxicity assay was conducted but the first mutation assay served to select doses for the subsequent assays.

B. MUTAGENICITY ASSAY: Five concentrations of Hallcomid M-8-10 ranging from 8 to 5000 µg/plate were tested with and without S9-mix in the first assay. All plating was in quadruplicate. Excess cytotoxicity was seen at 1000 and 5000 µg/plate; therefore, the dose range for the second and third assays was 25 to 800 µg/plate. No increase in the number of revertants per plate was seen at any test material concentration in any bacterial strain with or without S9-mix in any assay. The concentrations of S9-fraction used were 30%, 10% and 4% in the first, second and third assay, respectively. The solvent and positive control values were appropriate for the respective strains and within the historical control ranges. Results of the first, second and third mutation assays are summarized in Tables 1, 2 and 3, respectively.

TABLE 1. Summary of the First Mutation Assay with Hallcomid M-8-10

Treatment / Dose (µg/plate)	Number of revertant colonies per plate (mean ± standard deviation)			
	TA1535	TA100	TA1537	TA98
Without S9-mix				
Ethanol	11 ± 4	57 ± 8	6 ± 1	22 ± 3
Hallcomid M-8-10				
8	7 ± 4	37 ± 5	7 ± 2	22 ± 4
40	9 ± 3	44 ± 3	8 ± 2	25 ± 2
200	7 ± 3	50 ± 8	6 ± 1	28 ± 4
1000	4 ± 2 B	14 ± 11 B	t ± 2 B	6 ± 4 B
5000	0 ± 0 B	0 ± 0	0 ± 0	0 ± 0
Positive control (µg/plate)	NaN_3 (10)	NF (0.2)	4-NPDA (10)	4-NPDA (0.5)
	422 ± 60*	325 ± t4*	63 ± 19*	106 ± 31*
	TA1535	TA100	TA1537	TA98
With 30% S9-mix				
Ethanol	24 ± 8	80 ± 12	1t ± 4	36 ± 7
Hallcomid M-8-10				
8	19 ± 6	78 ± t2	9 ± 3	42 ± 11
40	21 ± 6	81 ± 14	t4 ± 5	35 ± 2
200	19 ± 4[1]	66 ± t4[1]	12 ± 2	33 ± 6[1]
1000	16 ± 3[1] B	49 ± 8[1] B	8 ± 3[1] B	34 ± 13[1] B
5000	0 ± 0[1] B	6 ± 5[1] B	3 ± 1[1] B	0 ± 1[1] B
	2-AA (3)	2-AA (3)	2-AA (3)	2-AA (3)

Positive control (µg/plate)	219 ± 33*	355 ± 32*	70 ± 9*	539 ± 35*

Data taken from Tables 1 - 4, pages 40 - 43 , MRID 45369728 for TA t535, TA100, TA1537 and TA98, respectively.
All plating in quadruplicate
[1] Bacteriotoxic (titer determinations made in the presence of S9-mix only)
B Background lawn reduced
* Met the criteria for a positive response
NaN_3 = Sodium Azide
NF = Nitrofurantoin
4-NPDA = 4-Nitro-1,2-phenylenediamine
2-AA = 2-Aminoanthracene

TABLE 2. Summary of the Second Mutation Assay with Hallcomid M-8-10				
Treatment / Dose (µg/plate)	Number of revertant colonies per plate (mean ± standard deviation)			
	TA1535	TA100	TA1537	TA98
Without S9-mix				
Ethanol	17 ± 3	81 ± 11	10 ± 3	23 ± 8
Hallcomid M-8-10				
25	16 ± 1	76 ± 7	8 ± 2	24 ± 4
50	11 ± 3	81 ± 13	11 ± 1	20 ± 4
100	12 ± 5	83 ± 11	13 ± 3	26 ± 3
200	7 ± 3	94 ± 5	7 ± 2	20 ± 5
400	7 ± 2 B	86 ± 21 B	8 ± 3 B	11 ± 4 B
800	2 ± 2 B	48 ± 4 B	5 ± 1 B	3 ± 4 B
Positive control (µg/plate)	NaN_3 (10)	NF (0.2)	4-NPDA (10)	4-NPDA (0.5)
	299 ± 38*	347 ± 26*	79 ± 18*	91 ± 28*
	TA1535	TA100	TA1537	TA98
With 10% S9-mix				
Ethanol	13 ± 1	101 ± 5	12 ± 4	32 ± 8
E 6876				
25	14 ± 4	100 ± 14	13 ± 5	27 ± 4
50	11 ± 2	84 ± 9	14 ± 2	34 ± 5
100	14 ± 3	85 ± 10	13 ± 3	35 ± 4
200	9 ± 4[1]	83 ± 10	11 ± 3	28 ± 9[1]
400	9 ± 1[1] B	83 ± 10[1] B	7 ± 2[1] B	20 ± 9[1] B
800	6 ± 2[1] B	63 ± 5[1] B	6 ± 2[1] B	7 ± 4[1] B
Positive control (µg/plate)	2-AA (3)	2-AA (3)	2-AA (3)	2-AA (3)
	275 ± 17*	845 ± 60*	443 ± 24*	916 ± 55*

Data taken from Tables 5 - 8, pages 44 - 47 , MRID 45369728 for TA1535, TA100, TA1537 and TA98, respectively.
All plating in quadruplicate
[1] Bacteriotoxic (titer determinations made in the presence of S9-mix only)
B Background lawn reduced
* Met the criteria for a positive response
NaN_3 = Sodium Azide
NF = Nitrofurantoin

4-NPDA = 4-Nitro-1,2-phenylenediamine
2-AA = 2-Aminoanthracene

TABLE 3. Summary of the Third Mutation Assay with Hallcomid M-8-10

Treatment / Dose (µg/plate)	Number of revertant colonies per plate (mean ± standard deviation)			
	TA1535	TA100	TA1537	TA98
With 4% S9-mix				
Ethanol	12 ± 5	83 ± 11	13 ± 5	47 ± 5
E 6876				
25	12 ± 1	98 ± 10	16 ± 4	40 ± 6
50	13 ± 1	94 ± 4	13 ± 4	44 ± 5
100	12 ± 2	90 ± 7	13 ± 4	36 ± 10
200	8 ± 2[1]	88 ± 9[1]	12 ± 4[1]	39 ± 4
400	11 ± 2[1] B	64 ± 12[1] B	10 ± 1[1] B	21 ± 2[1] B
800	5 ± 2[1] B	55 ± 8[1] B	8 ± 2[1] B	14 ± 6[1] B
Positive control (µg/plate)	2-AA (3)	2-AA (3)	2-AA (3)	2-AA (3)
	404 ± 35*	836 ± 14*	403 ± 61*	716 ± 95*

Data taken from Tables 9 - 12, pages 48 - 51 , MRID 45369728 for TA1535, TA100, TA1537 and TA98, respectively.
All plating in quadruplicate
[1] Bacteriotoxic (titer determinations made in the presence of S9-mix only)
B Background lawn reduced
* Met the criteria for a positive response
2-AA = 2-Aminoanthracene

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that Hallcomid M-8-10 was not mutagenic with or without S9-mix as tested in this study.

B. REVIEWER COMMENTS: The reviewer agrees with the investigators' conclusion. Hallcomid M-8-10 was tested to upper concentrations limited cytotoxicity, acceptable experimental protocol was followed and the solvent and positive control values were appropriate for the respective strains. The test material did not increase the number of revertants per plate over the respective solvent control values at any tested concentration, with or without S9-mix. Four plates per dose were used and repeat assays were conducted, two without S9-mix and three with S9-mix. A different concentration of S9 was used in each of the assays with S9-mix although the 4% concentration is below the recommended range of 5% to 30%. There was no hint of a mutagenic effect seen in the

study. The EPA Guideline recommends that five tester strains be used, and only four were used in this study. These four *S. typhimurium* strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidizing mutagens, crosslinking agents and hydrazines. Such substances may be detected by *Escherichia coli* WP2 strains or *S. typhimurium* TA102 which have an AT base pair at the primary reversion site. As the test material is not an oxidizer, shows no structural similarity to any known crosslinking agent, and does not include a hydrazine group, it is concluded that inclusion of *Escherichia coli* WP2(uvrA) would not have changed the conclusions. This is an **Acceptable/Guideline** study.

C. **STUDY DEFICIENCIES:** No study deficiencies were identified.

GENETIC TOXICITY IN VITRO (GENE MUTATIONS - Ames Test)

TEST SUBSTANCE

- **Identity**: Hallcomid – 8-10

‡ **Remarks:**

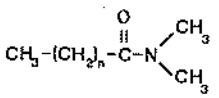
Description: Clear, yellowish liquid

Lot/Batch #: 002949

Purity: 98.08% a.i. (identified as 4.73% N,N-Dimethyl-hexanoic acid amide; 53.9% N,N-Dimethyl-octanoic acid amide; 38.9% N,N-Dimethyl-decanoic acid amide; 0.55% N,N-Dimethyl-dodecanoic acid amide; analyzed July 21, 1992

CAS # of TGAI:1118-92-9 (N,N-Dimethylcapramide) and 14433-76-2 (N,N-Dimethylcaprylamide)

Structure:



n = (4), 6, 8 or (10); ( ) = minor component

Solvent Used: Ethanol

METHOD

- **Method/guideline followed**: [OPPTS 870.5100 (§84-2)] OECD 471 (formerly OECD 471 & 472).
- **Test type**: *Salmonella*/microsome reverse gene mutation assay (Ames Test)
- **System of testing**: bacterial
- **GLP (Y/N)**: Yes
- **Year (study performed)**: 1992
- **Species/strain/cell type used**: TA98, TA100, TA1535 and TA1537
- **Metabolic activation**:
  - Male Sprague-Dawley rat liver
  - Quantity: 4%, 10% and 30% S9-fraction
  - Induced or not induced: Aroclor 1254 induced
- **Dose/concentration levels:**

  0, 8, 40, 200, 1000 and 5000 µg/plate without S9-mix;
  0, 8, 40, 200, 1000 and 5000 µg/plate with 30% S9;
  0, 25, 50, 100, 200, 400 and 800 µg/plate without S9-mix;
  0, 25, 50, 100, 200, 400 and 800 µg/plate with 10% S9;
  0, 25, 50, 100, 200, 400 and 800 µg/plate with 4% S9
- **Statistical methods**: None used.

‡ **Remarks:**

– **Test protocol:** A standard plate assay was conducted using quadruplicate plating. Two independent assays were conducted in the absence of S9-mix and three in the presence of S9-mix. Criteria for a positive response were a reproducible, dose-related increase in the number of revertants per plate over the corresponding solvent control value, reaching at least a doubling of the control rate with TA98, TA100 and TA1535 and at least a three-fold increase with TA1537. Four strains were tested in this study rather than the guideline recommendation of five strains; however, quadruplicate plating was used and at least two independent assays were conducted. The addition of a fifth strain would not have changed the conclusions. The 4% concentration of S9 used in the third assay is below the guideline recommended range of 5% to 30% but the positive control values were appropriate, indicating that the activation system was effective. Positive controls without S9-mix were Sodium azide at 10 µg/plate for TA1535, Nitrofurantoin at 0.2 µg/plate for TA100, 4-Nitro-1,2-phenylenediamine at 10 µg/plate for TA1537 and 4-Nitro-1,2-phenylenediamine at 0.5 µg/plate for TA98. The positive control with S9-mix was 2-Aminoanthracene at 3 µg/plate for all strains.

RESULTS

‡ **Result:** Negative. The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, in any strain, with or without S9-mix.

- **Cytotoxic concentrations**
  – With and without metabolic activation - 400 µg/plate and above in all strains and additionally at 200 µg/plate in TA98 and TA1535
- **Genotoxic effects:** None

Representative control results from Assay 2. (mean revertants/plate)

Without activation:	Solvent	Positive
TA1535	17 ± 3	299 ± 38
TA100	81 ± 11	347 ± 26
TA1537	10 ± 3	79 ± 18
TA98	23 ± 8	91 ± 28

With activation:	Solvent	Positive
TA1535	13 ± 1	275 ± 17
TA100	101 ± 5	845 ± 60
TA1537	12 ± 4	443 ± 24
TA98	32 ± 8	916 ± 55

CONCLUSIONS

‡ **Remarks:**

This is an Acceptable/Guideline study showing clear negative results.

DATA QUALITY

- **Reliability**: 1 - Reliable without restrictions

REFERENCES

Herbold, B.A.. (1992) Hallcomid M-8-10 *Salmonella*/microsome test. Bayer AG, Fachbereich Toxicology, Friedrich-Ebert-Straße 217-333, D-5600 Wuppertal 1, F.R.G. Laboratory Report No.: 21849, Study No.: T 3039100, November 17, 1992. MRID 45369728. Unpublished.

DATA EVALUATION RECORD

HALLCOMID M-8-10

STUDY TYPE: *IN VITRO* MAMMALIAN CELL GENE MUTATION TEST [OPPTS 870.5300 (§84-2)] OECD 476

MRID 45369729

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. RD-2003-4

Primary Reviewer:
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Date: _____

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Cheryl B. Bast, Ph.D., D.A.B.T.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

Template version 11/01

STUDY TYPE: In Vitro Mammalian Cells in Culture Gene Mutation assay in Chinese hamster V79 cells; OPPTS 870.5300 [§84-2]; OECD 476.

PC CODE: N/A

DP BARCODE: N/A
SUBMISSION NO.: N/A

TEST MATERIAL (PURITY): Hallcomid M-8-10 (98.26%, 98.08%, 98.17% a.i. as determined on January 24, 1992, July 21, 1992 and January 8, 1993, respectively. Mixture of N,N-Dimethyldecaneacidamide (39.6%), N,N-Dimethyloctaneacidamide (53.4%), N,N-Dimethylhexaneacidamide (4.59%) and N,N-Dimethyldodecaneacidamide (0.58%))

SYNONYMS: Mixture of Capronacid, Caprylacid, Caprinacid and Laurinacid

CITATION: Brendler-Schwaab, S. (1994) Mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay in vitro. Bayer AG, Fachbereich Toxicology, Friedrich-Ebert-Straße 217-333, D-5600 Wuppertal 1, F.R.G.. Laboratory Report No. 23536, Study No. T 0039125, December 9, 1994. MRID 45369729. Unpublished

SPONSOR: Bayer AG. (Submitted by The C.P. Hall Company, 311 South Wacker Drive, Suite 4700, Chicago, Illinois, 60606)

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the HGPRT locus (MRID 45369729), Chinese hamster V79 cells cultured in vitro were exposed for five hours to Hallcomid M-8-10, (98.26%, 98.08%, 98.17% a.i. depending on analysis date, batch # 002949) in ethanol at concentrations of 0, 25, 50, 100, 125, 150, 200 or 250 μg/mL in the presence and absence of mammalian metabolic activation (S9-mix). Two independent assays were conducted and duplicate cultures were used at each test material concentration. The S9-fraction was obtained from Aroclor 1254 induced male Wistar rat liver.

Hallcomid M-8-10 was tested up to cytotoxic concentrations. In the absence of S9-mix, a dose-dependent decrease in survival relative to the solvent control values was seen in both assays. Relative survivals at 150 μg/mL were 42.4% and 72.9% in the first and second assays, respectively. Higher concentrations were excessively cytotoxic. Cytotoxicity was reduced in the presence of S9-mix with relative survivals exceeding 100% at all test material concentrations up to 200 μg/mL in the first assay. Essentially no viable cells were seen at 250 μg/mL. A dose-dependent reduction in relative survival was seen in the second assay with values of 69.2% at 200 μg/mL and 17.6% at 250 μg/mL. No biologically or statistically significant increase in the mutant frequency was seen at any test material concentration with or without S9-mix in either assay. The solvent control values were within the testing laboratory's historical solvent control ranges and positive controls (Ethyl methanesulfonate in the absence of S9-mix and Dimethylbenzanthracene with S9-mix) induced clear and statistically significant increases in the mutant frequencies. There was no evidence of induced mutant colonies over background in Hallcomid M-8-10 treated cells.

This study is classified as Acceptable/Guideline and satisfies the requirement for Test Guideline OPPTS 870.5300, OECD 476 for in vitro mutagenicity (mammalian forward gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:

Hallcomid M-8-10

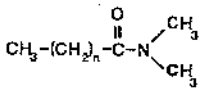
Description: Clear, light-yellow liquid

Lot/Batch #: 002949

Purity: 98.26%, 98.08%, 98.17% a.i. as determined on January 24, 1992, July 21, 1992 and January 8, 1993, respectively

CAS # of TGAI: 1118-92-9 (N,N-Dimethyloctanamide or N,N-Dimethyloctaneacidamide) and 14433-76-2 (N,N-dimethyldecanamide or N,N-Dimethyldecaneacidamide)

Structure:



n = (4), 6, 8, (10); ( ) = minor component

Major components: N,N-Dimethyldecaneacidamide (39.6%); N,N-Dimethyloctaneacidamide (53.4%); Minor components: N,N-Dimethylhexaneacidamide (4.59%); N,N-Dimethyldodecaneacidamide (0.58%).

Solvent Used: Ethanol

2. Control materials:

Negative control: Culture medium

Solvent control (final conc.): Ethanol / 1%

Positive control: (concentrations / solvent) Nonactivation: Ethyl methanesulfonate / 900 µg/mL / unspecified

Activation: Dimethylbenzanthracene / 20 µg/mL / DMSO

3. Activation: S9 derived from male Wistar rats:

x	Induced	x	Aroclor 1254	x	Rat	x	Liver
	Non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

Describe S9 mix composition: The S9-fraction was purchased from Cytotest Cell Research, FRG
8 mM $MgCl_2 \cdot 6H_2O$
33 mM KCl
5 mM Glucose-6-phosphate
t mM NADP
40% S9-fraction

4. Test cells: mammalian cells in culture:

	Mouse lymphoma L5178Y cells	x	V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells		list any others

Media: Cells were cultured in hypoxanthine-free Eagle's Minimal Essential Medium (MEM) supplemented with nonessential amino acids, L-glutamine (2 mM), MEM-vitamins, $NaHCO_3$, penicillin (50 units/mL), streptomycin (50 µg/mL) and heat-inactivated fetal calf serum (FCS) at 10%. The FCS concentration was reduced to 2% during treatment. Selection medium was culture medium containing 10 µg/mL of 6-thioguanine (6-TG).

Properly maintained?	x Yes	No
Periodically checked for Mycoplasma contamination?	x Yes	No
Periodically checked for karyotype stability?	x Yes	No
Periodically "cleansed" against high spontaneous background?	x Yes	No

5. Locus examined:

Thymidine kinase (TK) HGPRT Na^+/K^+ ATPase

Selection agent:		Bromodeoxyuridine (BrdU)		8-Azaguanine (8-AG)		Ouabain
		Fluorodeoxyuridine (FdU)	x	6-Thioguanine (6-TG) (10 µg/mL)		
		Trifluorothymidine (TFT)				

HGPRT = Hypoxanthine-guanine-phosphoribosyl transferase

6. Test compound concentrations used:

Preliminary cytotoxicity test:
Nonactivated conditions: 7.9, 15.7, 31.3, 62.5, 125.0, 250.0, 500.0, 750.0, 1000.0 µg/mL
Activated conditions: 7.9, 15.7, 31.3, 62.5, 125.0, 250.0, 500.0, 750.0, 1000.0 µg/mL
Mutation assay:
Nonactivated conditions: 25, 50, 100, 125, 150, 200, 250 µg/mL
Activated conditions: 25, 50, 100, 125, 150, 200, 250 µg/mL

B. TEST PERFORMANCE:

1. Cell treatment:

a. Cells were exposed to test compound, negative/solvent or positive controls for 5 hours (nonactivated) 5 hours (activated).

b. After washing, cells were cultured for 6 days (expression period) before cell selection.

c. After expression, 3×10^5 cells/dish (8 dishes/ group) were cultured for 7 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 7 days without selective agent to determine cloning efficiency.

2. **Statistical methods:** Mutation frequencies were subjected to a weighted analysis of variance and to a weighted recursive regression. Poisson derived weights were used in both cases. Pairwise comparisons of the treated groups to the solvent control were made using the Dunnett test at $p < 0.05$. The statistical approach is acceptable.

3. **Evaluation criteria**: An acceptable assay required an average cloning efficiency of the negative and solvent controls of at least 50%, a relative survival at the highest test material dose of 0% to 30% unless the highest dose was limited by solubility and an average mutant frequency of the solvent control not greater than 25×10^{-6}. A mutant frequency is considered acceptable only if the absolute cloning efficiency is 10% or higher and the frequency was determined from at least five dishes. The positive control must induce an average mutant frequency at least three times that of the concurrent solvent control.

   Criteria for a positive response were a reproducible, dose-dependent statistically significant increase in mutant frequency in test material treated cells. The mutant frequency should be at least two to three times that of the concurrent negative or solvent control and, preferably the dose response should be seen over at least three doses. A reproducibility increase in mutant frequency greater than two times the respective solvent control value seen at a single dose near the highest tested concentration is also considered a positive response. Results are considered equivocal if a reproducible, statistically significant increase in mutant frequency is seen at one or more doses in the absence of dose-dependency.

II. **REPORTED RESULTS**: The test material was shown to be stable in the vehicle for at least 24 hours at room temperature. Hallcomid M-8-10 concentrations determined analytically (procedure not specified) after 0 and 24 hours of storage at room temperature were 95.7% and 99.6%, respectively, of a nominal 0.05mg/mL concentration and 101.0% and 95.0%, respectively, of a nominal 100 mg/mL concentration.

A. **PRELIMINARY CYTOTOXICITY ASSAY:** Nine concentrations of Hallcomid M-8-10 ranging from 7.9 to 1000 μg/mL were tested with and without S9-mix in the preliminary cytotoxicity test. The upper dose in the cytotoxicity test was limited by solubility as the test material precipitated in culture medium at 1500 μg/mL. The test material reacts with plastic at high concentrations, therefore, test material solutions were prepared in glass tubes. Hallcomid M-8-10 was not cytotoxic at up to 125 μg/mL with or without S9-mix but no viable cells were present at the next highest concentration, 250 μg/mL. An upper concen-tration of 250 μg/mL was selected for the mutation assays both with and without S9-mix.

B. **MUTAGENICITY ASSAY:** Seven concentrations of Hallcomid M-8-10 of from 25 to 250 μg/mL were tested with and without S9-mix in two independent assays. Duplicate cultures were used at

each concentration. In the absence of S9-mix, a dose-dependent decrease in survival relative to the solvent control values was seen in both assays. Relative survivals at 150 μg/mL were 42.4% and 72.9% in the first and second assays, respectively. Higher concentrations were excessively cytotoxic. No statistically significant increase in mutant frequency was seen at any test material concentration without S9-mix in either assay. An increase in mutation frequency seen in one culture at 100 μg/mL in the second assay was not reproducible and no dose-response was seen; thus the increase was not considered biologically significant. The solvent and positive control values without S9-mix were appropriate. Results of the first and second assays without S9-mix are summarized in Tables 1 and 2, respectively.

In the first assay in the presence of S9-mix, the relative survival of Hallcomid M-8-10 treated cells exceeded 100% at all concentrations up to 200 μg/mL but essentially no viable cells were seen at 250 μg/mL. A dose-dependent reduction in relative survival was seen in the second assay with values of 69.2% at 200 μg/mL and 17.6% at 250 μg/mL. No biologically or statistically significant increase in mutant frequency was seen at any test material dose with S9-mix in either assay. The solvent and positive control values were appropriate. Results of the first and second assays with S9-mix are summarized in Tables 3 and 4.

TABLE 1. First Mutation Assay without S9-mix

Treatment (μg/mL)	Survival to treatment (% vehicle control)	Total mutant colonies (total of 8 dishes)	Cloning efficiency (CE(%))	Mutant frequency (per 10^6 clonable cells)
Untreated	t07.8	3	56.0 ± 8.8	2.2
Untreated	–	8	68.5 ± 15.6	4.9
Ethanol (t%)	t00.0	9	86.5 ± 5.8	4.3
Ethanol (t%)	–	5	64.3 ± 15.6	3.2
Hallcomid				
25	94.0	7	51.7 ± 4.7	5.6
25	–	4	54.3 ± 4.2	3.t
50	83.5	t0	66.7 ± 5.6	6.2
50	–	4	63.8 ± 11.5	2.6
t00	88.8	2	49.3 ± 2.5	1.7
t00	–	0	60.7 ± 8.6	0.0
125	62.4	6	75.3 ± 17.2	3.3
125	–	2	82.2 ± 8.9	t.0
t50	42.4	5	77.3 ± 6.0	2.7

150	–	7	122.0 ± 7.4	2.4
200	0.8	N	N	–
200	–	N	N	–
250	N	N	N	–
250	–	N	N	–
EMS (900)	78.9	744	52.7 ± 4.7	588.2*
EMS (900)	80.0	930	44.0 ± 2.5	880.7*

Data summarized from MRID 45369729, Table 3, page 33
N = Not cloned due to cytotoxicity; * = Statistically significant at $p < 0.05$ using the Dunnett test.
EMS = Ethyl methanesulfonate

TABLE 2. Second Mutation Assay without S9-mix

Treatment (µg/mL)	Survival to treatment (% vehicle control)	Total mutant colonies (total of 8 dishes)	Cloning efficiency (CE(%))	Mutant frequency (per 10^6 clonable cells)
Untreated	112.8	1	71.5 ± 10.6	0.6
Untreated	–	2	88.0 ± 4.3	0.9
Ethanol (1%)	100.0	6	96.3 ± 3.2	3.0
Ethanol (1%)	–	5	71.3 ± 26.1	2.9
Hallcomid				
25	139.6	0	81.8 ± 10.2	0.0
25	–	3	67.2 ± 7.7	1.9
50	101.1	9	60.2 ± 15.7	6.2
50	–	1	97.3 ± 12.6	0.5
100	125.1	11	49.0 ± 6.1	9.4
100	–	5	101.3 ± 11.3	2.1
125	90.9	3	53.8 ± 2.5	2.3
125	–	4	86.2 ± 6.0	1.9
150	72.9	12	66.0 ± 6.8	7.6
150	–	5	71.3 ± 7.4	2.9
200	0.0	N	N	–
200	–	N	N	–
250	N	N	N	–

250	–	N	N	–
EMS (900)	60.4	881	66.5 ± 8.9	552.0*
EMS (900)	–	879	64.3 ± 7.6	569.6*

Data summarized from MRID 45369729, Table 4, page 34
N = Not cloned due to cytotoxicity
* = Statistically significant at $p < 0.05$ using the Dunnett test.
EMS = Ethyl methanesulfonate

TABLE 3. First Mutation Assay with S9-mix

Treatment (µg/mL)	Survival to treatment (% vehicle control)	Total mutant colonies (total of 8 dishes)	Cloning efficiency (CE(%))	Mutant frequency (per 10^6 clonable cells)
Untreated	103.9	8	77.0 ± 5.2	4.3
Untreated	–	7	73.7 ± 3.5	4.0
Ethanol (1%)	100.0	6	66.3 ± 4.5	3.8
Ethanol (1%)	–	6	67.5 ± 2.6	3.7
Hallcomid				
25	100.0	2	80.3 ± 3.9	1.0
25	–	0	73.0 ± 6.8	0.0
50	109.5	8	66.8 ± 4.1	5.0
50	–	1	112.7 ± 10.4	0.4
100	138.7	6	65.3 ± 1.8	3.8
100	–	5	90.7 ± 3.1	2.3
125	120.9	1	49.0 ± 7.1	0.9
125	–	6	93.2 ± 18.0	2.7
150	102.3	2	77.3 ± 11.0	1.1
150	–	11	89.7 ± 8.3	5.1
200	120.9	1	83.0 ± 26.4	0.5
200	–	1	97.3 ± 6.7	0.4
250	N	N	N	–
250	–	N	N	–
DMBA (20)	101.8	237	78.8 ± 5.3	125.3*

DMBA (20)	--	196	89.8 ± 11.5	90.9*

Data summarized from MRID 45369729, Table 5, page 35
N = Not cloned due to cytotoxicity
* = Statistically significant at p < 0.05 using the Dunnett test.
DMBA = Dimethylbenzanthracene

TABLE 4. Second Mutation Assay with S9-mix

Treatment (µg/mL)	Survival to treatment (% vehicle control)	Total mutant colonies (total of 8 dishes)	Cloning efficiency (CE(%))	Mutant frequency (per 10^6 clonable cells)
Untreated	115.6	4	69.0 ± 5.9	2.4
Untreated	--	4	68.3 ± 5.0	2.4
Ethanol (1%)	100.0	5	71.8 ± 4.3	2.9
Ethanol (1%)	--	2	68.5 ± 12.7	1.2
Hallcomid				
25	130.0	8	111.0 ± 9.0	3.0
25	--	1	80.0 ± 3.3	0.5
50	96.4	10	100.3 ± 8.3	4.2
50	--	4	88.3 ± 15.6	1.9
100	105.6	4	82.7 ± 6.2	2.0
100	--	5	79.2 ± 17.2	2.6
125	131.6	4	96.7 ± 9.8	1.7
125	--	2	90.3 ± 2.6	0.9
150	88.4	9	82.3 ± 4.9	4.6
150	--	2	70.3 ± 25.0	1.2
200	69.2	5	63.3 ± 9.0	3.3
200	--	12	75.5 ± 6.1	6.6
250	17.6	0	71.5 ± 18.1	0.0
250	--	0	75.7 ± 14.9	0.0
DMBA (20)	129.2	100	88.2 ± 13.5	47.2*
DMBA (20)	--	107	77.8 ± 4.3	57.3*

Data summarized from MRID 45369729, Table 6, page 36
N = Not cloned due to cytotoxicity
* = Statistically significant at p < 0.05 using the Dunnett test.
DMBA = Dimethylbenzanthracene

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that Hallcomid M-8-10 was not mutagenic at the HGPRT locus of Chinese hamster V79 cells as tested in this study.

B. REVIEWER COMMENTS: The reviewer agrees with the investigators' conclusion. Hallcomid M-8-10 was tested to a sufficiently high concentration, proper experimental protocol was followed and the solvent and positive control values were appropriate. Although the investigators did not present data on pH and osmolality of the treatment medium, their protocol does call for determination of the osmolality of the tested concentration range in culture medium if a positive result is seen in the mutation assay. There was no evidence of a biologically and statistically significant increase in mutant frequency seen in this study. This is an Acceptable/Guideline study.

C. STUDY DEFICIENCIES: No study deficiencies were identified.

GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

TEST SUBSTANCE

- Identity: Halicomid – 8-10

‡ Remarks:

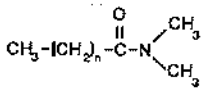
Description: Clear, light-yellow liquid

Lot/Batch #: 002949

Purity: 98.26%, 98.08%, 98.17% a.i. as determined on January 24, 1992, July 21, 1992 and January 8, 1993, respectively

CAS # of TGAI: 1118-92-9 and 14433-76-2

Structure:



n = 6 or 8 (major constituents); 4 or 10 (minor constituents)

Solvent Used: Ethanol

METHOD

- Method/guideline followed: [OPPTS 870.5300 (§84-2)] OECD 476.
- Test type: *In vitro* mammalian cell gene mutation test (HGPRT locus)
- System of testing: mammalian cell culture
- GLP (Y/N): Yes
- Year (study performed): 1993 (report is dated 1994)
- species/strain/cell type used: Chinese hamster lung V79 cells
- Metabolic activation:
  - Male Wistar rat liver
  - Quantity: 5% S9-mix
  - Induced or not induced: Aroclor 1254 induced
- Dose/concentrations levles:

  Nonactivated conditions: 0, 25, 50, 100, 125, 150, 200 or 250 µg/mL

  Activated conditions: 0, 25, 50, 100, 125, 150, 200 or 250 µg/mL
- Statistical methods: Mutation frequencies were subjected to a weighted analysis of variance and to a weighted recursive regression. Poisson derived weights were used in both cases. Pairwise comparisons of the treated groups to the solvent control were made using the Dunnett test at $p < 0.05$. The statistical approach is acceptable.

‡ Results:

– Test protocol: V79 cells were cultured in hypoxanthine-free Eagle's Minimal Essential Medium (MEM) supplemented with nonessential amino acids, L-glutamine (2 mM), MEM-vitamins, $NaHCO_3$, penicillin (50 units/mL), streptomycin (50 µg/mL) and heat-inactivated fetal calf serum (FCS) at 10%. The FCS concentration was reduced to 2% during treatment. Selection medium was culture medium containing 10 µg/mL of 6-thioguanine (6-TG). Cells were treated for five hours with the desired concentration of test material or control. Duplicate cultures were used at each dose. All incubation was at 37°C in a humidified atmosphere with 5% CO_2. Cells were cultured for a six-day expression period at which

time 3 x 10^5 cells/dish (8 dishes/ group) were cultured for 7 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 7 days without selective agent to determine cloning efficiency.

Positive controls:

Without activation: Ethyl methanesulfonate (900 µg/mL)

With activation: Dimethylbenzanthracene (20 µg/mL)

Solvent: Ethanol

Criteria for evaluating results: An acceptable assay required an average cloning efficiency of the negative and solvent controls of at least 50%, a relative survival at the highest test material dose of 0% to 30% unless the highest dose was limited by solubility and an average mutant frequency of the solvent control not greater than 25 x 10^{-6}. A mutant frequency is considered acceptable only if the absolute cloning efficiency is 10% or higher and the frequency was determined from at least five dishes. The positive control must induce an average mutant frequency at least three times that of the concurrent solvent control. Criteria for a positive response were a reproducible, dose-dependent statistically significant increase in mutant frequency in test material treated cells. The mutant frequency should be at least two to three times that of the concurrent negative or solvent control and, preferably the dose response should be seen over at least three doses. A reproducibility increase in mutant frequency greater than two times the respective solvent control value seen at a single dose near the highest tested concentration is also considered a positive response. Results are considered equivocal if a reproducible, statistically significant increase in mutant frequency is seen at one or more doses in the absence of dose-dependency.

RESULTS

- Result: Negative
- Cytotoxic concentration:
  - With metabolic activation: In one assay there was essentially no reduction in relative survival up to 200 µg/mL and virtually no survival at 250 µg/mL. In the second assay there was a dose-related decrease in relative survival to 17.6% at 250 µg/mL.
  - Without metabolic activation: Dose-related decrease in relative survival, virtually no survival at 200 µg/mL and higher.
- Genotoxic effects:
  - With metabolic activation: None
  - Without metabolic activation: None

  Statistical results, as appropriate: No statistically significant increases in mutant frequency at any test material concentration.
- Remarks field for results: An increase in mutant frequency from the concurrent solvent control value of approximately 3.0 x 10^{-6} to 9.4 x 10^{-6} was seen in one of the duplicate cultures at 100 µg/mL in the second assay but not in the second culture in this assay or in either culture in the first assay. This increase was not considered biologically significant.

CONCLUSIONS

‡ Remarks:

Halicomid M-8-10 was not mutagenic as tested in this study.

DATA QUALITY

- Reliability = 1, reliable without restriction

REFERENCES

Brendler-Schwaab, S. (1994) Mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay *in vitro*. Bayer AG, Fachbereich Toxicology, Friedrich-Ebert-Straße 217-333, D-5600 Wuppertal 1, F.R.G.. Laboratory Report No. 23536, Study No. T 0039125, December 9, 1994. MRID 45369729. Unpublished

DATA EVALUATION RECORD

HALLCOMID M-8-10

STUDY TYPE: *IN VITRO* MAMMALIAN CYTOGENETICS [OPPTS 870.5375(§84-2)]
MRID 45369730

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. RD-2003-4

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Robert H. Ross, M.S., Group Leader

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Quality Assurance:
Susan Chang, M.S.

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Mammalian Cytogenetics Assay in Chinese hamster CHO cells; OPPTS 870.5375 (§84-2): OECD 473

PC CODE: N/A

DP BARCODE: N/A
SUBMISSION NO.: N/A

TEST MATERIAL (PURITY): Hallcomid M-8-10 (98.08% a.i. identified as 53.9% N,N-Dimethylcaprylamide;38.9% N,N-Dimethylcapramide; 4.73% N,N-Dimethylcaproamide; 0.55% N,N-Dimethyllauramide); analyzed July 2t, 1992))

SYNONYMS: Mixture of: 4.73% N,N-Dimethyl-hexanoic acid amide; 53.9% N,N-Dimethyl-octanoic acid amide; 38.9% N,N-Dimethyl-decanoic acid amide; 0.55% N,N-Dimethyl-dodecanoic acid amide

CITATION: Gahlmann, R. (1995) *In vitro* mammalian chromosome aberration test with Chinese hamster ovary (CHO) cells. Bayer AG, Fachbereich Toxicology, Friedrich-Ebert-Straße 2t7-333, D-42096 Wuppertal, F.R.G.. Laboratory Report No.: 24450, Study No.: T 70391 t3, November 7, t995 [Note: study termination date is given as January 8, t993]. MRID 45369730. Unpublished.

SPONSOR: Bayer AG. (Submitted by The C.P. Hall Company, 3t1 South Wacker Drive, Suite 4700, Chicago, Illinois, 60606)

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics (chromosome aberrations) assay (MRID 45369730), Chinese hamster ovary CHO cell cultures were exposed to Hallcomid —8-10 (98.08% a.i. identified as 4.73% N,N-Dimethyl-hexanoic acid amide; 53.9% N,N-Dimethyl-octanoic acid amide; 38.9% N,N-Dimethyl-decanoic acid amide; 0.55% N,N-Dimethyl-dodecanoic acid amide; analyzed July 21, t992), Batch # 002949) at concentrations of 0, t0, 40 or 160 μg/mL for 4 hours without metabolic activation (S9-mix) and to concentrations of 0, 7.2, 36 or t80 μg/mL with S9-mix. The vehicle was ethanol. The S9-fraction was obtained from Aroclor t254 induced male F344 rat liver. Cells were harvested 20 hours post-exposure at all concentration with and without S9-mix and additionally at 4 hours and 26 hours post-exposure at 160 μg/mL without S9-mix and at t80 μg/mL with S9-mix. Cells were evaluated for structural aberrations.

Hallcomid M-8-t0 was tested up to cytotoxic concentrations. Upper concentrations which caused about a 50% reduction in the relative mitotic index were chosen based on the results of a preliminary cytotoxicity test. There were no statistically or biologically significant increases in the percentage of cells with structural aberrations, including or excluding gaps, at any test material concentration or harvest time without S9-mix. A statistically significant increase in the percentage of aberrant cells was seen at the four-hour post-exposure sampling time in cells exposed at 180 μg/mL with S9-mix but at no other dose or sampling time with S9-mix. The actual value of 3.5% seen at 180 μg/mL was within the historical solvent control range and not considered biologically

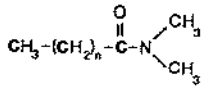
significant. The four-hour post-exposure harvest time is too short to evaluate chromosomal aberration induction; however, the 20-hour post-exposure sampling time meets the guideline recommendation of sampling at 1.5 x the average generation time of the cells used (14 hours for the CHO cells in this study). The solvent and positive control (Mitomycin C without activation and Cyclophosphamide (Endoxan) with activation) values were appropriate. There was no evidence of chromosome aberrations induced over background.

This study is classified as Acceptable/Guideline and satisfies the Test Guideline *in vitro* mammalian cytogenetics (chromosomal aberrations) OPPTS 870.5375; OECD 473.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

Test material:	llcomid M-8-10
scription:	ear, light-yellow liquid
t/Batch #:	2949
rity:	.08% a.i. identified as 53.9% N,N-Dimethylcaprylamide;38.9% N,N-Dimethylcapramide; 4.73% N,N-Dimethylcaproamide; 0.55% N,N-Dimethyllauramide) ; analyzed July 21, 1992)
AS # of TGAI:	18-92-9 (N,N-Dimethyloctanamide) and 14433-76-2 (N,N-Dimethyldecanamide)
ucture	
	=(4), 6, 8 or (10); ( ) = minor components
lvent Used:	anol; (DMSO was used in the preliminary cytotoxicity test)

Control materials:

gative control:	treated
lvent control (final conc.):	anol / 0.2 mL/culture
sitive control: (concentrations / solvent)	nactivation: Mitomycin C / 2 µg/mL / HBSS
	tivation: Cyclophosphamide / 10 µg/mL / HBSS

Activation: S9- fraction from Wistar rats was purchased from CCR, Roßdorf, Germany (lot # 091291, protein content 40.0 mg/mL)

	ced		lor 1254				r
	-induced		nobarbitol		se		g
			e		ster		er

			er:		er		

S9 mix composition: (per 1 mL)

S9-fraction	0.400 mL
$MgCl_2 \cdot 6\ H_2O$	1.626 mg
KCl	2.460 mg
Glucose-6-phosphate, disodium salt	1.520 mg
NADP, disodium salt	0.788 mg
Sodium phosphate buffer (pH 7.4)	0.600 mL

4. Test cells: mammalian cells in culture

9 cells (Chinese hamster lung fibroblasts)

man lymphocytes

inese hamster ovary (CHO -WB-1) cells (Received August 21, 1990 from Dr. Sheldon Wolff, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco.

inese hamster lung fibroblast

dia: Growth medium was composed of 500 mL of Ham's F12 medium, 2.5 mL of 200 mM L-glutamine, 5 mL of Penicillin (5000 I.U./mL)/Streptomycin (5000 µg/mL) solution and either 25 mL or 50 mL of fetal calf serum (5% or 10%final concentration).

oper ly m ai nt ai ne d?	s	or not stated
riod ic all y ch ec ke d fo r *M yc op la s m* a co nt a mi na tio n?	s	or not stated

riod ic all y ch ec ke d fo r ka ry ot yp e st ab ilit y?	s	or not stated

Test compound concentrations used:

/totoxicity assay: (mitotic index and survival index)

1st	nactivated and activated conditions:	, 50, 100, 250, 500, 750, 1000 µg/mL
2nd	nactivated and activated conditions:	0, 130, 160, 190, 220, 250 µg/mL

/togenetic assay:

nactivated conditions:	0 µg/mL (8-hour and 30-hour harvest times); , 40, 160 µg/mL (24-hour harvest time)
tivated conditions:	0 µg/mL (8-hour and 30-hour harvest times); :, 36, 180 µg/mL (24-hour harvest time)

B. TEST PERFORMANCE:

1. Preliminary cytotoxicity assay: CHO cells, which had been passaged on the day before treatment, were seeded at 1 x 10^6 cells in 20 mL of culture medium in 75 cm^2 flasks and incubated at 37°C in an atmosphere of 5% CO_2 in air. Duplicate cultures were used. For treatment with S9-mix, the growth medium was removed and replaced with 19 mL medium containing 5% FCS, 1 mL of S9-mix and 0.2 mL of the test material solution. For treatment without S9-mix, the growth medium was replaced with 20 mL of medium containing 10% FCS and 0.2 mL of test material solution. Cell were exposed to the test material for four hours at 37°C, the treatment medium then removed, the cells washed with pre-warmed (about 37°C) phosphate buffered saline (PBS), 20 mL of fresh medium containing 10% FCS were added to each flask and the cell then incubated at 37°C with 5% CO_2 until cell harvest.

   Cell survival following treatment was determined by detaching the cells from the flasks using trypsin, preparing a known dilution and counting the cells using a hemocytometer.

The mitotic index was determined from the number of cells in mitosis in a total of 1000 cells per culture.

2. Cytogenetic assay:

ell exposure time:	st material	olvent control	ositive control
n-activated:	ir	ir	ir
livated:	ir	ir	ir

indle inhibition:

ibition used/concentration:	lcemid / 0.4 µg/mL
ministration time:	ours (before cell harvest)

ell harvest time after termination of treatment:	st material	olvent control	ositive control
n-activated:	20, 26 hr	20, 26 hr	hr
tivated:	20, 26 hr	20, 26 hr	hr

d. Details of slide preparation: Following Colcemid treatment, the growth medium was removed and the cells dissociated with a trypsin solution. The cell suspension was centrifuged at 700 rpm for 5 minutes, the supernatant removed, 1 - 2 mL of a hypotonic solution (0.56% KCl) at 37°C was added and within 4 minutes the volume was increased to 6 mL with additional hypotonic solution and the cells then resuspended. The cells were recentrifuged, the supernatant removed and a few drops of a cold (4°C) ethanol/acetic acid solution (3:1) were added, the cells were mixed in the fixative and the volume brought to 6 mL with the same solution and remixed. Following a 20 - 30 minute holding period at room temperature, the cells were centrifuged, the supernatant discarded, the cell pellet resuspended in fixative and again centrifuged. The cell pellet was then resuspended in a small volume of fresh fixative. Aliquots of the cell suspension were dropped onto cool, clean slides and allowed to dry for at least two hours. The dried slides were stained in 5% Giemsa solution for about five minutes and rinsed twice in water. The slides were then dried and covered. An alternative procedure was described in which slides were submerged in pure methanol for three minutes, stained for 20 - 30 minutes in 5% Giemsa solution, rinsed twice in water and once in acetone and finally held in xylene for about 30 minutes. The slides were covered after drying. The investigators did not report which procedure was used in the present study. At least two slides were prepared from each culture and slides were coded prior to analysis.

e. Metaphase analysis

. of cells examined per dose: 200 (100 per culture) (1000 cells/culture for mitotic index determinations)

ored for structural?	s	
ored for numerical?	s	
ded prior to analysis?	s	

‡ Evaluation criteria: Cells containing 21 chromosomes (the modal number) were scored for structural aberrations (chromosome and chromatid breaks, chromosome and chromatid exchanges, gaps, deletions, fragments and multiple aberrations (≥ five aberrations/cell but excluding gaps)). Metaphases showing chromosome disintegration were also recorded but not included with the cells scored for other types of aberrations. A cell with one or more aberrations was classified as an aberrant cell. The frequency of aberrant metaphases was calculated both including and excluding gaps but gaps were not included in the statistical analysis. Criteria for a positive response were a dose-dependent, statistically significant increase in the frequency of aberrant metaphases in test material treated cells over the concurrent solvent control value, excluding gaps. The actual value must be outside the historical control range. A test was considered equivocal if there was a statistically significant but not dose-dependent increase in the frequency of aberrant metaphases or if there was a dose-dependent but not statistically significant increase. Appropriate positive and solvent control values were required for the assay to be considered acceptable.

g. Statistical analysis: Pair-wise comparisons of the number of metaphases with aberrations, including and excluding gaps, and of metaphases with exchanges among 200 cells in test material treated and solvent control treated cells were done using the Fischer exact test. Statistical significance was determined at $p < 0.05$ or $p < 0.01$. This statistical approach was appropriate.

II. REPORTED RESULTS: The test material was shown to be stable in the vehicle for at least 24 hours at room temperature. Concentrations of Hallcomid M-8-10 determined analytically (procedure not specified) after 0 and 24 hours of storage at room temperature were 95.7% and 99.6%, respectively, of a nominal 0.05mg/mL concentration and 101% and 95.0%, respectively, of a nominal 100 mg/mL concentration.

A. PRELIMINARY CYTOTOXICITY ASSAY: In the first pre-test, seven concentrations of Hallcomid M-8-10 ranging from 10 to 1000 µg/mL were tested with and without S9-mix using a 4-hour exposure time and a 20-hour post-exposure cell harvest time. Concentrations of 250 µg/mL and higher were excessively cytotoxic both with and without S9-mix. A second pre-test was performed at six concentrations ranging from 100 to 250 µg/mL with and without S9-mix using a four-hour exposure and an a 20-hour post-exposure cell harvest time. Results of the second pre-test are summarized in Table 1.

BLE 1. Summary of the second preliminary cytotoxicity test[1]

Concentration (µg/mL)	S9-Mix	Survival index (%)[2]	Mitotic index (%)[2]
	–	94.2	75.9
	–	99.4	75.9
	–	58.5	59.5
	–	22.8	5.1

	–	3.0	0
	–	0.3	0
	+	88.2	70.4
	+	95.3	109.9
	+	76.4	97.2
	+	52.0	53.5
	+	10.7	0
	+	0.2	0

Data were summarized from pages 19 and 20, MRID 45369730
[1] Cells were exposed to the test material for four hours and harvested 20 hours post-exposure
[2] Relative to the solvent control

Based on the results of the second preliminary cytotoxicity test, upper concentrations of 160 µg/mL and 180 µg/mL were selected for the cytogenetic assay without S9-mix and with S9-mix, respectively.

B. CYTOGENETIC ASSAY: The concentrations of Hallcomid M-8-10 evaluated at the various cell harvest times without S9-mix are given in Table 2 and those evaluated with S9-mix are given in Table 3. Duplicate cultures were used at each test point. There were no statistically or biologically significant increases over the solvent control values in the percentage of cells with structural aberrations at any dose without S9-mix. A statistically significant increase in the number of aberrant metaphases was seen at 180 µg/mL with S9-mix at the 4-hour post-exposure time; however, the number was within the historical solvent control range. The statistical significance was reached due to the unusually low number of aberrant cells seen in the concurrent solvent control. The solvent and positive control values were within the historical control ranges. Results of the cytogenetic assay without and with S9-mix are summarized in Tables 2 and 3, respectively.

TABLE 2. Chromosomal aberration test with Hallcomid M-8-10 without S9-mix

Treatment (µg/mL)	Number of structural aberrations								otic index (%)	Aberr. metaphase s - gaps (%)	xchange (%)
	g	ctb	ctf	ctd	csb	csf	csd	other			
our exposure - 4-hour post-exposure harvest (total in 200 cells, 100 per culture)											
anol									0.0		
lcomid											
160									2		
our exposure, 20-hour post-exposure harvest (total in 200 cells, 100 per culture)											

anol									0.0		
reated									0.1		
lcomid											
t0									4		
40									9		
t60									8		
mycin C									0.t	0**	0**
our exposure - 26-hour post-exposure harvest (total in 200 cells, t00 per culture)											
anol									0.0		
lcomid											
t60									5		

Data summarized from MRID 45369730, Tables 1, 2, 3, 4, 5, pages 3 t, 32, 33, 34, 35, respectively.
g = gaps (combined chromatid and chromosome)
ctb = chromatid break
ctf = chromatid fragment
ctd = chromatid deletion
csb = chromosome break
csf = chromosome fragment
csd = chromosome deletion
** Statistically significant ($p < 0.0$ t)
Other = exchanges, multiple aberrations and chromosome disintegration

TABLE 3. Chromosomal aberration test with Hallcomid M-8-10 with S9-mix

Treatment (µg/mL)	Number of structural aberrations								otic index (%)	Aberr. Metaphase s - gaps (%)	xchange (%)
	g	ctb	ctf	ctd	csb	csf	csd	other			
our exposure - 4-hour post-exposure harvest (total in 200 cells, 100 per culture)											
anol									0.0		
lcomid											
180									7	*	
our exposure, 20-hour post-exposure harvest (total in 200 cells, 100 per culture)											
anol									0.0		
reated									7		
lcomid											
7.2									4.0		

36									5		
180									3.8		
doxan									7	5*	*
our exposure - 26-hour post-exposure harvest (total in 200 cells, 100 per culture)											
anol									0.0		
lcomid											
180									5.5		

Data summarized from MRID 45369730, Tables 1, 2, 3, 4, 5, pages 31, 32, 33, 34, 35, respectively.
g = gaps (combined chromatid and chromosome)
ctb = chromatid break
ctf = chromatid fragment
ctd = chromatid deletion
csb = chromosome break
csf = chromosome fragment
csd = chromosome deletion
* Statistically significant ($p < 0.05$)
Other = exchanges, multiple aberrations and chromosome disintegration
Endoxan = Cyclophosphamide

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that Hallcomid M-8-10 did not induce structural chromosomal aberrations in Chinese hamster CHO cells with or without S9-mix as tested in this study.

B. REVIEWER COMMENTS: The reviewer agrees with the investigator's conclusions. Hallcomid M-8-10 was tested to cytotoxic concentrations, the experimental protocol was acceptable and the solvent and positive control values were appropriate. There was no evidence for a biologically and statistically significant increase in the number of aberrant cells in test material treated cells over the respective solvent control value at any tested concentration, with or without S9-mix. The four-hour post-exposure harvest time used at the 160 μg/mL concentration without S9-mix and at the 180 μg/mL concentration with S9-mix is too short to effectively evaluate chromosomal aberration induction; however, both 20-hour and 26-hour post-exposure harvest times were also used at these concentrations. The average generation time of the CHO cells was approximately 14 hours, thus an appropriate sampling time would be about 21 hours after the start of treatment (1.5 x the generation time). This is an Acceptable/Guideline study.

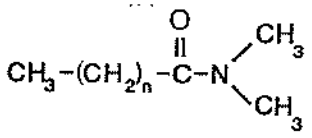
C. STUDY DEFICIENCIES: The investigators did not comment on the pH or osmolality of treatment medium but this omission does not invalidate the study.

GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

TEST SUBSTANCE

- Identity: Hallcomid – 8-10

‡ Remarks:
Description: Clear, light-yellow liquid
Lot/Batch #: 002949
Purity: 98.08% a.i. (identified as 4.73% N,N-Dimethyl-hexanoic acid amide; 53.9% N,N-Dimethyl-octanoic acid amide; 38.9% N,N-Dimethyl-decanoic acid amide; 0.55% N,N-Dimethyl-dodecanoic acid amide; analyzed July 21, 1992
CAS # of TGAI: 1118-92-9 (N,N-Dimethylcapramide) and 14433-76-2 (N,N-Dimethylcaprylamide)
Structure:



Solvent Used: Ethanol

METHOD

- Method/guideline followed: [OPPTS 870.5375 (§84-2)] OECD 473.
- Test type: *In vitro* mammalian cytogenetics assay
- System of testing: mammalian cell culture
- GLP (Y/N): Yes
- Year (study performed): 1995
- Species/strain/cell type used: Chinese hamster CHO cells
- Metabolic activation:
  - Male Wistar rat liver
  - Quantity: 1 mL of S9-mix (40% S9-fraction) in 19 mL medium
  - Induced or not induced: Aroclor 1254 induced
- Dose/concentrations levels:
  Nonactivated conditions: 0, 10, 40, 160 µg/mL
  Activated conditions: 0, 7.2, 36, 180 µg/mL
- Statistical Methods: Fischer exact test. Determined at $p < 0.05$ or $p < 0.01$.

‡ Remarks:
- Test protocol: CHO cells in Ham's F12 medium containing 5% fetal calf serum (FCS) for tests without S9-mix or 10% FCS for studies with S9-mix were treated at the desired test material concentration, solvent control or positive control for four hours. Duplicate cultures were used at each concentration. Positive controls were 2 µg/L Mitomycin C without activation and 10 µg/mL Cyclophosphamide with activation. Cells were harvested at 20 hours post-treatment at all test material concentrations and additionally at four hours and 26 hours post-treatment at 160 µg/mL and 180 µg/mL. Colcemid at 0.4 µg/mL was added two hours before cell harvest. Two-hundred cells (100 per culture) were evaluated at each test material concentration and control group. Cells containing 21 chromosomes (the modal number) were were scored for structural aberrations (chromosome and chromatid breaks, chromosome and chromatid exchanges, gaps, deletions, fragments and multiple aberrations (≥ five aberrations/cell but excluding gaps)). Criteria for a positive response were a dose-dependent, statistically significant increase in the frequency of aberrant metaphases in test material treated cells over the concurrent solvent control value, excluding gaps.

The actual value must be outside the historical control range.

RESULTS

- Result: Negative with and without activation
- Cytotoxic concentration
  - With metabolic activation - In the preliminary cytotoxicity assay, the mitotic index was reduced to 53.5% of the solvent control value at 190 µg/mL and to 0% at 220 µg/mL.
  - Without metabolic activation - The mitotic index was reduced to 5.1% of the solvent control value at 190 µg/mL and to 0% at 220 µg/mL.
- Genotoxic effects:
  - With metabolic activation - None
  - Without metabolic activation - None

Statistical results, as appropriate: No statistically significant increase in the percentage of cells with structural aberrations at any experimental point without S9-mix. A statistically significant increase ($p < 0.05$) was seen at 180 µg/mL with S9-mix at the four-hour post-exposure harvest time. The actual value of 3.5% was within the range of the historical controls and not considered biologically significant.

CONCLUSIONS

‡ Remarks:
This is an Acceptable/Guideline study showing clear negative results.

DATA QUALITY

- Reliability = 1, reliable without restrictions

The investigators did not mention pH or osmolality of the treatment medium; however, cell growth and control results did not indicate any problem in this area. The four-hour post-harvest time is too short for evaluating chromosomal aberration induction but the 20-hour post-exposure harvest time was acceptable.

REFERENCES

Gahlmann, R. (1995) *In vitro* mammalian chromosome aberration test with Chinese hamster ovary (CHO) cells. Bayer AG, Fachbereich Toxicology, Friedrich-Ebert-Straße 217-333, D-42096 Wuppertal, F.R.G.. Laboratory Report No.: 24450, Study No.: T 7039113, November 7, 1995. MRID 45369730. Unpublished.

DATA EVALUATION RECORD

HALLCOMID M-8-10

STUDY TYPE: OTHER GENOTOXICITY: UNSCHEDULED DNA SYNTHESIS IN MAMMALIAN CELL CULTURES [OPPTS 870.5550 (§84-2)] OECD 482
MRID 45369731

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Arlington, VA 22202

Prepared by

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Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes/Mammalian Cell Cultures [OPPTS 870.5550 (*in vitro*) (§84-2)] OECD 482 (*in vitro*)

PC CODE: N/A

DP BARCODE: N/A
SUBMISSION NO.: N/A

TEST MATERIAL (PURITY): Hallcomid M-8-10 (98.3%, 98.08% a.i. as determined on January 24, 1992 and July 21, 1992, respectively. Mixture of N,N-Dimethyldecaneacidamide (39.5%), N,N-Dimethyloctaneacidamide (53.3%), N,N-Dimethylhexaneacidamide (3.45%) and N,N-Dimethyldodecaneacidamide (1.4%))

SYNONYMS: Mixture of Capronacid, Caprylacid, Caprinacid and Laurinacid

CITATION: Brendler-Schwaab, S. (1994) Hallcomid M-8-10: Test on unscheduled DNA synthesis in rat liver primary cell cultures *in vitro*. Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-233, D-42096 Wuppertal, F.R.G. Laboratory Report No.: 22924, Study No.: T 7039096, March 3, 1994. MRID 45369731. Unpublished

SPONSOR: Bayer AG. (Submitted by The C.P. Hall Company, 311 South Wacker Drive, Suite 4700, Chicago, Illinois, 60606)

EXECUTIVE SUMMARY: In an unscheduled DNA synthesis assay (MRID 45369731), primary rat hepatocyte cultures were exposed to Hallcomid M-8-10 (Batch No. 002949, 98.3% and 98.08% a.i.) in ethanol at concentrations of 0, 29.8, 39.6, 49.4, 59.5, 79.1, 98.8 or 118.6 µg/mL for 18 hours in the presence of 10 µCi/mL ^{3}H-thymidine. UDS was determined using the autoradiographic procedure. Hepatocytes were obtained from young adult male Sprague-Dawley rats.

Hallcomid M-8-10 was tested up to cytotoxic concentrations. It is reported that the highest dose was excessively cytotoxic (relative survival reduced to 52.1%) and no slides could be evaluated at this dose. Relative survival in the remaining dose groups ranged from 79.3% to 90.1% in a non dose-dependent manner. Mean grain counts were based on evaluation of 50 cells per slide, three slides per dose with the exception of the 79.1 µg/mL dose groups where cells on only two slides were evaluated. Duplicate cultures were used at each dose. The mean net nuclear grain count (-1.15 ± 0.30) and the average number of cells in repair (0.00%), defined as cells with five or more net nuclear grains, in the solvent control were within the historical control ranges. No statistically significant increase in the mean net nuclear grain count or the percentage of cells in repair over the solvent control values were seen at any test material concentration and no dose-dependency was apparent. The highest number of net nuclear grains seen in test material treated cells was 0.20 ± 0.56 at the lowest tested dose. The 2-acetylaminofluorene positive control values of 7.79 ± 1.22 net nuclear grains and 82.67% of cells in repair were appropriate. There was no evidence

that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced.

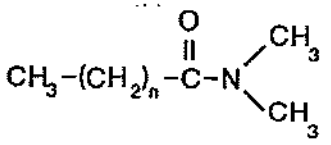
This study is classified as Acceptable/Guideline and satisfies the guideline requirement for Test Guideline OPPTS 870.5550; OECD 482 for other genotoxic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

Test material: allcomid M-8-10

scription:	ht yellow fluid
t/Batch #:	2949
rity:	.3% and 98.08% a.i. as measured on January 24, 1992 and July 21, 1992, respectively
AS # of TGAI:	18-92-9 (N,N-Dimethyldecanamide) and t4433-76-2 (N,N-Dimethyloctanamide)
ucture:	
lvent Used:	anol

Control materials:

gative control:	t used		
lvent:	anol	ncentration:	not exceeding 1% v/v
sitive control /solvent:	Acetylaminofluorene	ncentration:	0.25 µg/mL

3. Test compound concentrations used:

Preliminary cytotoxicity test:
1.78, 3.6, 7.1, 14.2, 28.4, 56.8, 114.0, 227.0, 455.0, 909.0 µg/mL

UDS assay:
29.8, 39.6, 49.4, 59.5, 79.1, 98.8 or 119.6 µg/mL

4. Media: The culture medium was Williams Medium E supplemented with L-glutamine (2 mM), gentamycin sulfate (50 µg/mL) and 10% heat-inactivated fetal calf serum (WEC). During treatment, the fetal calf serum concentration was reduced to 1% and the gentamycin was omitted. ^{3}H-thymidine at 10 µCi/mL was present during treatment.

5. Test cells: Mammalian cells in culture/primary rat hepatocytes. Hepatocytes were obtained from young adult male Sprague-Dawley rats.

6. Cell preparation:

a. Perfusion technique: The rat was anesthetized by i.p. injection of 0.05 mL Nembutal Sodium solution (60 mg/mL) per 100 g body weight 10 minutes prior to perfusion. The abdomen was shaved, wetted with 70% ethanol, opened and the liver perfused *in situ* through the vena cava for 1.5 minutes with EGTA (ethylene glycol-bis (β-aminoethylether) N,N,N',N'-tetraacetic acid) solution at 10 mL/minute, then for 2 minutes at 20 mL/minute at which time the perfusate was switched without interrupting the flow to a collagenase solution for 10 minutes at 20 mL/minute. The EGTA solution was composed of 19 mg EGTA in 0.1 mL 2N NaOH, 0.5 mL of 2 M HEPES (4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid), 0.1 mL of gentamycin sulfate (50 mg/mL) all brought to a final volume of 100 mL with Hanks balanced salt solution without Ca^{2+} or Mg^{2+}. The collagenase solution was composed of 487 mL Williams Medium E without serum, 2.5 mL 2 M HEPES, 0.5 mL of gentamycin sulfate (50 mg/mL), 0.1 mL 2 N NaOH and 40,000 to 50,000 units Type IV collagenase. All solutions were at 37°C.

b. Hepatocyte harvest/culture preparation: Following perfusion, the hepatocytes were isolated using the protocol of Butterworth et al. (1987). Although no details of the hepatocyte harvest were provided in the present study, the general procedure used in the testing laboratory is more or less as follows. The liver is removed, incisions are made in each liver lobe and the cells collected using a metal comb. The cell suspension obtained is filtered through gross gauze and the volume brought to 50 mL with cold WEI (Williams Medium E without serum) and the cells held on ice for 5 - 10 minutes. The supernatant is removed, presumably following centrifugation, and the cell pellet resuspended in 50 mL of cold WEI. Following filtration through fine gauze, the volume is brought to 50 mL and the cell suspension centrifuged for three minutes at 50 x g at < 15°C. The cell pellet is resuspended in WEI and centrifuged as before, the cell pellet resuspended in 25 mL of WEI and the cell concentration and viability determined by trypan blue exclusion.

Cultures for cytotoxicity determinations were prepared by seeding 7.5×10^5 viable cells per dish into two 60 mm Petri dishes precoated with collagen. Two additional dishes were prepared to determine cell viability, attachment rate and morphology about two hours after the cultures were established. Cultures for the UDS assay were prepared by placing a Thermanox 25 mm round plastic coverslip precoated with collagen into each well of a six-well culture dish and adding approximately 3.75×10^5 cells in 2.5 mL of WEC to each well. Cultures were incubated for 90 - 150 minutes at 37°C in a humidified atmosphere with 5% CO_2.

B. TEST PERFORMANCE

1. Cytotoxicity assay: Following the attachment period, unattached cells were washed off the dishes with phosphate buffered saline (PBS). Hepatocyte viability and number were determined on two of the dishes using the trypan blue exclusion method. The culture medium in the remaining two six-well dishes per treatment was replaced with medium containing 1% FCS, the desired concentration of test material or control agent and 10 μCi/mL of ^{3}H-thymindine and incubated for 18 to 24 hours. Viable cell counts were determined by the trypan blue exclusion method 20 - 24 hours after the start of treatment and cell survival relative to the solvent control was calculated.

2. UDS assay:

   a. Treatment: Growth medium in the six-well dishes was replaced with medium containing 1% FCS, the desired concentration of test material and 10 μCi/mL ^{3}H-thymidine. Cultures were then incubated as before for 18 - 24 hours, washed twice with PBS and held for 5 - 10 minutes in a 1% sodium citrate solution to swell the nuclei. The cells (on coverslips) were fixed by three changes of acetic acid : absolute ethanol solution (3 : 1) for a total time of at least 30 minutes. The wells were washed two to six times with deionized distilled water and air dried.

   b. Preparation of autoradiographs/grain development: All procedures were done in the dark. The dried coverslips were mounted cell-side-up on microscope slides, the slides dipped in Kodak NTB-2 photographic emulsion (either undiluted or diluted 1 : 1 with distilled water) in a darkroom and air-dried overnight. The coated slides were then stored in light-tight boxes with a drying agent for 4 - 10 days at -20°C. Following the exposure period, the emulsion was developed for two to four minutes in Kodak D-19 developer at <15°C. The slides were rinsed with distilled water, fixed in Kodak Fixer for 5 - 8 minutes, air-dried and stained with hematoxylin and eosin.

   c. Grain counting: Fifty cells per slide, 150 cells per dose were evaluated for UDS activity. Counting was done by hand using a Zeiss microscope at 100x under oil emersion interfaced through a high resolution TV camera to a color TV. Cells counted were viable at the time of fixation. Isolated nuclei and cells with abnormal morphology were not evaluated. The net nuclear grain count was determined by counting the number of grains over the nucleus and subtracting the average number of grains in three nuclear-sized regions of the corresponding cytoplasm. The mean net nuclear grain count and the percentage of cells in repair, defined as nuclei with five or more net nuclear grains, was calculated for each test material dose and for the solvent and positive controls.

   d. Evaluation criteria: Criteria for an acceptable assay include hepatocyte viability of at least 50%, viability of the monolayer cell cultures used for the UDS assay of at least 75%, viability of cells in the solvent control cultures of at least 60% after 16 - 24 hours and appropriate responses to the solvent and positive controls. Grain counts from at least two replicate cultures per dose are required for the results to be considered acceptable. The net nuclear grain count of the solvent control should be between -8 to +0.5 with no more than 10% of the cells in repair. The 2-AAF positive control should induce mean net nuclear grain counts in the

range of 7 - 20 with 70% to 100% of the cells in repair. At least four to five doses should be analyzed for grain counts but not necessarily all in the same trial. The highest dose should reduce the relative survival to about 50% or be limited by test material solubility or be the limit dose of 5 mg/mL for the assay. A mean net nuclear grain count of two or more with 20% or more of the cells responding is considered a positive response. A count between 0.5 and 2 is considered a marginal response but can be considered a positive response if a positive dose-response in seen in both the net nuclear grain count and the percentage of cells in repair. A cytoplasmic background count exceeding 30 grains per nuclear-sized area in control cultures invalidates the experiment. Cells undergoing replicative repair are recorded but not analyzed for nuclear grains.

e. Statistical analysis: The percentage of cells in repair for each dose group was compared to the solvent control using a one-sided 2 x 2 chi-squared test corrected for continuity. The statistical significance of a result was assessed by comparing the square root of the test statistic to the upper 95% quantile ($p \leq 0.05$) of the normal standard distribution.

II. REPORTED RESULTS: The test material was shown to be stable in the vehicle for at least 24 hours at room temperature. Hallcomid M-8-10 concentrations determined analytically (procedure not specified) after 0 and 24 hours of storage at room temperature were 95.7% and 99.6%, respectively, of a nominal 0.05 mg/mL concentration and 101.0% and 95.0%, respectively, of a nominal 100 mg/mL concentration.

A. PRELIMINARY CYTOTOXICITY ASSAY: A clear solution of Hallcomid M-8-10 in ethanol was obtained up to a concentration of 100 mg/mL. Solutions were prepared in glass tubes rather than in plastic tubes because the test material reacts with plastic at high concentrations. Ten concentrations of Hallcomid M-8-10 ranging from 1.78 to 909.0 µg/mL were tested in the preliminary cytotoxicity test using primary rat hepatocytes with viability of 75.8% following isolation. Cells were exposed to the test material for 18 hours. Cell survival relative to the solvent control value was unaffected at 1.78 µg/mL, varied randomly from 80.0% to 91.3% in the dose range of 3.6 to 114.0 µg/mL and was 0% at 227.0 µg/mL and higher. An upper test material dose of 118.6 µg/mL was selected for the UDS assay.

B. UDS assay: Seven concentrations of Hallcomid M-8-10 ranging from 29.8 to 118.6 µg/mL were tested in the UDS assay using an 18 hour exposure time. The primary rat hepatocytes used for the assay had a viability of 74.0% after isolation and 78.4% after a 1.5 hour attachment period. Average viability of the solvent control cultures following the 18 hour treatment period was 72.6%.
Mean grain counts were based on evaluation of 50 cells per slide, three slides per dose with the exception of the 79.1 µg/mL dose group where cells on only two slides were evaluated. Relative survival was reduced to 52.1% at 118.6 µg/mL and it is stated that no slides could be evaluated (no further information) at this concentration. The net nuclear grain count and the average number of cells in repair in the solvent control were within the historical control range. No statistically significant increase in the mean net nuclear grain count or the percentage of cells in repair over the solvent control values was seen at any test material concentration and no dose-response was apparent. The positive control values were appropriate. Results of the UDS assay are summarized in Table 1.

TABLE 1. Summary of the UDS Assay with Hallcomid M-8-10

Treatment	let grains per nucleus ± SD [1]	lean grains per nucleus ± SD [1]	ean cytoplasmic grain count ± SD [1]	erage % cells in repair [1]	% Relative survival [3]
anol	5 ± 0.30	t ± 0.51	6 ± 0.63	0	0.0
H 6561 (µg/mL)					
29.8	0 ± 0.56	7 ± 0.91	7 ± 1.30	3	6
39.6	78 ± 0.78	9 ± 1.29	7 ± 1.13	7	3
49.4	3 ± 0.40	2 ± 0.21	9 ± 0.44	0	3
59.5	20 ± 0.35	5 ± 0.15	5 ± 0.36	0	1
79.1[2]	21 ± 0.70	1 ± 0.04	2 ± 0.74	0	1
98.8	05 ± 0.28	7 ± 0.34	1 ± 0.30	0	9
118.6	slides were evaluable			0	1
AF (0.25 µg/mL)	9 ± 1.22	3 ± 0.74	4 ± 0.54	67*	4

Data from Table 2, MRID 4536973t, page 30
* Statistically significant at $p \leq 0.05$
[1] Data based on average of triplicate slides except where noted
[2] Data based on average of two slides
[3] Survival relative to the solvent control
2-AAF = 2-Acetylaminofluorene

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that Hallcomid M-8-10 was negative in the primary rat hepatocyte UDS assay.

B. REVIEWER COMMENTS: The reviewer agrees with the investigators' conclusion. Hallcomid M-8-10 was tested to cytotoxic concentrations, proper experimental protocol was followed and the solvent and positive control values were appropriate. There was no evidence that Hallcomid M-8-10 induced UDS as tested in this study. This is an Acceptable/Guideline study.

C. STUDY DEFICIENCIES: No study deficiencies were identified.

REFERENCES:

Butterworth, B.E., J. Ashby, E. Bermudez et al. (1987) A protocol and guide for the *in vitro* rat hepatocyte DNA-repair assay. Mutation Research 189:113-121.

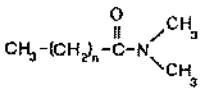
GENETIC TOXICITY IN VITRO (Unscheduled DNA Synthesis)

TEST SUBSTANCE

- Identity: Hallcomid – 8-10

‡ Remarks:
Description: Light-yellow fluid
Lot/Batch #: 002949
Purity: 98.3% and 98.08% a.i. as determined on January 24, 1992 and July 21, 1992, respectively (Mixture of N,N-Dimethyldecaneacidamide (39.5%), N,N-Dimethyloctaneacidamide (53.3%), N,N-Dimethylhexaneacidamide (3.45%) and N,N-Dimethyldodecaneacidamide (1.4%)
CAS # of TGAI: 1118-92-9 (N,N-Dimethyloctanamide) and 14433-76-2 (N,N-Dimethyldecanamide)

Structure:



n = (4), 6, 8 or (10); ( ) = minor constituents

Solvent Used: Ethanol

METHOD

- Method/guideline followed: OPPTS 870.5550 [§84-2]; OECD 482.
- Test type: *In vitro* mammalian cell unscheduled DNA synthesis (UDS) assay
- System of testing: Primary rat hepatocytes
- GLP (Y/N): Yes
- Year (study performed): 1994
- Species/strain/cell type used: Sprague-Dawley rats
- Metabolic activation: Not required with rat hepatocytes.
- Concentrations tested:
  0, 29.8, 39.6, 49.4, 59.5, 79.1, 98.8 and 118.6 µg/mL
- Statistical methods: One-sided 2 x 2 Chi-squared test corrected for continuity

‡ Remarks:

– Test protocol: Cultures for the UDS assay (in duplicate) were prepared by placing a Thermanox 25 mm round plastic coverslip precoated with collagen into each well of a six-well culture dish and adding approximately 3.75 x 10^5 cells in 2.5 mL of Williams Medium E to each well. Cultures were incubated for 90 - 150 minutes at 37°C in a humidified atmosphere with 5% CO_2. Growth medium in the six-well dishes was replaced with medium containing 1% FCS, the desired concentration of test material and 10 µCi/mL ^{3}H-thymidine. Cultures were then incubated as before for 18 hours, washed twice with PBS and held for 5 - 10 minutes in a 1% sodium citrate solution to swell the nuclei. The cells (on coverslips) were fixed by three changes of acetic acid : absolute ethanol solution (3 : 1) for a total time of at least 30 minutes. The wells were washed two to six times with deionized distilled water and air dried. The dried coverslips were mounted cell-side-up on microscope slides, the slides dipped in Kodak NTB-2 photographic emulsion (either undiluted or diluted 1 : 1 with distilled water) in a darkroom and air-dried overnight. The coated slides were then stored in light-tight boxes with a drying agent for 4 - 10 days at -20°C. Following the exposure period, the emulsion was developed for two to four minutes in Kodak D-19 developer at <15°C. The slides were rinsed with distilled water, fixed in Kodak Fixer for 5 - 8 minutes, air-dried and stained with

hematoxylin and eosin.

Fifty cells per slide, t50 cells per dose were evaluated for UDS activity. Cells counted were viable at the time of fixation. Isolated nuclei and cells with abnormal morphology were not evaluated. The net nuclear grain count was determined by counting the number of grains over the nucleus and subtracting the average number of grains in three nuclear-sized regions of the corresponding cytoplasm. The mean net nuclear grain count and the percentage of cells in repair, defined as nuclei with five or more net nuclear grains, was calculated for each test material dose and for the solvent and positive controls. A mean net nuclear grain count of two or more with 20% or more of the cells responding is considered a positive response. A count between 0.5 and 2 is considered a marginal response but can be considered a positive response if a positive dose-response in seen in both the net nuclear grain count and the percentage of cells in repair. A cytoplasmic background count exceeding 30 grains per nuclear-sized area in control cultures invalidates the experiment.

RESULTS

- Result: Negative
- Cytotoxic concentration: 118.6 µg/mL was excessively cytotoxic and no slides could be evaluated although the relative survival at this dose was 52.1%.
- Genotoxic effects: Relative survival in the remaining dose groups ranged from 79.3% to 90. t% in a non dose-dependent manner. Mean grain counts were based on evaluation of 50 cells per slide, three slides per dose with the exception of the 79. t µg/mL dose groups where cells on only two slides were evaluated. Duplicate cultures were used at each dose. The mean net nuclear grain count (- t. t5 ± 0.30) and the average number of cells in repair (0.00%), defined as cells with five or more net nuclear grains, in the solvent control were within the historical control ranges. No statistically significant increase in the mean net nuclear grain count or the percentage of cells in repair over the solvent control values were seen at any test material concentration and no dose-dependency was apparent. The highest number of net nuclear grains seen in test material treated cells was 0.20 ± 0.56 at the lowest tested dose. The 2-acetylaminofluorene positive control values of 7.79 ± t.22 net nuclear grains and 82.67% of cells in repair were appropriate.
- Statistical results, as appropriate: No statistically significant increases in net nuclear grain count or in the percentage of cells in repair were seen in Hallcomid M-8-10 treated cells.

CONCLUSIONS

‡ Remarks:
There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced.

DATA QUALITY

- Reliability = 1, reliable without restriction

REFERENCES

Brendler-Schwaab, S. (1994) Hallcomid M-8-10: Test on unscheduled DNA synthesis in rat liver primary cell cultures *in vitro*. Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-233, D-42096 Wuppertal, F.R.G. Laboratory Report No.: 22924, Study No.: T 7039096, March 3, 1994. MRID 45369731. Unpublished.