**ROBUST SUMMARY**
**OF INFORMATION ON**

**Substance Group** **GREASE THICKENERS**

**Summary prepared by** American Petroleum Institute

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

### 1.1.1 GENERAL SUBSTANCE INFORMATION

**Substance type** : Petroleum product
**Physical status** : Solid

**Remark** : Lubricating greases are solid or semi-solid materials made by thickening lubricating oils with soaps.
The soaps are formed in-situ in the lubricating oil by the chemical reaction of an alkali and the respective fatty acid.
This robust summary covers the calcium and lithium greases in which calcium and lithium soaps respectively have been used as the thickening agent.

Information on several greases as well as on lithium stearate, a soap commonly used as a thickener, is included in this robust summary.
In addition some information is included on magnesium stearate (closely related to calcium stearate) and on castor oil (mostly ricinoleic acid) which is closely related to the larger fatty acids used to make the salts in this category.

09.09.2004

### 1.13 REVIEWS

**Memo** : Leonard et al

**Remark** : Leonard et al reviewed the available information on the teratogenicity, mutagenicity and carcinogenicity of lithium compounds.
Their conclusions were:
"Such effects would be highly unlikely in an occupational setting but might be a risk to the considerable percentage of the population treated for manic depressive disorders.
It was concluded that lithium compounds have no significant clastogenic and, based on studies in microorganisms, only doubtful mutagenic activity.
Information on teratogenic effects is contradictory. While some observations in man and a few animal studies suggest that lithium in concentrations in the order of those given to patients may cause malformations, other observations do not support this claim and the risk with carefully controlled therapy is probably small. .....
No information is available on cancer caused by treatment with lithium, and it is highly unlikely that lithium is carcinogenic."

24.12.2003 (14)

**Memo** : Cosmetic ingredient review panel

**Remark** : A cosmetic ingredients review panel concluded that stearate compounds are safe as cosmetic ingredients.

23.12.2003 (4)

### 2.1 MELTING POINT

**Method** : Calculated by MPBPWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test substance** : Grease thickeners

**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The melting point estimates given here are for fatty acid salts covering this range of carbon atoms. The data represent a potential melting point range for all substances in the grease thickeners category.

**Result** :

| Molecular Weight | No. C Atoms | MP Values, (°C) Estimated | Measured |
|---|---|---|---|
| **Lithium Salts** | | | |
| nonanedioic acid, dilithium salt | | | |
| 200.09 | 9 | 186 | |
| octadecanoic acid, lithium salt | | | |
| 290.42 | 18 | 249 | |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | | | |
| 306.42 | 18 | 264 | |
| docosanoic acid, lithium salt | | | |
| 346.53 | 22 | 271 | |
| **Calcium Salts** | | | |
| octadecanoic acid, 12-hydroxy, calcium salt | | | |
| 639.03 (1) | 36 | 320 | |
| stearic acid, calcium salt | | | |
| 607.04 (1) | 36 | | 179 |

(1) Compound composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
Melting points were measured values or calculated using a validated computer model.

09.09.2004 (32)

### 2.2 BOILING POINT

**Method** : Calculated by MPBPWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test substance** : Grease thickeners

**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The boiling point estimates given here are for fatty acid salts covering this range of molecular weights and number of carbon atoms. The data represent a potential boiling point range for all substances in the grease thickeners category.

**Result** :

| Molecular Weight | No. C Atoms | Estimated BP Value, (°C) |
|---|---|---|
| **Lithium Salts** | | |
| nonanedioic acid, dilithium salt | | |
| 200.09 | 9 | 484 |
| octadecanoic acid, lithium salt | | |
| 290.42 | 18 | 578 |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | | |
| 306.42 | 18 | 611 |
| docosanoic acid, lithium salt | | |
| 346.53 | 22 | 624 |
| **Calcium Salts** | | |
| octadecanoic acid, 12-hydroxy, calcium salt | | |
| 639.03 (1) | 36 | 730 |
| stearic acid, calcium salt | | |
| 607.04 (1) | 36 | 661 |

(1) Compound composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
Estimated boiling points were calculated using a validated computer model.

09.09.2004 (32)

## 2.4 VAPOUR PRESSURE

**Decomposition** :
**Method** : Calculated by MPBPWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test substance** : Grease thickeners

**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The vapor pressure estimates given here are for fatty acid salts covering this range of molecular weights and number of carbon atoms. The data represent a potential vapor pressure range for all substances in the grease thickeners category.

**Result** :

| Molecular Weight | No. C Atoms | Estimated VP Value, (hPa) |
|---|---|---|
| **Lithium Salts** | | |
| nonanedioic acid, dilithium salt | | |
| 200.09 | 9 | $2 \times 10^{-9}$ |
| octadecanoic acid, lithium salt | | |
| 290.42 | 18 | $1 \times 10^{-12}$ |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | | |
| 306.42 | 18 | $2 \times 10^{-16}$ |
| docosanoic acid, lithium salt | | |
| 346.53 | 22 | $5 \times 10^{-14}$ |
| **Calcium Salts** | | |
| octadecanoic acid, 12-hydroxy, calcium salt | | |
| 639.03 (1) | 36 | $1 \times 10^{-21}$ |

stearic acid, calcium salt
607.04 (1) 36 $6 \times 10^{-14}$
(1) Compound composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
Estimated vapor pressures were calculated using a validated computer model.

17.11.2004 (32)

### 2.5 PARTITION COEFFICIENT

**Method** : Calculated by KOWWIN, V1.66 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test substance** : Grease thickeners

**Remark** : Because fatty acids are ionizable compounds, Kow measurements (hence log P) can vary greatly with pH. The variation depends upon pH and the pKa of the compound. In general, Kow values of a compound are lower when it exists predominantly in the ionized form as compared to existing primarily in the non-ionized form. The KOWWIN V1.66 model handles ion pairs in a special way and gives Kow estimates that are an estimate for the ionized acid. Many fatty acids have pKa values circumneutral, and they would exist predominantly in the molecular form at environmentally relevant pHs. Therefore, the estimates given here are potentially lower than what would be expected for the salt form at typical environmental pHs.

**Result** :

| | **Molecular Weight** | **No. C Atoms** | **Estimated Log Kow** |
|---|---|---|---|
| **Lithium Salts** | | | |
| nonanedioic acid, dilithium salt | 200.09 | 9 | -3.56 |
| octadecanoic acid, lithium salt | 290.42 | 18 | 4.13 |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | 306.42 | 18 | 2.60 |
| docosanoic acid, lithium salt | 346.53 | 22 | 6.10 |
| **Calcium Salts** | | | |
| octadecanoic acid, 12-hydroxy, calcium salt | 639.03 (1) | 36 | 11.7 |
| stearic acid, calcium salt | 607.04 (1) | 36 | 14.3 |

(1) Compound composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
Estimated partition coefficients were calculated using a validated computer model.

17.11.2004 (32)

### 2.6.1 SOLUBILITY IN DIFFERENT MEDIA

**Solubility in** : Water
**Method** : Calculated by WSKOWWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)

**Test substance** : other TS: Grease thickeners

**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The water solubility estimates given here are for fatty acid salts covering this range of molecular weights and number of carbon atoms. The data represent a potential water solubility range for all substances in the grease thickeners category.

**Result** :

| Molecular Weight | No. C Atoms | Solubility, mg/l [(1)] Estimated | Measured |
|---|---|---|---|
| **Lithium Salts** | | | |
| nonanedioic acid, dilithium salt | | | |
| 200.09 | 9 | (877) | |
| octadecanoic acid, lithium salt | | | |
| 290.42 | 18 | 4.1 | |
| | | (0.002) | |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | | | |
| 306.42 | 18 | 222 | |
| | | (0.1) | |
| docosanoic acid, lithium salt | | | |
| 346.53 | 22 | 0.04 | |
| | | ($2.0 \times 10^{-5}$) | |
| **Calcium Salts** | | | |
| octadecanoic acid, 12-hydroxy, calcium salt | | | |
| 639.03 [(2)] | 36 | $9.7 \times 10^{-9}$ | |
| | | ($6.4 \times 10^{-7}$) | |
| stearic acid, calcium salt | | | |
| 607.04 [(2)] | 36 | | 40 @ 15°C |

[(1)] Estimated solubility values determined by the relationship with Kow and by the fragment constant method (in parenthesis).

[(2)] Compound composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
Water solubility estimates were measured values or calculated using a validated computer model.

09.09.2004 (32) (34)

### 2.14 ADDITIONAL REMARKS

**Memo** : Physico-chemical properties of grease thickeners

**Remark** : Greases are formed through a chemical reaction of a mineral oil, a fatty acid, and a metal caustic (typically calcium or lithium hydroxide). This reaction occurs in the mineral oil matrix when a fatty acid or its methyl ester is dissolved in the mineral oil followed by the addition of the caustic. The caustic and fatty acid molecules react to form an insoluble metal salt of the fatty acid. Because the thickener is synthesized in situ during the manufacture of the finished grease, secondary interactions between the fatty acid salt and the mineral oil matrix also result, creating the physical consistency of grease (see also Section 1.1.1). The byproducts of this reaction are either water or methanol depending on whether the fatty acid or its methyl ester, respectively, was used as the reactant. When fatty acids are reacted with caustic outside of a mineral oil matrix, the resulting

compounds are called soaps (NLGI, 1996).

Computer predictions for melting point, boiling point, vapor pressure, partition coefficient, and water solubility were made for the salts as if they existed outside the grease matrix. However, the endpoint values should be qualified with the understanding that the thickening agent is created by a chemical reaction in situ and does not exist as a separate entity outside of the grease matrix.

09.09.2004 (17)

### 3.1.1 PHOTODEGRADATION

**INDIRECT PHOTOLYSIS**

**Sensitizer** : OH
**Method** : Calculated by AOPWIN V1.90 (EPIWIN V3.10; EPA 2000)
**Year** : 2000
**GLP** : No
**Test substance** : Grease thickeners

**Remark** : Due to the extremely low vapor pressure of these substances plus the fact that these compounds are made within a mineral oil matrix, there is essentially no opportunity for these substances to enter the atmosphere. However, the modeling results show that if any vapors entered the atmosphere, these molecules would undergo indirect photolysis reactions and not persist.

**Result** : Concentration of sensitizer: $1.5 \times 10^6$ OH/cm³

See table of half-lives below (values given in days):

| Test Substance | Molecular Weight | No. C Atoms | Estimated Half-life, (days) |
|---|---|---|---|
| **Lithium Salts** | | | |
| nonanedioic acid, dilithium salt | 200.09 | 9 | 1.4 |
| octadecanoic acid, lithium salt | 290.42 | 18 | 0.5 |
| octadecanoic acid, 12- hydroxystearate, lithium salt | 306.42 | 18 | 0.4 |
| docosanoic acid, lithium salt | 346.53 | 22 | 0.4 |
| **Calcium Salts** | | | |
| octadanoic acid, 12-hydroxy, calcium salt | 639.03 | 36 (1) | 0.2 |
| stearic acid, calcium salt | 607.04 | 36 (1) | 0.2 |

(1) Composed of two fatty acid molecules attached to calcium

**Reliability** : (2) valid with restrictions
The endpoint was estimated using a validated computer model.

17.11.2004 (31)

### 3.1.2 STABILITY IN WATER

**GLP** : No
**Test substance** : Grease thickeners various

**Remark** : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. The chemical components that comprise the grease thickener category are salts of fatty acids that are not subject to hydrolysis because they lack functional groups that hydrolyze.

**Reliability** : (1) valid without restriction
17.11.2004 (9)

### 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

**Method** : Calculations by Level 1, Version 2.02, a fugacity-based environmental equilibrium partitioning model (Mackay 1991).

**Remark** : Grease thickening agents are created by a chemical reaction within a mineral oil matrix resulting in the formation of grease. The distribution estimates given here are for pure compounds representing the range of molecular weights of substances in the grease thickeners category. When these substances exist in their pure state, fugacity modeling showed them to partition mostly to either soil or water. The degree of partitioning to either of these environmental compartments was related to the water solubility of the compound. These estimates should be used with the knowledge that such thickening agents are entrained within a grease matrix and such entrainment would limit environmental exposure.

**Result** :

Air, Water, Soil, Sediment, Suspended Sediment, Fish.

**PERCENT DISTRIBUTION**

| Number C Atoms | Air | Water | Soil | Sed. | Susp. Sed. | Fish |
|---|---|---|---|---|---|---|
| **Lithium Salts** | | | | | | |
| nonanedioic acid, dilithium salt | | | | | | |
| 9 | <0.1 | 100 | <0.1 | <0.1 | <0.1 | <0.1 |
| octadecanoic acid, lithium salt | | | | | | |
| 18 | <0.1 | 8 | 90 | 2 | <0.1 | <0.1 |
| octadecanoic acid, 12-hydroxy-, lithium salt | | | | | | |
| 18 | <0.1 | 73 | 26 | 0.6 | <0.1 | <0.1 |
| docosanoic acid, lithium salt | | | | | | |
| 22 | <0.1 | <0.1 | 98 | 2 | <0.1 | <0.1 |
| **Calcium Salts** | | | | | | |
| octadecanoic acid, 12-hydroxy-, calcium salt | | | | | | |
| 18 | <0.1 | <0.1 | 98 | 2 | <0.1 | <0.1 |

stearic acid, calcium salt
18 <0.1 <0.1 98 2 <0.1 <0.1

**Reliability** : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model. The estimates given are for pure substances and not likely to reflect the disposition from a grease matrix.

17.11.2004 (16)

### 3.5 BIODEGRADATION

| | |
|---|---|
| **Test Substance:** | Calcium Stearate; CAS No. 1592-23-0 |
| **Method/Guideline:** | Ready Biodegradability by OECD 301B: $CO_2$ Evolution (Modified Sturm Test) (OECD, 1981) |
| **Year (guideline):** | 1981 |
| **Type (test type):** | Ready Biodegradability |
| **GLP:** | Not Stated |
| **Year (study performed):** | 1987 |
| **Inoculum:** | Sludge from sewage treatment plant receiving predominantly domestic waste water |
| **Exposure Period:** | 20 or 24 days |
| **Test Conditions: (FT-TC)**<br><br>**Note: Concentration prep., vessel type, replication, environmental conditions.** | Six independent 301B tests were conducted with calcium stearate for the purpose of evaluating the following conditions of the experimental design:<br><br>1. Effect of agitation versus no agitation during the test period, and<br><br>2. Effect of test substance distribution technique, which included<br>- no dispersion of test substance,<br>- dispersion via application of test substance on a glass filter,<br>- dispersion via application of ultrasound.<br><br>The different combinations of the above treatments were as follows:<br><br>**Test** / **Duration (days)** / **Agitation** / **Method of Dispersion**<br>A / 24 / – / –<br>B / 24 / + / –<br>C / 24 / – / glass filter<br>D / 20 / + / ultrasound<br>E / 20 / + / glass filter |

| | |
|---|---|
| | F 24 + glass filter<br><br>The authors report that installations and equipment were as described in the OECD 301B method (1981) with the modifications that the size of the carboys was 3 liters and they contained 1.5 to 2 liters of solution. Agitation, when used, was created by a magnetic stirrer with a PTFE-coated rod of 6 cm length rotating at approximately 60 rpm. A constant temperature of 23°C was maintained by immersion of the carboys in a water bath.<br><br>The authors indicated that the inoculum and mineral solutions were prepared according to the OECD guideline.<br><br>Direct dispersion was done either by adding calcium stearate as a powder directly to the carboys containing the inoculum, or as a suspension in water prepared by ultrasonic dispersion. When a solid carrier was used (glass filter paper), calcium stearate was first melted then applied to glass filter papers. The filter papers were cut into small pieces then added to the carboys. The concentration of calcium stearate used in all tests was 20 mg/L.<br><br>Biodegradation was determined by comparing the evolution of carbon dioxide to the theoretical carbon dioxide ($ThCO_2$) that could potentially be evolved during mineralization. The $ThCO_2$ for calcium stearate was cited as 2.61 g $CO_2$/g. |
| **Results: (FT-RE)**<br><br>**Units/Value:**<br><br>**Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.** | **Test** / **Degradation (%)** / **10% lag time (approx.) (days)** / **Days to 60% Biodegradation (approx.)**<br>A 91 2 11<br>B 99 1 7<br>C 55 5 —<br>D 72 2 8<br>E 84 2 12<br>F 88 5 12<br><br>Calcium stearate was biodegraded by the $CO_2$ evolution test, fulfilling the criteria of ready biodegradability (60% within a 10-day window once 10% biodegradation has been achieved) in all tests except in one case where it was applied on a glass filter in a non-agitated carboy. |
| **Conclusion: (FT-CL)** | Calcium stearate was found to be readily biodegradable. |
| **Reliability: (FT-RL)** | (2) Reliable with restrictions. The authors cited a standardized method for measuring ready biodegradability, but the report did not provide complete details of the test methodology used in the study. |
| **Reference: (FT-RE)** | de Morsier, A., J. Blok, P. Gerike, L. Reynolds, H. Wellens, and W.J. Bontinck. 1987. Biodegradability tests for poorly-soluble compounds. Chemosphere, 16(4):833-847. |
| **Other (source): (FT-SO)** | American Petroleum Institute, Petroleum HPV Technical Work Group. |

| | |
|---|---|
| **Test Substance:** | Calcium Stearate; CAS No. 1592-23-0 |
| **Method/Guideline:** | Ready Biodegradability by OECD 301C: Modified MITI Test (I) (OECD, 1981) |
| **Year (guideline):** | 1981 |
| **Type (test type):** | Ready Biodegradability |
| **GLP:** | Not Stated |
| **Year (study performed):** | 1987 |
| **Inoculum:** | Sludge from a domestic sewage treatment plant |
| **Exposure Period:** | 32 days |
| **Test Conditions: (FT-TC)**<br><br>**Note: Concentration prep., vessel type, replication, environmental conditions.** | Two independent 301C tests were conducted with calcium stearate for the purpose of evaluating the following conditions of the experimental design:<br><br>Effect of test substance distribution technique, which included<br><br>- no dispersion of test substance, and<br><br>- dispersion using a carrier.<br><br>The different combinations of the above treatments were as follows:<br><br>**Test — Duration (days) — Agitation — Method of Dispersion**<br><br>A — 32 — + — —<br><br>B — 32 — + — nonylphenol (10EO.5PO)<br><br>The authors state that the inoculum and mineral solutions were prepared according to the OECD guideline. The test was carried out in a HACH manometric respirometer at 22±3°C according to OECD Guideline 301C.<br><br>Sludge was washed twice by centrifugation and re-suspended in the test medium. The final concentration of sludge in the test medium was 30 mg/L. Prior to adding test chemical, the inoculum was incubated for one week at the test temperature to reduce the endogenous respiration rate.<br><br>Direct dispersion was done either by adding calcium stearate as a powder directly to the bottles containing the inoculated medium, or prior emulsification in water with nonylphenol (10EO.5PO) used as the emulsifier. A blank test was set up with the emulsifier at the concentration used in the calcium stearate test and used to correct for oxygen uptake. Agitation was created by a magnetic stirrer with a PTFE-coated rod of 6 cm length rotating at approximately 60 rpm.<br><br>Biodegradation was determined by comparing the oxygen consumption in the test bottles to the theoretical oxygen demand (ThOD) determined for the test substance. |

| | |
|---|---|
| | The ThOD for calcium stearate was cited as 2.74 g $O_2$/g. |
| **Results: (FT-RE)**<br><br>**Units/Value:**<br><br>**Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.** | **Test** / **Degradation (%)** / **10% lag time (approx.) (days)** / **Days to 60% Biodegradation (approx.)**<br>A 91 4 18<br>B 93 4 13<br><br>Calcium stearate was biodegraded by the MITI (I) test when the test substance was either added with an emulsifier or when added neat in powdered form to the test bottles. In both tests, the condition of ready biodegradability (60% within a 10-day window once 10% biodegradation has been achieved) was met. |
| **Conclusion: (FT-CL)** | Calcium stearate was found to be readily biodegradable. |
| **Reliability: (FT-RL)** | (2) Reliable with restrictions. The authors cited a standardized method for measuring ready biodegradability, but the report did not provide complete details of the test methodology used in the study. |
| **Reference: (FT-RE)** | de Morsier, A., J. Blok, P. Gerike, L. Reynolds, H. Wellens, and W.J. Bontinck. 1987. Biodegradability tests for poorly-soluble compounds. Chemosphere, 16(4):833-847. |
| **Other (source): (FT-SO)** | American Petroleum Institute, Petroleum HPV Technical Work Group. |

| | |
|---|---|
| **Substances:** | 12-Hydroxy stearic acid/Calcium Salt |
| **Method/Guideline:** | Similar to EPA 560/6-82-003, CG 2000 |
| **Year (guideline):** | 1982 |
| **Type (test type):** | Ready Biodegradability: Shake Flask Test |
| **GLP (Y/N):** | No |
| **Year (study performed):** | 1990 |
| **Inoculum:** | Activated sludge and soil (unacclimated inoculum) |
| **Exposure Period (Contact Time):** | 28 Days for two trials (the test was run twice) |

| | |
|---|---|
| **Test Conditions:** | Solutions of the test and reference materials were prepared by adding an appropriate amount of the test material (or reference material) to a volumetric flask and dissolving it in methylene chloride. These solutions were added to the appropriate flasks to a final concentration of 10 mg/L carbon and the methylene chloride was allowed to volatilize under a gentle stream of air before the test medium was added.<br><br>The test and reference materials were added to duplicate 2 liter Erlenmeyer flasks followed by enough volume of test medium to yield a 1 liter final volume after inoculum addition. Enough mixed liquor was added to each flask to give a final dry sludge solids concentration of 30 mg/L. In addition, 0.1 g of soil was added to each flask followed by 1 mL of yeast extract solution (0.15 g/L). The blank flasks were treated in an identical manner, except they received methylene chloride without an added substrate. The flasks were tightly closed with rubber stoppers which contained a 10 mL glass KOH trap and an inlet and outlet port both made from glass tubing. The flasks were then placed on a rotary shaker at approximately 150 RPM and at 25 ± 3°C.<br><br>Flask traps were sampled at 1 - 7 day intervals depending on microbial activity. The KOH was removed from the trap and placed in a 50 mL Erlenmeyer flask. The trap was washed with approximately 10 mL of distilled water which was placed in the same 50 mL Erlenmeyer flask as the KOH. The trap contents and wash received 2 mL of a saturated barium chloride solution and 0.1 mL of phenolphthaline indicator. The solution was then titrated to a colorless endpoint with 0.2N HCl. The trap was recharged with 10 mL of fresh KOH and inserted back into the flask. Prior to being placed onto the shaker, the flask was flushed for several minutes with $CO_2$-free air.<br><br>One day prior to the final sampling, the medium was acidified with 1 mL of concentrated sulfuric acid and left on the shaker for a minimum of 12 hours. |
| **Results:** | In 28 days, 61.5% and 67.6% of the carbon in the test substance was converted to $CO_2$-in test #1 and test #2, respectively. In the same time period, 82.2% and 79.0% of the carbon in the positive control (Rapeseed oil) was converted to $CO_2$-in test #1 and test #2, respectively.<br><br>12-Hydroxy stearic acid/Calcium Salt did not meet one of the criteria necessary for classification in the OECD biodegradability category of "Ready Biodegradable". To be placed in this category, greater than 60% of a test substance's carbon must be converted to $CO_2$-in 28 days and in addition, once 10% of the material has been converted to $CO_2$, the 60% mark must be reached in 10 days. 12-Hydroxy stearic acid/Calcium Salt exceeded the 60% in 28 days but did not reach the 60% mark within the 10 day window. On day 4, 10% of the carbon in the test material was converted to $CO_2$. On day 14, only 51% of the carbon in the test material was converted to $CO_2$. |
| **Conclusion:** | 12-Hydroxy stearic acid/Calcium Salt is not ready biodegradable. |
| **Reliability:** | 2 - Test not conducted under full GLP regulations. |
| **Reference:** | Stonybrook Laboratories, Inc. 1994. Aerobic Biodegradation Study of 12-Hydroxy stearic acid/Calcium Salt, Study # 64043 |
| **Other (source):** | Aerobic Aquatic Biodegradation, Method CG-2000, Chemical Fate Testing Guidelines, Office of Toxic Substances, Office of Pesticides and Toxic Substances, U.S Environmental Protection Agency, August 1982,<br>EPA 560/6-82-003. |

| | |
|---|---|
| **Substances:** | Lithium Hydroxystearate |
| **Method/Guideline:** | Similar to EPA 560/6-82-003, CG 2000 |
| **Year (guideline):** | 1982 |
| **Type (test type):** | Ready Biodegradability: Shake Flask Test |
| **GLP (Y/N):** | No |
| **Year (study performed):** | 1991 |
| **Inoculum:** | Activated sludge, soil (unacclimated inoculum) |
| **Exposure Period (Contact Time):** | 28 Days |
| **Test Conditions:** | Solutions of the test and positive control materials were prepared by adding an appropriate amount of the test material (or positive control) to a volumetric flask and dissolving it in methylene chloride. These solutions were added to the appropriate flasks to a final concentration of 10 mg/L carbon. The methylene chloride was allowed to volatilize under a gentle stream of air before the test medium was added.<br><br>The test and positive control materials were added to duplicate 2 liter Erlenmeyer flasks followed by 975 mL of test medium, 25 mL of inoculum, and 0.1 g of soil addition. Also, 1 mL of yeast extract solution (0.15 g/L) was added. The flasks were tightly closed with rubber stoppers which contained a 10 mL glass KOH trap and an inlet and outlet port both made from glass tubing. The flasks were then placed on a rotary shaker at approximately 150 RPM and at 25 ± 3°C.<br><br>Flask traps were sampled at 1 - 7 day intervals depending on microbial activity. The KOH was removed from the trap and placed in a 50 mL Erlenmeyer flask. The trap was washed with approximately 10 mL of distilled water which was placed in the same 50 mL Erlenmeyer flask as the KOH. The trap contents and wash received 2 mL of a saturated barium chloride solution and 0.1 mL of phenolphthaline indicator. The solution was then titrated to a colorless endpoint with 0.2N HCl. The trap was recharged with 10 mL of fresh KOH and inserted back into the flask. Prior to being placed onto the shaker, the flask was flushed for several minutes with $CO_2$-free air.<br><br>One day prior to the final sampling, the medium was acidified with 1 mL of concentrated sulfuric acid and left on the shaker for a minimum of 12 hours. |

| | |
|---|---|
| **Results:** | In 28 days, 74.7% of the carbon in the test substance was converted to $CO_2$. In the same time period, 77.7% of the carbon in the positive control (Rapeseed oil) was converted to $CO_2$.<br><br>Lithium Hydroxystearate met both of the criteria necessary for classification in the OECD biodegradability category of "Ready Biodegradable". To be placed in this category, greater than 60% of a test substance's carbon must be converted to $CO_2$-in 28 days and in addition, once 10% of the material has been converted to $CO_2$, the 60% mark must be reached in 10 days. On day 2, 25.2% of the carbon in the test material was converted to $CO_2$. On day 9, 64.2% of the carbon in the test material was converted to $CO_2$. |
| **Conclusion:** | Lithium Hydroxystearate is ready biodegradable. |
| **Reliability:** | 2 - Test not conducted under full GLP regulations. |
| **Reference:** | Stonybrook Laboratories, Inc. 1992. Aerobic Biodegradation Study of Lithium Hydroxystearate, Study # 64539 |
| **Other (source):** | Aerobic Aquatic Biodegradation, Method CG-2000, Chemical Fate Testing Guidelines, Office of Toxic Substances, Office of Pesticides and Toxic Substances, U.S Environmental Protection Agency, August 1982,<br>EPA 560/6-82-003. |

### 3.8 ADDITIONAL REMARKS

**Memo** : Biodegradability of grease and grease thickeners

**Remark** : In order to assess the biodegradability of grease and grease thickeners, it is necessary to have an understanding of the components and manufacture of greases. As described in Section 1.1.1, the principle components making up grease are 1) mineral oil base fluid, 2) alkali metals such as lithium or calcium hydroxides, and 3) various fatty acids. When these individual components are combined in their proper proportions, the mineral oil thickens due to formation of the thickener (i.e., calcium or lithium salts of the fatty acids) and the affinity of the thickener for the base oil (NLGI, 1996). Proportions of the different reactants vary, but thickeners typically contribute 1% to 14% by weight, with the balance being made up of mineral oil and performance additives. Some residual water is generally present (approximately 10% of the thickener, or 0.1% to 1.4%) and indeed necessary for uniform dispersion of the thickener in the oil (NLGI, 1996).

Attempts to produce environmentally friendly greases that are biodegradable have focused primarily on alternatives for the mineral base oil (Grives, 1999; Faci, et al., 2003; Stempfel and Baumann, 2003). With base oil being greater than 65% of greases, it comprises a major component affecting biodegradability of the product. As described in the lubricating base oil HPV test plan and robust summaries (API, 2003), mineral base oils would not be classified as readily biodegradable, but since they consist primarily of hydrocarbons, which are ultimately assimilated by micro-organisms, they are considered to be inherently biodegradable. In ready biodegradation testing, these substances degraded from 1.5% to 29% when tested by the OECD 301B procedure and 31% to 50% when tested by the OECD 301F method (API, 2003).

Faci et al. (2003) compared the biodegradation potential of mineral oil grease with one that had been formulated with vegetable oil. Using the OECD 301F method, biodegradation of the grease formulated with vegetable oil ranged from 62% to 75%, whereas the mineral oil grease achieved 5% to 8% biodegradation. The type of thickener used in the Faci et al. (2003) study was not specified, but Grives (1999) evaluated the biodegradability of vegetable oil-based greases thickened by inorganic clay with two preparations of a lithium hydroxystearate thickener. That study found essentially no difference in biodegradation of vegetable oil greases prepared with the inorganic thickener (75% biodegradation) to those thickened with the organic fatty acid soap (75% and >85% biodegradation).

The thickeners in and of themselves would not be expected to persist in the environment except as part of the grease matrix. This is because they are preparations of fatty acids that are derived from edible animal fats or vegetable oils. Included in this category are stearic acid (C18), 12-hydroxystearic acid (C18), docosanoic acid (C22), hydrogenated castor oil (comprised of ricinoleic and similar acids, C18), and methyl esters of oxidized hydrocarbon waxes (=C18). One lithium salt of a dicarboxylic acid (azelaic, C9) is included in the category as it is commonly used in lithium complex greases. Azelaic acid is manufactured from ricinoleic acid (castor oil). The following biodegradation data include various analogs of some of the fatty acids in this category. These data show that fatty acids similar to those used in grease thickeners may be considered readily biodegradable or at least inherently biodegradable. . Fatty acids undergo aerobic biodegradation by the process of beta-oxidation. Beta-oxidation of the parent fatty acid forms acetate and a new fatty acid of two less carbon atoms. This process repeats itself until the compound is completely broken down. The hydrocarbon will eventually be degraded to $CO_2$ and $H_2O$ (Atlas and Bartha, 1993). For this reason, the length of the fatty acid chain does not preclude biodegradation, but it may take longer to achieve complete mineralization. The beta-oxidation sequence does not necessarily require the presence of molecular oxygen, and fatty acid biodegradation may proceed under anaerobic conditions (Atlas and Bartha, 1993).

Substances in the grease thickeners category are composed of calcium or lithium salts of fatty acids. These fatty acids range in size from 9 to 22 carbon atoms in length and represent substances of plant and animal origin. The following biodegradation data are intended to serve as surrogate estimates of the biodegradation potential of these grease thickeners.

| Substance | No. C atoms | Biodeg % | Method | Source |
|---|---|---|---|---|
| **Surrogates for >C18 Fatty Acid Salts (CAS 4499-91-6; 68603-11-2)** | | | | |
| Docosanoic Acid CAS# 112-85-6 | 22 | 48 - 56 | OECD 301C | UNEP (2001) |
| | | 79 - 96 | OECD 302C | |
| **Surrogates for C16-C18 Fatty Acid Salts (CAS 3159-62-4; 4485-12-5; 5342-16-5; 64754-95-6; 68783-36-8; 7620-77-1; 1592-23-0; 64755-01-7)** | | | | |
| Sodium Stearate | 18 | 89 | Modified Sturm | P&G Chemicals (2003) |

| | | | | |
|---|---|---|---|---|
| Tall Oil CAS# 8002-26-4 | | | | |
| | 16 - 18 | 60 | OECD 301D | Pine Chemical Association (2001) |
| | | 73 | OECD 301F | |
| Tall Oil Fatty Acids CAS# 61790-12-3 | | | | |
| | 16 - 18 | 56 | OECD 301D | Pine Chemical Association (2001) |
| | | 84 | OECD 301F | |
| | | 74 | OECD 301C | |
| Fatty Acids, C16-18 unsaturated branched & linear CAS# 68955-98-6 | | | | |
| | 16 - 18 | 67 | EPA OPPTS 853.110 | Pine Chemical Association (2001) |
| Tall Oil Fatty Acids, K-salt CAS# 61790-44-1 | | | | |
| | 16 - 18 | 79 | EPA OPPTS 853.110 | Pine Chemical Association (2001) |

**Surrogates for C9 Fatty Acid salt (CAS No. 38900-29-7)**

No surrogate data was found for this CAS number. Biodegradation is expected to achieve similar rates to longer chained fatty acids via beta-oxidation metabolic pathway (see technical discussion).

03.12.2004 (1) (2) (5) (8) (17) (24) (25) (26) (30)

## 4.1 ACUTE/PROLONGED TOXICITY TO FISH

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | Oncorhynchus mykiss (Fish, fresh water) |
| **Exposure period** | : | 7 day(s) |
| **Analytical monitoring** | : | No |
| **Method** | : | Reference method for determining acute lethality of effluents to rainbow trout. Environment Canada, EPS 1/RM/13 |
| **Year** | : | 2003 |
| **GLP** | : | No |
| **Test substance** | : | Grease with calcium soap thickener and performance additives |
| **Result** | : | There was 30% fish mortality in the grease treatment and 10% mortality in the control after 7 days. Control and treatment fish group weights were 5.8 and 6.0 g, respectively. |
| **Test condition** | : | Test substance was prepared for testing by spreading 250 g onto 20.3 cm x 25.3 cm glass sheet at a thickness of 1.0 cm.<br>The sheet was placed into a 22 L plastic pail with polyethylene liner and filled with 20 L of dechlorinated tap water as dilution water (loading rate = 12,500 mg grease/l).<br>Dilution water chemistry was not provided in the test report. A test vessel containing only dilution water was used as a control. A separate control with dilution water and an empty glass sheet was also used. There was one replicate per treatment. Ten fish were added to each test vessel (loading density <0.5 g/l) and fish survival was monitored daily for 7 days. At the end of the exposure period, the test fish were weighed. All treatments were pre-aerated at 6.5 ml/min/l. Temperature, pH, conductivity, and dissolved oxygen in the test vessels were monitored daily. During the test, temperature ranged from 14 to 15 °C, pH was 7.9 to 8.0, conductivity was 393 to 457 µS/cm, and dissolved oxygen was 8.6 to 9.1 mg/l. Fish used in testing were obtained from Ackenberry Trout Farms and held 22 days before testing. Fish mortality 7 days before test was <2%.<br>Control fish length ranged from 3.0 to 4.9 cm and fish weight ranged from 0.5 to 0.9 g. |
| **Reliability** | : | (2) valid with restrictions<br>Acceptable study following Environmental Canada test method and conducted by laboratory that is accredited by the Canadian Association of Environmental and Analytical Laboratories. Test report contained sufficient documentation except for dilution water quality parameters. Study lacked analytical monitoring of the test solution and only one unreplicated treatment was used. |

11.01.2005 (13)

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | Oncorhynchus mykiss (Fish, fresh water) |
| **Exposure period** | : | 7 day(s) |
| **Analytical monitoring** | : | No |
| **Method** | : | Reference method for determining acute lethality of effluents to rainbow trout. Environment Canada, EPS 1/RM/13 |
| **Year** | : | 2003 |
| **GLP** | : | No |
| **Test substance** | : | Grease with mixed calcium 12-hydroxystearate and tallow thickener and performance additives |
| **Result** | : | There was no fish mortality in the grease treatment and the control after 7 |

days. Control and treatment fish group weights were 4.5 and 5.6 g, respectively.

**Test condition** : Test substance was prepared for testing by spreading 250 g onto 20.3 cm x 25.3 cm glass sheet at a thickness of 1.0 cm. The sheet was placed into a 22 L plastic pail with polyethylene liner and filled with 20 L of dechlorinated tap water as dilution water (loading rate = 12,500 mg grease/l).
Dilution water chemistry was not provided in the test report. A test vessel containing only dilution water was used as a control. A separate control with dilution water and an empty glass sheet was also used. There was one replicate per treatment. Ten fish were added to each test vessel (loading density <0.5 g/l) and fish survival was monitored daily for 7 days. At the end of the exposure period, the test fish were weighed. All treatments were pre-aerated at 6.5 ml/min/l. Temperature, pH, conductivity, and dissolved oxygen in the test vessels were monitored daily. During the test, temperature ranged from 14 to 15 °C, pH was 7.7 to 8.0, conductivity was 412 to 458 µS/cm, and dissolved oxygen was 8.8 to 9.2 mg/l. Fish used in testing were obtained from Ackenberry Trout Farms and held 22 days before testing. Fish mortality 7 days before test was <2%.
Control fish length ranged from 3.2 to 4.8 cm and fish weight ranged from 0.3 to 0.9 g.

**Reliability** : (2) valid with restrictions
Acceptable study following Environmental Canada test method and conducted by laboratory that is accredited by the Canadian Association of Environmental and Analytical Laboratories. Test report contained sufficient documentation except for dilution water quality parameters. Study lacked analytical monitoring of the test solution and only one unreplicated treatment was used.

11.01.2005 (13)

| | |
|---|---|
| **Test Substance:** | Lithium Hydroxystearate |
| **Method/Guideline:** | EEC Guideline, Acute Toxicity For Fish |
| **Year (guideline):** | 1984 |
| **Type (test type):** | Static 96-Hour Acute Toxicity to Rainbow Trout: Oil / Water Dispersion (OWD) |
| **GLP (Y/N):** | No |
| **Year (study performed):** | 1990 |
| **Species:** | *Oncorhycnhus mykiss* (Rainbow Trout) |
| **Analytical Monitoring:** | Yes |
| **Exposure Period:** | 96 hours |
| **Statistical Method:** | Binomial Probability Analysis |
| **Test Conditions:** | Juvenile rainbow trout used in this study were purchased in a single batch. Acclimation prior to experimentation lasted 5 days; the fish were fed a commercial fish food *ad libitum* and were held at 12 ± 2°C. Mortality was <10% in the 48 hours prior to study initiation. The trout were not fed in the 24 hours preceding the study nor during the conduct of the study.<br>The study was conducted in 10 gallon glass aquaria each containing 30 liters of water. Test chambers were held in a recirculating water bath maintained at 12 ± 2°CThe photoperiod during testing was the same as |

| | |
|---|---|
| | provided during acclimation (16-hour light/8-hour dark).<br><br>The test exposure chambers were dosed within one hour following fish addition and remained uncovered for the duration of the test. The test system was designed to keep the test material in suspension throughout the water column. Each test chamber was equipped with a vertically mounted, motor driven propeller assembly that was housed in a 2-inch diameter PVC cylinder with 2 horizontal apertures near the bottom. The motor speed was adjusted to create a vortex extending 1/4 to 1/2 inch below the water surface. Water and test substance spilling into the top of the column was expelled through the apertures in the cylinder bottom. The solution in each chamber was not renewed during the study's duration.<br><br>The test was initiated by randomly assigning 20 fish to each test chamber. OWD motors were turned on prior to fish addition. The test substance was added within one hour following fish addition. Test fish were exposed to a control and 5 nominal concentrations of Lithium Hydroxystearate (100, 250, 500, 1000, and 2000 ppm). The fish in each test chamber was observed for mortality and/or abnormal behavior at 24-hour intervals following study initiation. |
| **Results:** | Test substance accumulation on the surface of treatments hindered accurate mortality counts until test termination. One mortality was observed in each of the following concentrations at 96 hours: 250 ppm, 1000 ppm, 2000 ppm.<br><br>The 96-hour computer-estimated $LC_{50}$ for Lithium Hydroxystearate was > 2000 ppm, calculated using binomial probability. |
| **Results:** | Throughout the study in the water bath, the temperature ranged from 11.5 - 12.2 °C; the mean pH per test concentration ranged from 7.83 - 7.90 units, the mean dissolved oxygen per test concentration ranged from 8.6 - 9.2 mg/L. Water quality parameters were measured at test initiation and at 24 hour intervals.<br><br>The mean standard length of the fish was 36 mm. The mean weight of the fish was 0.82 g |
| **Conclusion:** | Lithium Hydroxystearate, with an $LC_{50}$ estimated to be > 2000 ppm, is in the "Slightly Toxic" category ($LC_{50}$ 1000 - 5000 ppm). |
| **Reliability:** | 2 - Test not conducted under full GLP regulations. |
| **Reference:** | Stonybrook Laboratories, Inc. 1992. A Static 96-Hour Acute Toxicity Study of Lithium Hydroxystearate to Rainbow Trout, Study # 64580 |
| **Other (source):** | Official Journal of the European Communities, 1984. No. L 251/146-154. C.1. Acute Toxicity for Fish. |

### 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

| | | |
|---|---|---|
| **Type** | **:** | Static |
| **Species** | **:** | Acartia tonsa |
| **Exposure period** | **:** | 48 hour(s) |
| **Analytical monitoring** | **:** | No |
| **Method** | **:** | MAFF/U.K.OCNS/PARCOM |
| **Year** | **:** | 1994 |
| **GLP** | **:** | Yes |

| | | |
|---|---|---|
| **Test substance** | : | Grease with Calcium 12-hydroxystearate (CAS No. 3159-62-4) thickener and performance additives |
| **Result** | : | 48-h $EL_{50}$ >1000 mg/l WAF.<br>Immobilization in the 1000 mg/l WAF of the grease was 13% after 48 h. Control immobilization was 3%. Exposure of A. tonsa to the 1.0 mg/l 3,5-DCP solution resulted in 34% (32 organisms tested) immobilization. Numbers of immobilized A. tonsa were 1, 4, and 11 in the control, 1000 mg/l WAF, and 3,5-DCP reference, respectively.<br>The temperature range recorded during the test was 0.2 °C outside the recommended levels of 18 to 22 °C. This deviation was not considered significant. |
| **Test condition** | : | A 1000 mg/l water accommodated fraction was prepared by stirring 2 g of the grease in 2 L of artificial seawater for 24 h. The solution was allowed to stand for at least 1 h before the aqueous phase was drawn off and used undiluted as the test medium. Artificial seawater was prepared by dissolving artificial seasalts (Tropic Marin, Aquatechnik, Wartenberg, W. Germany) in reverse osmosis purified water to a salinity of 34 ± 2o/oo. Triplicate groups with 10 A. tonsa were exposed to 100 ml of test solution held in 200 ml glass crystallizing dishes. Three dishes containing artificial seawater only served as controls. Three dishes containing 1 mg/l solution of a reference compound 3,5-dichlorophenol (DCP) were also prepared. Aged A. tonsa (23 days old) from laboratory cultures were used. Original stocks were supplied by the Vandkvalitetsinstituttet, Copenhagen, Denmark.<br>Immobilized A. tonsa were recorded and removed from test vessels after 24 and 48 h. At the end of the test a few drops of formalin were added to the test vessels to preserve the organisms for subsequent counting. Test was carried out in temperature controlled room set at 20 ± 2 °C with a 16h light, 8h dark illumination cycle. Test solutions were not renewed or aerated during test. Salinity ranged from 34 to 35o/oo. pH ranged from 8.2 to 8.3. Dissolved oxygen was 7.2 to 7.6 at 0 h and 6.8 to 7.0 at 48 h. Temperature throughout the test was 17.8 to 19.3 °C. |
| **Reliability** | : | (2) valid with restrictions<br>Only one concentration of the grease was tested. Analytical monitoring of the test solutions was not performed. |

11.01.2005 (28)

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | Acartia tonsa |
| **Exposure period** | : | 48 hour(s) |
| **Unit** | : | |
| **Analytical monitoring** | : | No |
| **Method** | : | MAFF/U.K.OCNS/PARCOM |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | Grease with Calcium 12-hydroxystearate (CAS No. 3159-62-4) thickener and performance additives |
| **Result** | : | 48-h $EL_{50}$ >1000 mg/l WAF.<br>Immobilization in the 1000 mg/l WAF of the grease was 20% after 48 h. Control immobilization was 3%. Exposure of A. tonsa to the 1.0 mg/L 3,5-DCP solution resulted in 34% (32 organisms tested) immobilization. Numbers of immobilized A. tonsa were 1, 6, and 11 in the control, 1000 mg/l WAF, and 3,5-DCP reference, respectively.<br>The temperature range recorded during the test was 0.2 °C outside the recommended levels of 18 to 22 °C. This deviation was not considered |

significant.

**Test condition** : A 1000 mg/l water accommodated fraction was prepared by stirring 2 g of the grease in 2 L of artificial seawater for 24 h. The solution was allowed to stand for at least 1 h before the aqueous phase was drawn off and used undiluted as the test medium. Artificial seawater was prepared by dissolving artificial seasalts (Tropic Marin, Aquatechnik, Wartenberg, W. Germany) in reverse osmosis purified water to a salinity of 34 ± 2o/oo. Triplicate groups with 10 A. tonsa were exposed to 100 ml of test solution held in 200 ml glass crystallizing dishes. Three dishes containing artificial seawater only served as controls. Three dishes containing 1 mg/l solution of a reference compound 3,5-dichlorophenol (DCP) were also prepared. Aged A. tonsa (23 days old) from laboratory cultures were used. Original stocks were supplied by the Vandkvalitetsinstituttet, Copenhagen, Denmark.
Immobilized A. tonsa were recorded and removed from test vessels after 24 and 48 h. At the end of the test a few drops of formalin were added to the test vessels to preserve the organisms for subsequent counting. Test was carried out in temperature controlled room set at 20 ± 2 °C with a 16h light, 8h dark illumination cycle. Test solutions were not renewed or aerated during test. Salinity ranged from 34 to 35o/oo. pH ranged from 8.2 to 8.4. Dissolved oxygen was 7.2 to 7.6 at 0 h and 6.8 to 7.0 at 48 h. Temperature throughout the test was 17.8 to 19.3 °C.

**Reliability** : (2) valid with restrictions
Only one concentration of the grease was tested. Analytical monitoring of the test solutions was not performed.

11.01.2005 (27)

### 4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

**Species** : Skeletonema costatum (Algae)
**Exposure period** : 72 hour(s)
**Analytical monitoring** : No
**Method** : MAFF/U.K.OCNS/PARCOM, ISO
**Year** : 1994
**GLP** : Yes
**Test substance** : Grease with Calcium 12-hydroxystearate (CAS No. 3159-62-4) thickener and performance additives

**Method** : Williams test for NOELs
**Result** : In the limit test, the 1000 mg/l WAF of the grease produced significant adverse effects (>90%) on both average specific growth rate and area under the growth curve compared to the control over 72 h.
In the definitive test, the 72-h $EbL_{50}$ was between 100 and 1000 mg/l WAFs (close to 320 mg/l which resulted in 42% reduction).
The 72-h $ErL_{50}$ was between 320 and 1000 mg/l WAFs.
72-h NOEbL = 32 mg/l WAF.
72-h NOErL = 320 mg/l WAF.

| Nominal Conc. (mg/l) | 0-72 h % reduction (area) | 0-72h % reduction (growth rate) | 72 h mean chain conc (million chains/ml) |
|---|---|---|---|
| Control | -- | -- | 0.3 |
| 32 | 5 | -1.1 | 0.3 |
| 100 | 16 | 0.31 | 0.26 |
| 320 | 42 | 1.8 | 0.22 |

| | | | |
|---|---|---|---|
| 1000 | 100 | 100 | 0.007 |
| 3,5-DCP | 22 | -1.8 | 0.3 |

In the definitive test, the mean chain length in the starter culture was 2.6 cells/chain. At test termination, the mean chain lengths were 5.3, 5.3, 4.8, and 4.6 in the control, 32, 100, and 320 mg/l WAFs. No chains were visible in the 1000 mg/l WAF. Mean chain length in a 3,5-DCP flask was 3.7.

The reference compound 3,5-DCP produced reductions in growth rate and area under the growth curve that met the recommended MAFF criteria at 48 h. However, after 72 h only the area under the growth curve met the recommended criteria, since no inhibitory effects on growth rate relative to the control were observed. These results were not considered to invalidate the test as the response criteria was under regulatory review.

The pH change after 72 h in one of the control flask in the definitive test was 1.1 units which exceeded the increase of 1 unit recommended by ISO, but it was not considered to have affected the integrity of the study.

**Test condition** : Two growth inhibition tests were performed. In the initial limit test, the 1000 mg/l WAF was prepared by stirring 2 g of the grease in 2 L of algal media for 24 h. The solution was allowed to stand for at least 1 h before the aqueous phase was drawn off and used undiluted as the test medium. Stock algal media was prepared by adding Analar grade salts in reverse osmosis water. The stock media were added to filtered, autoclaved seawater collected from an oyster hatchery at Reculver on the North Kent coast, UK, to prepare the culture and testing media following ISO recommendations (ISO/TC 147/SC5/WG 5 N/20, 1988). The second and definitive test consisted of WAFs prepared at loading rates of 32, 100, 320, and 1000 mg/l. Test vessels were 250 or 300 ml Erlenmeyer flasks containing 100 ml of test solution, inoculated with S. costatum to give an initial nominal cell concentration of 10,000 cells/ml. Three flasks were prepared for each loading rate and six flasks containing algal media only served as controls. Three flasks containing 100 ml of a 1.5 mg/l solution of 3,5-dichlorophenol (DCP) were also prepared. One uninoculated flask of each loading rate and control served as blanks. Flasks were covered with aluminum foil caps and incubated in a cooled, orbital incubator (100 cycles/min) under constant illumination (~3000 lux).
Chain/particle counts were made from all of the flasks at the start of the test and at 24-h intervals using a Coulter Counter. At test termination, mean chain length of S. costatum was estimated from a subsample from a control flask and one flask from each loading rate by microscopic examination in a Sedgewick Rafter cell. Effects of algal growth were evaluated by comparisons of areas under the growth curve and comparisons of average specific growth rates of the treatments relative to control. S. costatum used in the studies were laboratory cultures derived from a culture obtained from the culture collection maintained at the Scottish Marine Biological Association Laboratory in Oban, Scotland. Temperature ranged from 19.7 to 21.1 °C. pH ranged from 7.8 to 8.4 at 0 h and 8.6 to 9.1 at 72 h.

**Reliability** : (1) valid without restriction
11.01.2005 (28)

**Species** : Skeletonema costatum (Algae)
**Exposure period** : 72 hour(s)
**Analytical monitoring** : No
**Method** : MAFF/U.K.OCNS/PARCOM, ISO
**Year** : 1994

**GLP** : Yes
**Test substance** : Grease with Calcium 12-hydroxystearate (CAS No. 3159-62-4) thickener and performance additives

**Method** : Williams test for NOELs
**Result** : In the initial test and limit test, the 72-h $EbL_{50}$ > 1000 mg/l WAF.
The 72-h $ErL_{50}$ > 1000 mg/l WAF.
72-h NOEbL = 1000 mg/l WAF.
72-h NOErL = 1000 mg/l WAF.

**INITIAL TEST**:

| Nominal Conc. (mg/l) | 0-72 h % reduction (area) | 0-72h % reduction (growth rate) | 72 h mean chain conc (million chains/ml) |
|---|---|---|---|
| Control | ---- | ---- | 0.22 |
| 10 | -40 | -0.36 | 0.3 |
| 32 | -51 | -6.1 | 0.3 |
| 100 | -30 | -3 | 0.28 |
| 320 | -19 | -3.9 | 0.26 |
| 1000 | -18 | -12 | 0.25 |
| 3,5-DCP | 35 | 16 | 0.13 |

**LIMIT TEST**:

| Nominal Conc. (mg/l) | 0-72 h % reduction (area) | 0-72h % reduction (growth rate) | 72 h mean chain conc (million chains/ml) |
|---|---|---|---|
| Control | ---- | ---- | 0.24 |
| 1000 | 27 | 8.4 | 0.19 |

**FINAL DCP TEST**:

| Nominal Conc. (mg/l) | 0-72 h % reduction (area) | 0-72h % reduction (growth rate) | 72 h mean chain conc (million chains/ml) |
|---|---|---|---|
| Control | ---- | ---- | 0.3 |
| 3,5-DCP | 22 | -1.8 | 0.3 |

In the initial test, the mean chain length in the starter culture was 3.3 cells/chain. At test termination, the mean chain lengths were 4.4 and 4.7 in the control and 1000 mg/l WAF. Mean chain length in the starter culture was 3.2 cells/chain in the limit test and 5.3 and 4.8 in the control and 1000 mg/l WAF at the end of the test. Mean chain length in the starter culture was 2.6 cells/chain in the final DCP test and 5.3 and 3.7 in the control and 1.5 mg/l DCP at the end of the test.

The effects of the reference compound 3,5-DCP on average specific growth rate were outside the recommended criteria of 20 to 80% reduction. These results were not considered to affect the validity of the test as the response criteria was under regulatory review.

Temperature range in the initial test was outside the recommended limits although there were no adverse effects on control growth. The pH change after 72 h in the control in the final DCP test was 1.1 units which exceeded the increase of 1 unit recommended by ISO, but it was not considered to have affected the integrity of the study.

**Test condition** : Two growth inhibition tests were performed. In the initial test, WAFs with loading rates of 10, 32, 100, 320, and 1000 mg/l were prepared by adding the appropriate weight of grease to 2L of algal media and stirring for 24 h. The solutions were allowed to stand for at least 1 h before the aqueous phase was drawn off and used undiluted as test media. Stock algal media was prepared by adding Analar grade salts in Millipore Milli-Q filtered water. The stock media were added to filtered, autoclaved seawater collected from an oyster hatchery at Reculver on the North Kent coast, UK, to prepare the culture and testing media following ISO recommendations (ISO/TC 147/SC5/WG 5 N/20, 1988). Test vessels were 250 ml Erlenmeyer flasks containing 100 ml of test solution, inoculated with S. costatum to give an initial nominal cell concentration of 10,000 cells/ml. Three flasks were prepared for each loading rate and six flasks containing algal media only served as controls. Three flasks containing 100 ml of a 1.5 mg/l solution of 3,5-dichlorophenol (DCP) were also prepared. One uninoculated flask of each loading rate and control served as blanks. Flasks were covered with aluminum foil caps and incubated in a cooled, orbital incubator (100 cycles/min) under constant illumination (~3000 lux). Chain/particle counts were made from all of the flasks at the start of the test and at 24-h intervals using a Coulter Counter. At test termination, mean chain length of S. costatum was estimated from a subsample from a control flask and one flask from the 1000 mg/l WAF by microscopic examination in a Sedgewick Rafter cell. Owing to concerns regarding the validity of the first test because of an exceedance in test temperature, a second limit test consisted of control, 1000 mg/l WAF, and 1 mg/l 3,5-DCP was conducted. The 3,5-DCP test was subsequently repeated at the correct concentration of 1.5 mg/l. Effects of algal growth were evaluated by comparisons of areas under the growth curve and comparisons of average specific growth rates of the treatments relative to control.
S. costatum used in the studies were laboratory cultures derived from a culture obtained from the culture collection maintained at the Scottish Marine Biological Association Laboratory in Oban, Scotland. Temperatures ranged from 19.8 to 23.9 °C, 20.0 to 20.4 °C, and 19.7 to 21.1 °C in the initial test, limit test, and the final 3,5-DCP test . In the initial test, pH was 8.1 at 0 h and 8.3 to 8.5 at 72 h. pH ranged from 8.2 to 8.3 at 0 h and 8.9 to 9.0 at 72 h in the limit test. In the final DCP test, control pH was 7.8 at 0 h and 8.9 at 72 h and DCP solution pH was 8.4 at 0 h and 9.0 at 72 h.

**Reliability** : (1) valid without restriction
11.01.2005 (27)

## 4.9 ADDITIONAL REMARKS

**Memo** : Aquatic toxicity of dissociation products of grease thickeners

**Remark** : The physical consistency of grease thickeners (Section 1.1.1), the manner in which they are produced (Section 1.1.1), and their low solubility all contribute to a low risk of exposure to aquatic organisms (Sections 2.14 and 2.6.1). When aquatic organisms were tested against whole grease thickened with calcium soap, calcium 12-hydroxystearate, or mixed tallow and calcium 12-hydroxystearate, either no toxicity was observed, or effects were in the 100 to 1000 mg/l range (see Sections 4.1, 4.2 and 4.3). These values can also be used as read-across data for lithium salts. As shown below for ECOSAR estimates of aquatic toxicity, lithium salts of fatty acids C9 (nonanedioc dilithium salt) and C22 (docosanoic lithium salt) would be expected to show no toxicity at the limit of these compound's water solubility. 12-hydroxy-octadecanoic lithium salt is expected to show only

slight toxicity.

The low hazard of these products extends to their dissociation products, as noted below for various components of grease thickeners and surrogate structures. Although it is not likely that aquatic exposure to dissociation products of grease thickeners will occur, if such instances arise, the toxicity of the fatty acid moiety is expected to be low. These data should be qualified in that they were derived from secondary sources or as modeled estimates and should be considered not reliable until confirm by empirical studies.

| Substance<br>Test<br>Animal | Endpoint | Value<br>mg/l | Source |
|---|---|---|---|
| **Fatty Acids and salts** | | | |
| C9 nonanedioic acid, dilithium salt<br>[water solubility 877 mg/l (WSKOWWIN V1.41)] | | | |
| Fish | $LC_{50}$ | (a) | US EPA 2000b |
| Invertebrate | $EC_{50}$ | (a) | ECOSAR V0.99 |
| Algae$EC_{50}$ | $EC_{50}$ | (a) | |
| C16 palmitic, Na salt<br>[water solubility 33 mg/l (WSKOWWIN V1.41)] | | | |
| Goldfish | Lethal dose | 11 | P & G, 2003 |
| Red killifish | 96-h $LD_{50}$ | (a) | |
| Invertebrates | Lethal conc. | (a) | |
| C18 stearic, Na salt<br>[water solubility 3.3 mg/l (WSKOWWIN V1.41)] | | | |
| Goldfish | Lethal dose | (a) | P & G, 2003 |
| Red killifish | 96-h $LD_{50}$ | (a) | |
| Invertebrates | Lethal conc. | (a) | |
| Algae | $EC_{50}$ | (a) | |
| | NOEC | (a) | |
| C18 (x2) stearic, Ca salt<br>[water solubility <0.1 µg/l (WSKOWWIN V1.41)] | | | |
| Fish | $LC_{50}$ | (a) | US EPA 2000b |
| Invertebrate | $EC_{50}$ | (a) | ECOSAR V0.99 |
| Algae$EC_{50}$ | $EC_{50}$ | (a) | |
| C18 octadecanoic acid, 12 hydroxy- Li salt<br>[water solubility 222 mg/l (WSKOWWIN V1.41) | | | |
| Fish | $LC_{50}$ | 123 | US EPA 2000b<br>ECOSAR V0.99 |
| C22 docosanoic acid<br>[water solubility 0.016 mg/l (UNEP 2001)] | | | |
| Zebrafish | 96-h $LC_{50}$ | (a) | UNEP, 2001 |
| | 14-d $LC_{50}$ | (a) | |
| Invertebrates | 48-h $EC_{50}$ | (a) | |
| | 21-d $EC_{50}$ (repro) | (a) | |
| | 21-d NOEC (repro) | (a) | |

C22 docosanoic acid, Li salt
[water solubility 0.04 mg/l (WSKOWWIN V1.41)]

| | | | |
|---|---|---|---|
| Fish | $LC_{50}$ | (a) | |
| Invertebrates | $EC_{50}$ | (a) | US EPA 2000b |
| Algae | $EC_{50}$ | (a) | ECOSAR V0.99 |

(a) Not toxic at limits of solubility

11.01.2005 (25) (26) (32) (33)

### 5.1.1 ACUTE ORAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | > 5000 mg/kg bw |
| **Species** | : | Rat |
| **Strain** | : | Sprague-Dawley |
| **Sex** | : | Male/female |
| **Number of animals** | : | 10 |
| **Vehicle** | : | Undiluted |
| **Doses** | : | 5000 mg/kg only |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | Lithium complex Grease |
| **Method** | : | Five male and five female fasted rats were given a single oral dose (5000 mg/k) of the test material. The rats were observed 1, 4 and 24 hours after administration of the test material for clinical signs of toxicity and any other pharmacological signs. Body weights were recorded before administration of the test material and again on days 7 and 14.<br>All animals were sacrificed on day 14 and a gross necropsy was performed on each of them. Abnormal observations were recorded. |
| **Result** | : | No clinical signs were observed and no animal died during the study.<br>There was a body weight increase for all animals on the study. At necropsy there were no abnormal observations.<br>The $LD_{50}$ of the test material was greater than 5000 mg/kg. |
| **Test substance** | : | The grease had the following composition<br><br>Wt % base oil ~65<br><br>Thickeners<br>Li 12-hydroxy stearate 13.1%<br>Dilithium azelate 2.6%<br><br>Wt % other additives ~20 |
| **Reliability** | : | (1) valid without restriction |

11.01.2005 (20)

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | > 10000 mg/kg bw |
| **Species** | : | Rat |
| **Strain** | : | Albino |
| **Vehicle** | : | Corn oil |
| **Doses** | : | 0.05-10.0 g/kg |
| **Year** | : | 1982 |
| **GLP** | : | No data |
| **Test substance** | : | Magnesium stearate |
| **Result** | : | The publication states:<br>Given as 25% suspension in corn oil.<br><br>Animals fasted overnight and then given dose ranging from 0.05 to 10.0 g/kg. Animals observed daily for 14 days. All animals at 10.0 g/kg exhibited mild diarrhea. |
| **Reliability** | : | (4) not assignable<br>Information is taken from the report of a Cosmetic ingredient review panel. |

Original data not available.

09.12.2003 (4)

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | 5000 - 15000 mg/kg bw |
| **Species** | : | Rat |
| **Strain** | : | Albino |
| **Vehicle** | : | Propylene glycol |
| **Doses** | : | 0.05, 1, 3 & 15 g/kg |
| **Year** | : | 1982 |
| **GLP** | : | No data |
| **Test substance** | : | Lithium stearate |

**Result** : Lithium stearate was administered in propylene glycol (concentration unspecified) to 30 albino rats (sex not specified).

The publication states:

Animals fasted for 24 hrs. and then given dosages ranging from 0.05 to 15.0 g/kg. Animals dosed at 0.05, 1.0 and 3.0 g/kg showed no toxic effects; all animals administered 15.0 g/kg died within 16 hrs. having exhibited unkempt coats, impaired locomotion and lethargy prior to death.

**Reliability** : (4) not assignable
Information is taken from the report of a Cosmetic ingredient review panel.
Original data not available.

24.12.2003 (4)

### 5.1.3 ACUTE DERMAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | > 3000 mg/kg bw |
| **Species** | : | Rabbit |
| **Strain** | : | New Zealand white |
| **Sex** | : | Male/female |
| **Number of animals** | : | 10 |
| **Vehicle** | : | Undiluted |
| **Doses** | : | 300 mg/kg |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | Lithium complex Grease |

**Method** : Undiluted test material was applied to the shorn dorsal skin of five male and five female NZW rabbits. The applied grease was covered with an occlusive dressing which was left in place for 24 hours. Following the 24 hours exposure period the covering was removed and any residual test material was wiped from the skin using mineral oil and a gauze. Observations were recorded daily throughout the following 14 days. Body weights were recorded prior to application of the test material and again on days 7 and 14. All rabbits were killed by lethal injection and a gross necropsy was performed and a record made of any abnormalities.

**Result** : There were no clinical signs of toxicity during the study and no animals died. Erythema and edema was observed at the treated skin site when the occlusive covering was removed.
At this time average erythema and edema scores were 2.6 and 2 respectively (same average scores for each sex). The skin responses gradually subsided and by day 6 had completely disappeared. Animals

gained weight during the study and no abnormalities were observed at necropsy.
The dermal LD50 was therefore greater than 3000 mg/kg.

**Test substance** : The grease had the following composition

Wt % base oil ~65

Thickeners
Li 12-hydroxy stearate 13.1%
Dilithium azelate 2.6%

Wt % other additives ~20

**Reliability** : (1) valid without restriction
11.01.2005 (19)

### 5.2.1 SKIN IRRITATION

**Species** : Rabbit
**Concentration** : Undiluted
**Exposure** : Semiocclusive
**Exposure time** : 4 hour(s)
**Number of animals** : 6
**Vehicle** : None
**Year** : 1944
**GLP** : Yes
**Test substance** : Lithium complex Grease

**Method** : 0.5 ml of undiluted test material was applied to three separate sites on the shorn dorsal trunks of three male and three female NZW rabbits. Each site was covered with a semiocclusive dressing. One site was abraded, the other two were intact skin.
One of the intact skin sites was only covered for 4 hours and the other two sites were covered for 24 hours. At the end of the exposure periods, residual test material was removed from the skin using gauze and mineral oil.
After patch removal, the test site was examined for erythema and edema and the responses were scored immediately using the standard Draize scale. Skin responses were scored again at 1, 24, 48 and 72 hours after patch removal and again on days 4 through 6.
Body weights of animals were recorded before application of test material and again at the end of the study.

**Result** : No clinical signs of toxicity were observed and all animals gained weight over the course of the study.
Average scores for erythema and edema are as shown in the following table.

| **Time** | **4 hour** | | **24 hour exposure** | | | |
|---|---|---|---|---|---|---|
| | **Erythema** | **Edema** | **Erythema** | | **Edema** | |
| | | | **I*** | **A** | **I** | **A** |
| 0 hrs | 0.7 | 0 | 3.2 | 3.2 | 2.7 | 2.8 |
| 1 hr | 0.7 | 0 | 3.2 | 3.2 | 2.7 | 2.8 |
| 24 hrs | 0.2 | 0.2 | 3 | 3.2 | 2.3 | 2.3 |
| 48 hrs | 0.2 | 0.2 | 2 | 2.2 | 1.7 | 2 |
| 72 hrs | 0.2 | 0 | 1.5 | 1.7 | 1.3 | 1.5 |
| Day 4 | 0 | 0 | 1 | 1 | 0.5 | 0.7 |
| Day 5 | | | 0.2 | 0.2 | 0 | 0 |

| | | |
|---|---|---|
| Day 6 | 0 | 0 | 0 | 0 |

* I = Intact, A = Abraded

The four hour exposures resulted in only slight irritation which had cleared by day 4.

24 hour exposure caused moderate to severe erythema with well defined to severe edema. Skin responses had cleared by day 6 and there was no evidence that abraded skin was more irritated than intact skin.

The calculated Primary irritation indices were:
4 hour exposure 0.38
24 hour exposure 4.92

**Test substance** : The grease had the following composition

Wt % base oil ~65

Thickeners
Li 12-hydroxy stearate 13.1%
Dilithium azelate 2.6%

Wt % other additives ~20

**Reliability** : (1) valid without restriction
11.01.2005 (21)

**Species** : Rabbit
**Concentration** : Undiluted
**Exposure time** : 4 hour(s)
**Number of animals** : 6
**Vehicle** : None
**PDII** : 0
**Result** : Not irritating
**Year** : 1982
**GLP** : No data
**Test substance** : Magnesium stearate

**Method** : Two studies were summarized:
A four hour study of acute dermal corrosion and a 24 hour study for skin irritation.
In both studies 6 albino rabbits were used.
The test material was applied under an occlusive dressing in both studies.
Also in both studies half the test sites were abraded while the other half were intact skin.

The corrosion study was conducted according to the procedure described in 49 CFR 173.240 (a) (1).

**Result** : The primary irritation index in both studies was 0.
**Reliability** : (4) not assignable
Information is taken from the report of a Cosmetic ingredient review panel. Original data not available.
09.12.2003 (4)

### 5.2.2 EYE IRRITATION

| | | |
|---|---|---|
| **Species** | : | Rabbit |
| **Concentration** | : | Undiluted |
| **Dose** | : | 0.1 ml |
| **Number of animals** | : | 6 |
| **Vehicle** | : | None |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | Lithium complex Grease |

**Method** : 0.1 ml of test material was placed in the conjunctival sac of the right eye of six female NZW rabbits. The left eye was untreated and served as control. The eyes were examined at 1, 24, 48 and 72 hours after treatment and again on day 7. Ocular reactions were scored according to the standard Draize scale.
Body weights were recorded at the beginning and the end of the study.

**Result** : Conjunctival redness was observed in all animals 1 hour after application of the test material and in three animals at 24 hours. This conjunctival response continued in one animal for 72 hours but was not seen in any animal after 7 days.
Iritis was observed in only one animal at 24 hours and corneal opacity also occurred at 24 hours in the same animal and this persisted for 24 hours. All eyes were normal after 7 days.

The average Draize scores for 6 rabbits are shown in the following table.

| Time after application of test material | Cornea | Iris | Conjunctivae |
|---|---|---|---|
| 1 hour | 0 | 0 | 10 |
| 24 hours | 0.8 | 0.8 | 3.3 |
| 48 hours | 0.8 | 0 | 2.7 |
| 72 hours | 0 | 0 | 1.3 |
| 7 Days | 0 | 0 | 0 |

**Test substance** : The grease had the following composition

Wt % base oil ~65

Thickeners
Li 12-hydroxy stearate 13.1%
Dilithium azelate 2.6%

Wt % other additives ~20

**Reliability** : (1) valid without restriction
11.01.2005 (22)

| | | |
|---|---|---|
| **Species** | : | Rabbit |
| **Concentration** | : | Undiluted |
| **Comment** | : | Not rinsed |
| **Number of animals** | : | 6 |
| **Vehicle** | : | None |
| **Result** | : | Not irritating |
| **Year** | : | 1982 |
| **GLP** | : | No data |
| **Test substance** | : | Magnesium stearate |

**Result** : The scores were zero on days 1, 2 and 3
**Reliability** : (4) not assignable
Information is taken from the report of a Cosmetic ingredient review panel. Original data not available.

09.12.2003 (4)

## 5.3 SENSITIZATION

| | | |
|---|---|---|
| **Type** | : | Buehler Test |
| **Species** | : | Guinea pig |
| **Concentration** | : | 1st: Induction undiluted occlusive epicutaneous<br>2nd: Challenge undiluted occlusive epicutaneous |
| **Number of animals** | : | 10 |
| **Vehicle** | : | None |
| **Result** | : | Not sensitizing |
| **Year** | : | 1997 |
| **GLP** | : | Yes |
| **Test substance** | : | Lithium complex grease |

**Method** : On the basis of the results of a preliminary irritation screen, it was decided to use undiluted test material for the induction and challenge dosing in the sensitization test.
The test material was applied under a Hilltop chamber to the shorn skin of 10 male and 10 female Guinea pigs. The patches were allowed to remain in place for six hours, after which they were removed and any residual test material was also removed from the skin using a gauze and mineral oil.
The treated sites were examine after each dosing day and scored for dermal irritation at 24 and 48 hours. This dosing and scoring procedure was performed once a week for three weeks.
A concurrent positive control group of five animals (3 males and 2 females) was treated with 0.3% 1-chloro-2,4-dinitrobenzene in 80% ethanol (ethanol in distilled water).
An additional group of ten animals (5 of each sex) was treated with vehicle (mineral oil).

Fourteen days after the last induction dose, the animals were challenged by applying material in the same manner as the induction applications but on a naive site.
The vehicle control group was challenged with mineral oil and test substance.
The positive control group animals were challenged with DNCB at 0.01% and 0.2% in acetone.
All animals were observed for local and systemic effects.
24 hours after challenge, the animals were depilated. After a minimum of 2 hours following depilation the test sites were assessed and graded (24

hour grade) and were graded again after a further 24 hours (48 hour grade).

When skin reactions were graded throughout the study scores were attributed to each test site on a scale of 0-3 for erythema.
After the sensitization doses a score of 1 or more was taken to indicate that sensitization had occurred. Furthermore if the test reactions exceeded the most severe control reactions, the animal was considered to be sensitized.

**Result** : A summary of the challenge scores is given in the following table.

| Test Group | % animals with score at 24 hours 0 | + | 1 | 2 | 3 |
|---|---|---|---|---|---|
| Vehicle control Induced with mineral oil | | | | | |
| Mineral oil challenge | 100 | 0 | 0 | 0 | 0 |
| Test material challenge | 100 | 0 | 0 | 0 | 0 |
| Test material induced with neat test material | | | | | |
| Test material challenge | 100 | 0 | 0 | 0 | 0 |
| Positive control animals induced with 0.3% DNCB | | | | | |
| 0.01% DNCB challenge | 60 | 20 | 20 | 0 | 0 |
| 0.2% DNCB challenge | 0 | 0 | 20 | 0 | 80 |

The positive control data clearly demonstrate the sensitivity of the test method. The test material itself did not cause skin sensitization in this study.

**Test substance** : The grease had the following composition

| | |
|---|---|
| Wt % base oil | ~80 |
| Thickeners | |
| Li 12-hydroxy stearate | 8.8% |
| Dilithium azelate | 1.8% |
| Wt % other additives | ~10 |

**Reliability** : (1) valid without restriction
11.01.2005 (23)

## 5.4 REPEATED DOSE TOXICITY

| | | |
|---|---|---|
| **Type** | : | Sub-chronic |
| **Species** | : | Rat |
| **Sex** | : | Male/female |
| **Strain** | : | Wistar |
| **Route of admin.** | : | Oral feed |
| **Exposure period** | : | 3 Months |
| **Frequency of treatm.** | : | Daily in the diet |
| **Doses** | : | 5, 10 & 20% in the diet |
| **Control group** | : | Yes |
| **NOAEL** | : | 5 % |
| **Year** | : | 1980 |
| **GLP** | : | No data |
| **Test substance** | : | Magnesium stearate |

**Method** : Groups of 20 male and 20 female six week old rats were fed diets containing 5, 10 or 20 magnesium stearate. The diets were semi synthetic

in which sodium caseinate replaced casein. The carbohydrates of the diet were substituted by magnesium stearate as follows:

| Group | Magnesium stearate % in diet | Carbohydrate % in diet |
|---|---|---|
| Contro l | 0 | 67.3 |
| | 5 | 62.3 |
| | 10 | 57.3 |
| | 20 | 47.8 |

The diets fed were considered isocaloric, as stearate has a calorific value of about 9, and a pilot study demonstrated that 35-40% of the stearate is absorbed at a 10% level in the diet. Acidified water (pH 3.5) was available ad libitum.
The animals were weighed once weekly and food utilization and weight gain was calculated for each sex of all groups of rats.
Blood samples were taken from 8 males and 8 females from each group prior to dosing and at 8 and 12 weeks. The following hematological and clinical chemistry determinations were made:

Hematology
Hemoglobin
packed cell volume (PCV)
red cell count
total white cell count
reticulocyte count
differential white cell count.

Clinical chemistry
Glucose
urea
aspartate amino transferase
alkaline phosphatase

At the termination of the study, the rats were sacrificed and the following organs were weighed: thymus, liver, kidneys, adrenals, testes/ovaries, heart, lungs, brain and pituitary.
Samples of the organs listed above and the following tissues were taken for light microscopy: urinary bladder, stomach, duodenum, pancreas, jejunum, cecum, colon, thyroid, parathyroid, triceps, brachial muscle, ischiadic nerve, axillar lymph node, uterus, sternum, eye, Harderian gland, skin and submandibular gland. Microscopic examination was undertaken on the high dose and control animals only.

**Result** : The weight gains of the 20% males were significantly less than he corresponding controls during the first 8 weeks of the study [No actual data given in the publication].
Concomitantly these animals were quiet with slow and unsteady movements. Four males in his group died within the first 2 months and all had stone formation in the lower urinary pathways and the deaths were considered to be related to this finding. One other male in this group was incontinent. In the remaining males, the symptoms receded during the following 4 weeks. There were no clinical effects in females in any group.
A reduction in PCV [P<0.01, but no data provided] was found in the 20% males compared to controls. No other hematological differences were reported.

In addition to the findings reported in the males that died in the 20% group, changes were also found in the renal pelvis and in the lower urinary pathways (due to stone formation) at autopsy in 4 males and one female in

the 20 % group.

The relative liver and kidney weights recorded were as follows:

| Dietary concentration | Sex | Liver g/100g body wt ±SD | Kidney g/100g body wt. ±SD |
|---|---|---|---|
| 0 | M | 3.25±0.21 | 633±48.6 |
| 5 | M | 3.13±0.21* | 614±51.5 |
| 10 | M | 2.99±0.23*** | 599±40.6* |
| 20 | M | 2.82±0.18*** | 640±80.7 |
| 0 | F | 3.30±0.24 | 768±103 |
| 5 | F | 3.33±0.18 | 661±86.5*** |
| 10 | F | 3.31±0.31 | 667±54.0*** |
| 20 | F | 3.16±0.23* | 646±55.8*** |

* P< 0.05
*** P<0.001

Nephrocalcinosis was seen in all females and in 12/20 males in the control group. In 18 of the females nephrocalcinosis was regarded as severe. Slight to moderate nephrocalcinosis was observed in 19/20 of the females in the 20% group and 7/20 of the males were affected only slightly. Deposition of iron was found in various amounts in kidney and in liver, the amount was increased in the liver of both sexes in the 20% group. Liver glycogen showed a marked decrease in males in the 20% group and no difference was found in the females.

The authors comment that :

the occurrence of nephrocalcinosis is a common finding in animals fed semi-synthetic diets. The increased magnesium content of the diet could explain the reduction of nephrocalcinosis in the 20% animals.
A high magnesium content of the diet has also been previously associated with a greater incidence of stone formation in the lower part of the urinary tract.

The authors concluded that:

when liver weight was used as a measure of adverse effect, the no effect level was estimated to be 5% magnesium stearate in the diet, corresponding to 2500 mg/kg body weight.

**Reliability** : (2) valid with restrictions
Few experimental details are provided and detailed results are not included in the publication.
However, the publication does provide useful information on the effects of repeated oral exposure to magnesium stearate.

24.12.2003 (29)

**Type** : Sub-chronic
**Species** : Rat
**Sex** : Male/female
**Strain** : Sprague-Dawley
**Route of admin.** : Gavage
**Exposure period** : 90 days
**Frequency of treatm.** : Daily, seven days each week

| | | |
|---|---|---|
| **Doses** | : | 250, 500 & 1000 mg/kg/day |
| **Control group** | : | Yes, concurrent vehicle |
| **NOAEL** | : | 1000 mg/kg |
| **Year** | : | 1977 |
| **GLP** | : | Yes |
| **Test substance** | : | R960002575 |

**Method** :

Sprague-Dawley rats were used in this study. The animals (males and females) were aged 6 weeks at the beginning of the study.
The test material was administered orally by gavage at doses of 250, 500 or 1000 mg/kg/day in a dose volume of 4 ml/kg to groups of ten male and ten females for each dose level.
Additionally, a group of ten male and ten females served as vehicle controls and for these corn oil alone (4ml/kg) was administered. This treatment was continued daily, seven days each week for 90 days.

Animals were observed twice daily for clinical signs of toxicity. A more thorough examination was undertaken weekly and this included a detailed physical examination for signs of local or systemic toxicity, pharmacological effects and palpation for tissue masses.
Body weights and food intakes were recorded weekly.
At the end of the study, on day 91, and after overnight fasting, animals were killed and blood samples were collected for the following hematological and serum chemistry investigations.

<u>Hematology</u>
Hemoglobin concentration
Hematocrit
Erythrocyte count
Platelet count
Reticulocyte count
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Prothrobin time
Activated partial thromboplastin time
Total and differential leukocyte counts
Erythrocyte morphology
Reticulocyte count

<u>Clinical chemistry</u>
Aspartate aminotransferase
Alanine aminotransferase
Alkaline phosphatase
Blood urea nitrogen
Fasting glucose
Total protein
Albumin
Globulin (calculated)
A/G ratio (calculated)
Creatinine
Total bilirubin
Sodium
Potassium
Chloride
Calcium
Inorganic phosphorus

Gamma-glutamyl transferase

A complete post mortem examination was performed on all animals. This included an examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

The following organs were weighed:
Adrenal glands, brain, kidneys, testes with epididymides, liver and ovaries.

The following tissues were preserved and processed for histological examination.
Adrenal glands (2)
Aorta
Bone (sternum/femur with articular surface)
Brain (medulla/pons, cerebrum and cerebellum)
Epididymis (2)
Esophagus
Eye with optic nerve*
Heart
Kidneys (2)
Large intestine (cecum, colon and rectum)
Lacrimal gland*
Liver (2 sections)
Lung with mainstem bronchi
Lymph node (mediastinal)
Lymph node (mesenteric)
Mammary gland*
Muscle (biceps femoris)*
Nasal turbinates
Nerve (sciatic)
Ovaries (2)
Pancreas
Pituitary
Prostate
Salivary gland (submaxillary)
Seminal vesicles
Skin (treated and untreated)
Small intestine ( duodenum, ileum and jejunum)
Spinal cord (cervical, thoracic, lumbar)*
Spleen
Stomach
Testes
Thymic region
Thyroid (with parathyroids)
Trachea
Urinary bladder
Uterus (body/horns with cervix)
Zymbal's gland*
Macroscopic lesions
Target organs
All the above tissues from all the animals in the high dose group and the controls were examined microscopically, except those indicated * which were preserved but not examined.

Statistical analysis
Body weight, body weight change from week 0, food consumption, hematology and clinical chemistry parameters, terminal organ and body weights and organ/body weight ratios and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval.
A Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric parametric procedures were used.

The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case, standard regression techniques with a test for trend and lack of fit were used.
In the nonparametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1% two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

**Result** : There were no mortalities during the study and there were no treatment-related clinical signs of toxicity. There were no adverse effects of treatment observed during the ophthalmoscopic examinations. Body weights were unaffected by treatment. The food consumption values for the 500 and 1000 mg/kg groups were often higher than the controls. However, they were considered to be within normal ranges and not treatment-related.

All except the following hematological parameters were unaffected by treatment. Those listed below were within the normal range for the laboratory and were not considered to be of toxicological significance.
Prothrombin time increases in males only:
15% in 500 mg/kg/day group
19% in 1000 mg/kg/day group
Activated partial thromboplastin time increase
18% in 250 and 1000 mg/kg/day groups

The only difference in clinical chemistry was a 9% increase in the phosphate levels of the 500 mg/kg/day females. This difference was not considered to be a treatment-related effect.

There were no effects on either organ weights, organ/body weight ratios or organ/brain weight ratios.

There were no macroscopic findings at necropsy and no treatment-related microscopic findings.

The NOAEL was considered to be 1000 mg/kg/day.

**Test substance** : R960002575 is a Lithium complex grease with the following composition
Wt % base oil ~80

Thickeners
Li 12-hydroxy stearate 8.8%
Dilithium azelate 1.8%

Wt % other additives ~10

The test material was prepared as solutions in corn oil at the following concentrations to achieve the desired dose levels.

| Group | Dose group mg/kg/day | Concentration mg/ml | Volume ml/kg |
|---|---|---|---|
| I | 0 | 0 | 4 |
| II | 250 | 62.5 | 4 |
| III | 500 | 125 | 4 |
| IV | 1000 | 250 | 4 |

**Reliability** : (1) valid without restriction
03.12.2004 (10)

**Type** : Sub-acute
**Species** : Rat
**Sex** : Male/female
**Strain** : Sprague-Dawley
**Route of admin.** : Dermal
**Exposure period** : Six hours daily
**Frequency of treatm.** : Daily, five days each week for four weeks
**Post exposure period** :
**Doses** : 525, 1050 & 2100 mg/kg/day
**Control group** : Yes, concurrent vehicle
**NOAEL** : 2100 mg/kg bw
**Method** :
**Year** : 1977
**GLP** : Yes
**Test substance** : R960002575

**Method** : Male and female Sprague-Dawley rats aged approximately 7 and 9 weeks respectively were used in this study.
The test material was applied to the shorn skin of groups of five male and five females for each dose level.
Additionally, a group of five male and five females served as vehicle controls and for these mineral oil alone was applied. The test sites were covered with an occlusive dressing which was left in place for six hours. After this time, the dressings were removed and any residual test material was removed from the skin using a gauze and mineral oil. This treatment was continued daily, five days each week for four weeks.

Animals were observed twice daily for clinical signs of toxicity. A more thorough examination was undertaken weekly and this included a detailed physical examination for signs of local or systemic toxicity, pharmacological effects and palpation for tissue masses.
Body weights and food intakes were recorded weekly.
At the end of the study, and after overnight fasting, animals were killed and blood samples were collected for the following hematological and serum chemistry investigations.

Hematology
Hemoglobin concentration
Hematocrit

Erythrocyte count
Platelet count
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Prothrobin time
Activated partial thromboplastin time
Total and differential leukocyte counts
Erythrocyte morphology
Reticulocyte count

Clinical chemistry
Aspartate aminotransferase
Alanine aminotransferase
Alkaline phosphatase
Blood urea nitrogen
Fasting glucose
Total protein
Albumin
Globulin (calculated)
A/G ratio (calculated)
Creatinine
Total bilirubin
Sodium
Potassium
Chloride
Calcium
Inorganic phosphorus
Gamma-glutamyl transferase

A complete post mortem examination was performed on all animals. This included an examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

The following organs were weighed:
Adrenal glands, brain, kidneys, testes with epididymides, liver and ovaries.

The following tissues were preserved and processed for histological examination.
Adrenal glands (2)
Brain (medulla/pons, cerebrum and cerebellum)
Heart
Kidneys (2)
Liver (2 sections)
Ovaries (2)
Skin (treated and untreated)
Spleen
Testes with epididymides (2)

All the above tissues from all the animals in the high dose group and the controls were examined microscopically.

Statistical analysis
Body weight, body weight change from week 0, food consumption, hematology and clinical chemistry parameters, terminal organ and body

weights and organ/body weight ratios and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval.
A Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not nonparametric parametric procedures were used.

The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case, standard regression techniques with a test for trend and lack of fit were used.
In the nonparametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1% two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

**Result** : All animals survived throughout the study and there were no clinical signs of toxicity and no dermal irritation was observed in the treatment groups.
Body weights were unaffected by treatment except that at four weeks the 2100 mg/kg/day males weighed approximately 3% less than the corresponding controls. However, this difference was not statistically significant.
Food consumption of the treatment groups were generally similar to the controls. A slight increase in food consumption of the mid dose males and high dose females at weeks one and two respectively were not considered to be of biological relevance.
Hematological and clinical chemical parameters, organ weights and microscopic findings were all unaffected by treatment.
It was concluded that the NOAEL was 2100 mg/kg/day.

**Test substance** : R960002575 is a Lithium complex grease with the following composition

Wt % base oil ~80

Thickeners
Li 12-hydroxy stearate 8.8%
Dilithium azelate 1.8%

Wt % other additives ~10

The test material was prepared as solutions in mineral oil at the following concentrations to achieve the desired dose levels.

| Group | Dose group mg/kg/day | Concentration mg/ml | Volume ml/kg |
|---|---|---|---|
| I | 0 | 0 | 2.1 |
| II | 525 | 250 | 2.1 |
| III | 1050 | 500 | 2.1 |
| IV | 2100 | 1000 | 2.1 |

**Reliability** : (1) valid without restriction
03.12.2004 (11)

| | | |
|---|---|---|
| **Type** | : | Sub-chronic |
| **Species** | : | Rat |
| **Sex** | : | Male/female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | Dermal |
| **Exposure period** | : | Six hours daily |
| **Frequency of treatm.** | : | Daily, five days each week for 13 weeks |
| **Post exposure period** | : | |
| **Doses** | : | 525, 1050 & 2100 mg/kg/day |
| **Control group** | : | Yes, concurrent vehicle |
| **NOAEL** | : | 2100 mg/kg |
| **Year** | : | 1997 |
| **GLP** | : | Yes |
| **Test substance** | : | R960002575 |

**Method** :

Male and female Sprague-Dawley rats aged 7 and 9 weeks respectively were used in this study.
The test material was applied to the shorn skin of groups of ten male and ten females at doses of 525, 1050 or 2100 mg/kg/day. Additionally, a group of ten male and ten females served as vehicle controls and for these animals mineral oil alone was applied. The test sites were covered with an occlusive dressing which was left in place for six hours. After this time, the dressings were removed and any residual test material was removed from the skin using a gauze and mineral oil. This treatment was continued daily, five days each week for 13 weeks.

Animals were observed twice daily for clinical signs of toxicity. A more thorough examination was undertaken weekly and this included a detailed physical examination for signs of local or systemic toxicity, pharmacological effects and palpation for tissue masses. Examination of the skin for irritation was undertaken pre-test and then daily during the first week of exposure and weekly thereafter.
Body weights and food intakes were recorded weekly.
At the end of the study, and after overnight fasting, animals were killed and blood samples were collected for the following hematological and serum chemistry investigations.

<u>Hematology</u>
Hemoglobin concentration
Hematocrit
Erythrocyte count
Platelet count
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Prothrobin time
Activated partial thromboplastin time
Total and differential leukocyte counts
Erythrocyte morphology
Reticulocyte count

<u>Clinical chemistry</u>
Aspartate aminotransferase
Alanine aminotransferase
Alkaline phosphatase
Blood urea nitrogen

Fasting glucose
Total protein
Albumin
Globulin (calculated)
A/G ratio (calculated)
Creatinine
Total bilirubin
Sodium
Potassium
Chloride
Calcium
Inorganic phosphorus
Gamma-glutamyl transferase

A complete post mortem examination was performed on all animals. This included an examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

The following organs were weighed:
Adrenal glands, brain, kidneys, testes with epididymides, liver and ovaries.

The following tissues were preserved and processed for histological examination.
Adrenal glands (2)
Aorta
Bone (sternum/femur with articular surface)
Brain (medulla/pons, cerebrum and cerebellum)
Epididymis (2)
Esophagus
Eye with optic nerve*
Heart
Kidneys (2)
Large intestine (cecum, colon and rectum)
Lacrimal gland*
Liver (2 sections)
Lung with mainstem bronchi
Lymph node (mediastinal)
Lymph node (mesenteric)
Mammary gland*
Muscle (biceps femoris)*
Nasal turbinates
Nerve (sciatic)
Ovaries (2)
Pancreas
Pituitary
Prostate
Salivary gland (submaxillary)
Seminal vesicles
Skin (treated and untreated)
Small intestine ( duodenum, ileum and jejunum)
Spinal cord (cervical, thoracic, lumbar)*
Spleen
Stomach
Testes
Thymic region

Thyroid (with parathyroids)
Trachea
Urinary bladder
Uterus (body/horns with cervix)
Zymbal's gland*
Macroscopic lesions
Target organs
All the above tissues from all the animals in the high dose group and the controls were examined microscopically, except those indicated * which were preserved but not examined.

Statistical analysis
Body weight, body weight change from week 0, food consumption, hematology and clinical chemistry parameters, terminal organ and body weights and organ/body weight ratios and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval.
A Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not nonparametric parametric procedures were used.

The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case, standard regression techniques with a test for trend and lack of fit were used.
In the nonparametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1% two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

**Result** : There were no treatment-related deaths and there were no clinical signs of toxicity throughout the study. Although mild skin irritation was seen sporadically, it was not regarded as treatment-related. There were no treatment-related changes seen in the ophthalmoscopic examinations.
Apart from the mid dose males there were no treatment-related effects on body weight. In the case of the mid dose males, they were slightly lower than the controls throughout, but since animals in the higher dose group were unaffected this finding is not considered toxicologically significant.
Food consumption was unaffected by exposure to test material.
There were no biologically significant effects on either the hematology or clinical chemistry determinations that were undertaken.
Terminal organ weights, organ/body weight ratios and organ/brain weight ratios were unaffected by treatment.

There were no treatment-related macroscopic observations at necropsy and after histology, no microscopic changes were observed that were considered to be treatment-related.

**Test substance** : R960002575 is a Lithium complex grease with the following composition

Wt % base oil ~80

Thickeners
Li 12-hydroxy stearate 8.8%
Dilithium azelate 1.8%

Wt % other additives ~10

The test material was prepared as solutions in mineral oil at the following concentrations to achieve the desired dose levels.

| Group | Dose group mg/kg/day | Concentration mg/ml | Volume ml/kg |
|---|---|---|---|
| I | 0 | 0 | 2.1 |
| II | 525 | 250 | 2.1 |
| III | 1050 | 500 | 2.1 |
| IV | 2100 | 1000 | 2.1 |

**Reliability** : (1) valid without restriction
03.12.2004 (12)

**Type** : Sub-chronic
**Species** : Rat and Mouse
**Sex** : Male/female
**Strain** : Rat F344; Mouse B6C3F1
**Route of admin.** : Oral feed
**Exposure period** : 90 days
**Frequency of treatm.** : Continual in the diet
**Doses** : 0.62, 1.25, 2.5, 5 & 10 % in the diet
**Control group** : Yes
**Year** : 1992
**GLP** : Yes
**Test substance** : Castor oil

**Method** : 10 animals of each sex and of each species were used for each dose group.
The treatment groups were fed diets containing either 0.62, 1.25, 2.5, 5 or 10 % castor oil. In addition an extra 10 rats of each sex for each dietary level were fed for 21 days and these animals were used to provide blood samples for hematological and clinical chemical determinations on days 5 and 21, after which they were killed.
The main study animals were observed regularly throughout the study for clinical signs and were also weighed weekly.
Food consumption was also recorded throughout the study.
At the end of the study at 13 weeks, all animals underwent a complete necropsy. Blood samples were taken for the following hematological and clinical chemical measurements.

Hematology: Red blood cell count, examination of red blood cell morphology, hematocrit, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, differential white cell count, reticulocyte count (absolute) and platelet count (absolute).

Clinical chemistry: alkaline phosphatase, albumin, urea nitrogen, creatinine, alanine aminotransferase activity, total bile acids, sorbitol dehydrogenase activity, total protein and creatinine kinase activity.

The following organs were weighed: liver, right kidney, right testicle, heart, thymus and lungs.

The following tissues were examined histopathologically in all control and high dose rats and mice: Adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testis or ovaries/uterus, esophagus, eyes (if grossly abnormal), femur (including marrow), heart, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, preputial or clitoral glands, rectum, salivary glands, skin, spinal cord and sciatic nerve (if neurological signs present), spleen, forestomach and glandular stomach, thymus, thyroid gland, trachea, urinary bladder, Zymbal glands, all gross lesions and tissue masses including lymph nodes.
In addition the livers from male rats of all other dose groups were examined.

Reproductive toxicity screen
Sperm motility and sperm density was assessed at necropsy.
Additionally for the 12 days prior to necropsy, females were subject to a vaginal lavage with saline. The aspirate was stained and examined to enable an assessment to be made of the stages of the estrous cycle.

Statistical analysis
Body weight and organ weight data were examined within each sex by one-way analysis of variance followed by Dunnett's t-test if pair-wise comparisons were indicated ($P<0.05$).

**Result** : The following is taken from the abstract of the report:

Exposure to castor oil at dietary concentrations as high as 10% in 13-week studies did not affect survival or body weight gains of rats or mice (10 per sex and dose). There were no biologically significant effects noted in hematologic analyses in rats. Mild increases in total bile acids and in serum alkaline phosphatase were noted at various times during the studies in rats receiving the higher dietary concentrations of castor oil. Liver weights were increased in male rats receiving the 10% dietary concentration and in male and female mice receiving diets containing 5% or 10% castor oil. However, there were no histopathologic lesions associated with these liver changes, nor were there any compound-related morphological changes in any organ in rats or mice. No significant changes were noted in a screening for male reproductive endpoints, including sperm count and motility, and no changes were observed in the length of estrous cycles of rats or mice given diets containing castor oil. Thus, no significant adverse effects of castor oil administration were noted in these studies.

**Test substance** : USP AA grade castor oil was used.
It was incorporated in the diet and checks were made of actual dietary concentrations. These were as follows:

| **Target concentration (%)** | **Actual concentration (%)** |
|---|---|
| 0.62 | 0.62 |
| 1.25 | 1.26 |
| 2.5 | 2.64 |
| 5 | 4.91 |
| 10 | 9.67 |

**Reliability** : (1) valid without restriction
24.12.2003 (18)

## 5.5 GENETIC TOXICITY 'IN VITRO'

**Type** : Ames test
**Result** : Negative
**Test substance** : Magnesium stearate

**Remark** : A cosmetic ingredients review panel concluded that magnesium stearate was not a mutagen in microbial tests with Salmonella typhimurium TA-1535, TA-1537, TA-1538 and Saccharomyces cerevisiae D4 with or without metabolic activation by liver and lung preparations from rats, mice and monkeys.
The panel cited the following as the sources of the information:
FASEB (1976) and Litton Bionetics (1976)

**Reliability** : (4) not assignable
Information taken from a review report. No actual data are given.

03.12.2004 (6) (15)

## 5.7 CARCINOGENICITY

**Species** : Mouse
**Sex** : Male/female
**Strain** : C3H
**Route of admin.** : Dermal
**Exposure period** : 104 weeks
**Frequency of treatm.** : Twice weekly for 104 weeks
**Doses** : 50 mg/application
**Result** : Negative
**Control group** : yes
**GLP** : Yes
**Test substance** : PARL-3093-GR-81

**Method** : 50 mg undiluted test material was applied twice weekly to the shorn interscapular region of 50 male and 50 female C3H mice aged 6-8 weeks. Positive control groups of 50 mice of each sex had 50 mg of a 0.05% solution of BaP in toluene applied twice weekly and these groups served as the positive controls. In addition solvent control groups of 50 mice of each sex received twice weekly applications of 50 mg toluene and a further group of 50 mice of each sex were untreated. The latter groups comprised the solvent and untreated controls respectively.

Applications were continued for 104 weeks or until a horny lesion on the surface of the skin grew to 1 $mm^3$. The lesion was diagnosed as a papilloma and the week that it appeared was recorded. If the tumor grew rapidly, invaded surrounding tissues, or became ulcerated and/or necrotic, it was diagnosed as an "advanced tumor" and the week of the transition was recorded. If a tumor regressed, treatment was resumed and continued until the end of the study or until another papilloma developed. If no growth appeared before death, the animal was recroded as not developing a tumor. If however, a second neoplasm developed, the time of its appearance was used in the calculation of the average latency period for the group.

Animals were observed daily throughout the study for clinical signs of toxicity.
At the termination of treatment, all surviving animals were sacrificed. A complete post mortem examination was carried out on all animals sacrificed at the end of the study and on all animals that either died or were killed during the study because they were moribund.
At the post mortem examination the size and location of all skin neoplasms was recorded. Skin including the neoplasms and any other lesions was removed and placed in fixative for subsequent histopathological examination. Subcutaneous lymph nodes from the neck, ancillary region and groin areas were also removed from the same animals and prepared for subsequent microscopic examination. The chest, abdominal and cranial cavities were examined and all organs were removed and a note made of their gross appearance. Tissues from each organ were preserved for possible microscopic examination.
H & E sections of the skin and of the mammary glands were examined microscopically.

**Result** : The number of mice with histologically-confirmed tumors is shown in the following table.

| No. Mice | No. mice with tumors Malignant | Benign | Latent period (weeks) |
|---|---|---|---|
| Untreated controls | | | |
| 46 males | 0 | 0 | - |
| 50 females | 1 | 2 | - |
| Toluene controls | | | |
| 48 males | 3 | 3 | 87 |
| 50 females | 5 | 2 | 72 |
| Grease | | | |
| 47 males | 0 | 2 | 67 |
| 50 females | 1 | 0 | 82 |
| BaP | | | |
| 46 males | 21 | 5 | 48 |
| 49 females | 45 | 2 | 49 |

It was concluded that the test material was not a skin carcinogen.

**Test substance** : PARL-3093-GR-81 is a Lithium complex grease with the following composition

| | | |
|---|---|---|
| Base oil | approx | 80% wt |
| Li 12-hydroxystearate | | 7.5% wt |
| Other additives | approx | 12% wt |

**Reliability** : (2) valid with restrictions
It should be noted that this study was a study of skin carcinogenicity only.

03.12.2004 (3)

### 5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

**Species** : Rabbit
**Sex** : Female
**Route of admin.** : Gavage
**Frequency of treatm.** : Single dose given
**Doses** : 2.5 mg/kg
**Result** : Negative

| | | |
|---|---|---|
| **Year** | : | 1967 |
| **GLP** | : | No |
| **Test substance** | : | Vehicle containing 5.5% Magnesium stearate |
| **Result** | : | The CIR report states:<br>Fourteen females received the vehicle per os at a dose of 2.5 mg/kg 70 hours post coitus whereas 13 females were given the same dose 192 hours post coitus. Compared with anomalies in the fetuses from 16 untreated mothers (12 of 112 offspring had anomalies) the vehicle containing 5.5% magnesium stearate induced anomalies in 9 out of 86 and 11 out of 90 fetuses respectively, thus demonstrating the absence of teratogenic effect. |
| **Source** | : | Cosmetic Ingredient Panel review (1982) |
| **Test substance** | : | The test substance was a vehicle used to coat pharmaceutical tablets. The coating had the following composition:<br>Polyethylene glycol 27.5 mg<br>Starch 34 mg<br>Talc 27.5 mg<br>Silicon dioxide 5.5 mg<br>Magnesium stearate 5.5 mg |
| **Reliability** | : | (4) not assignable<br>Information is taken from the report of a Cosmetic ingredient review panel. The material tested contained only 5.5% magnesium stearate and the method was inadequate for an evaluation of developmental toxicity. |

03.12.2004 (7)

| | | |
|---|---|---|
| **Species** | : | Various |
| **Remark** | : | Leonard et al reviewed information on the teratogenic effect of lithium compounds.<br>They comment that results have varied in intact animals.<br>Whereas some authors have not demonstrated teratogenic effcects of lithium compounds, others have done so. The malformations reported have included reduced number and weight of the litter, more resorptions, wavy ribs and incomplete ossification.<br>These discrepancies might be due to a different sensitivity of the species and strains used, the stress of daily injections and/or differences in lithium concentrations present in serum during critical periods of development.<br><br>Lithium carbonate given to mice over several days yielding serum levels comparable to those in man treated for manic-depressive disorders did not show any effects, but six times higher doses caused malformations in the offspring.<br>Chronic exposure to lithium at doses that produced serum levels of the same order as seen in patients was toxic but did not affect the entire litter nor was it teratogenic to individual embryos.<br><br>Many authors have reported that lithium causes congenital defects, especially of the cardiovascular system when given to women during the first trimester of pregnancy. As a result registers of "Lithium babies" have been set up. Up till now, analysis of the limited data have demonstrated an effect.<br>The authors conclude that the question of the possible teratogenicity of lithium remains open until further work is done. |
| **Reliability** | : | (4) not assignable |

24.12.2003 (14)

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# High Production Volume Information System (HPVIS)

## Reproductive Toxicity

### TEST SUBSTANCE

| | |
|---|---|
| **Category Chemical:** | 7620-77-1, 53422-16-5, and 68783-36-8 |
| **Test Substance:** | Borated-Lithium 12-Hydroxystearate Complex Generic Grease |
| **Test Substance Purity/Composition and Other Test Substance Comments:** | Borated-Lithium 12-Hydroxystearate Complex Generic Grease was a complex lithium grease that contained approximately 7.9% lithium hydroxystearate (CAS No. 7620-77-1), 0.9% lithium salts of C16-C22 fatty acids (CAS No. 68783-36-8), 20.0% performance additives, and the remainder as base oil. It was the same formulation as “Envelope” Grease. Note that the fatty acids used to make the grease thickeners are not pure chemicals themselves, resulting in a range of carbon lengths for the resulting grease thickeners. Also note that this grease was not a commercial formulation; the percent of additives was intentionally doubled for the purpose of testing the toxicity of a grease with exaggerated levels of additives. |
| **Category Chemical Result Type :** | Measured |
| **Unable to Measure or Estimate Justification :** | |

### METHOD

| | |
|---|---|
| **Route of Administration:** | Dermal |
| **Type of Exposure:** | Non-occluded |
| **Species:** | Rat |
| **Mammalian Strain:** | F344 (VAF/Plus CDF(F344)/CrlBR) |
| **Other Strain:** | None |

| | |
|---|---|
| **Gender:** | Male |
| **Number of Animals per Dose:** | 15 |
| **Concentration:** | 100% |
| **Dose:** | 0, 500, or 2000 mg/kg |
| **Year Study Performed :** | 1993 |
| **Method/Guideline Followed:** | No specific guideline was followed. Study was performed to address a concern limited to effects on the male reproductive system. |
| **GLP:** | Study was conducted in accordance with EPA Good Laboratory Practices. |
| **Exposure Period:** | 10 weeks |
| **Frequency of Treatment:** | 5 days/week |
| **Post-Exposure Period:** | None |
| **Method/Guideline and Test Condition Remarks:** | Test material was applied to the clipped and intact dorsal skin on the animals. The grease was dispensed with a Tridak Dispense system and spread evenly onto the skin with a spatula. Exposure sites were not covered; the animals wore cardboard "Elizabethan" collars to minimize ingestion of the test material. Collars were lined with latex tubing to minimize irritation and were removed on weekends after residual test material was wiped off the back. Controls were handled in the same manner.<br><br>Clinical signs were recorded daily. Individual body weights were recorded weekly throughout the study. Individual food consumption was measured starting during week 5 of the study.<br><br>All animals were euthanized during week 11 of the study. A brief macroscopic examination of the thoracic and abdominal cavities was made. The left vas deferens was excised and the sperm contents were extracted and prepared for sperm motility. The testes, epididymides, left testicular parenchyma, and left cauda epididymis were weighed. Testicular spermatids and the sperm in the cauda epididymis were counted. A portion of the remaining sperm sample was used for evaluation of sperm morphology.<br><br>The right testis and epididymis were preserved. The testis of control and high-dose animals was evaluated histologically using PASH stain. Caput and cauda epididymal |

| | |
|---|---|
| | sections of the same groups were evaluated in H&E sections.<br>Statistical analysis: Quantitative data were analyzed by ANOVA followed by group comparisons using Tukey's test. |
| **Pre-Mating Exposure / Males :** | Not applicable |
| **Pre-Mating Exposure / Females:** | Not applicable |

## TEST RESULTS

**Concentration ( LOAEL / LOAEC / NOAEL / NOAEC )**

| Type | Population: | Value Description: | Value or Lower Concentration: | Upper Concentration: | Units: |
|---|---|---|---|---|---|
| NOAEL | Males | < | 500 | | mg/kg/day |

**Results:**

**Results Remarks:**

Evidence of stress (chromodacryorrhea and red nasal discharge) was observed in animals in all groups. Slight skin irritation was noted in several treated animals. After the first two weeks, body weights in the high-dose group were significantly lower than those of the controls. (See table.)

**Mean Body Weights (g) at Selected Intervals**

| Dose (mg/kg) | 0 | 500 | 2000 |
|---|---|---|---|
| Week 1 | 116 | 116 | 117 |
| Week 3 | 157 | 153 | 145* |
| Week 5 | 191 | 186 | 171* |
| Week 7 | 217 | 213 | 195* |
| Week 9 | 236 | 231 | 213* |
| Week 11 | 252 | 246 | 229* |

*Significantly different from controls (P<0.05)

The decrease in body weight occurred while mean food consumption was increased in the treated groups. (See table.)

**Mean Food Consumption (g/kg/day) at Selected Intervals**

| Dose (mg/kg) | 0 | 500 | 2000 |
|---|---|---|---|
| Day 39 to 43 | 85 | 90 | 94* |
| Day 46 to 50 | 83 | 87 | 92* |
| Day 53 to 57 | 77 | 80 | 87* |

| | | | |
|---|---|---|---|
| Day 60 to 64 | 73 | 77* | 84* |
| Day 67 to 71 | 71 | 75* | 81* |

*Significantly different from controls (P<0.05)

Weight of the epididymides was decreased in treated animals. Weight of the cauda epididymis was also decreased with an accompanying reduction in the number of sperm per cauda. The number of spermatids in the testes was not affected by treatment. Sperm motility and the number of abnormal sperm were also unaffected. No treatment-related changes were observed in the testis or epididymides microscopically. (See table.)

**Mean Values of Selected Endpoints**

| Dose (mg/kg) | 0 | 500 | 2000 |
|---|---|---|---|
| Epididymides weight (g) | 0.776 | 0.741* | 0.726* |
| Cauda epididymis weight (g) | 0.149 | 0.143 | 0.132* |
| No. sperm (x $10^6$)/cauda | 133.5 | 120.8 | 108.9* |
| No. sperm (x $10^6$)/g cauda | 896.8 | 844.0 | 829.1 |
| Testes weight (g) | 2.800 | 2.744 | 2.727 |
| Testis weight (g) | 1.331 | 1.303 | 1.299 |
| No. spermatids (x $10^6$)/testis | 196.6 | 195.8 | 196.6 |
| No. spermatids (x $10^6$)/g testis | 147.5 | 150.4 | 151.7 |

*Significantly different from controls (P<0.05)

**Conclusion:**

Dosing of the test material at 2000 mg/kg/day resulted in decreased weight of the epididymis and cauda epididymis with decreased number of sperm per cauda. Dosing at 500 mg/kg/day resulted in lower weight of the epididymides. Systemic toxicity was present with both doses, as evidenced by lower body weights with 2000 mg/kg/day and increased food consumption with both doses.
The formulation of the test material was the same as for “Envelope” Grease, tested in a separate 13-week dermal study in Sprague-Dawley rats. Neither body weight nor food consumption was significantly affected in that study, possibly indicating a different susceptibility between the two strains of rats.

## RELIABILITY/DATA QUALITY

**Reliability:**

1. Reliable without restriction

**Reliability Remarks:**

**Key Study Sponsor Indicator:**

Key study: Reproductive toxicity assessment in male F344 rats exposed dermally to borated lithium grease.

| REFERENCE | |
|---|---|
| **Reference:** | Reproductive toxicity assessment in male F344 rats exposed dermally to borated lithium grease. 1993. Mobil Environmental and Health Sciences Laboratory Report on Study 64718. |

# High Production Volume Information System (HPVIS)

## Repeated-Dose Toxicity

| Test Substance – Repeated-Dose Toxicity | |
|---|---|
| **Category Chemical:** | 3159-62-4 1592-23-0 64755-01-7 |
| **Test Substance:** | Generic Calcium Complex Grease |
| **Test Substance Purity/Composition and Other Test Substance Comments:** | Generic Calcium Complex Grease contained approximately 3.5% calcium acetate (CAS No. 62-54-4), 3.5% calcium salts of coco fatty acids (CAS No. 64754-97-8), 1.4% calcium salts of C6-C12 fatty acids (CAS No. 69012-90-4), 1.2% calcium salts of tallow fatty acids, hydrogenated (CAS No. 66071-81-6), and the remainder as performance additives and base oil. Note that the fatty acids used to make the grease thickeners are not pure chemicals themselves, resulting in a range of carbon lengths for the resulting grease thickeners. |
| **Category Chemical Result Type:** | Measured |
| **Method – Repeated-Dose Toxicity** | |
| **Route of Administration:** | Dermal |
| **Type of Exposure:** | Non-occluded |
| **Species:** | Rat |
| **Mammalian Strain:** | Sprague Dawley |
| **Gender:** | Male and female |
| **Number of Animals per Dose:** | 10 males/10 females |
| **Dose:** | Applied doses of grease were 0, 500, or 2000 mg/kg/day. In addition, a fourth group was included that received 2000 mg/kg/day of the specific mineral oil that comprised the majority of the grease formulation. |
| **Year Study Performed:** | 1986 |
| **Method/Guideline Followed:** | Similar to OECD 411 (Subchronic Dermal Toxicity: 90-Day Study), but with higher upper dose and additional endpoints |
| **GLP:** | Study was conducted in accordance with EPA Good Laboratory Practices (40 CFR 792) except for subpart referring to characterization of test and control substances. |
| **Exposure Period:** | 13 weeks |

| | |
|---|---|
| **Frequency of Treatment:** | 5 days/week |
| **Post-Exposure Period:** | None |
| **Method/Guideline and Test Condition Remarks:** | Hair was clipped from the dorsal trunk of each animal approximately 24 hours before initial dosing and periodically as necessary during the study. Dosing was performed on weekdays. The grease was delivered by a Tridak grease dispenser and spread evenly over the back with a spatula. Mineral oil was applied with a syringe. Exposure sites were not covered; the animals wore cardboard "Elizabethan" collars to minimize ingestion of the test material. Controls were handled in the same manner.<br><br>Clinical signs were recorded daily. Individual body weights and dermal irritation were recorded weekly throughout the study. Freshly voided urine was analyzed during weeks 5 and 13 for color, clarity, bilirubin, blood, glucose, ketone, protein, pH, specific gravity, and urobilinogen.<br><br>Blood was drawn from fasted animals during weeks 5 and 13 for determination of hematocrit, haemoglobin, and the number of platelets, red blood cells, and white blood cells. MCV, MCH, and MCHC were calculated. White blood cell differentials were performed and morphology of red blood cells was evaluated. Serum was analyzed for alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, globulin, glucose, inorganic phosphorus, potassium, sodium, sorbitol dehydrogenase, total protein, triglycerides, urea nitrogen, and uric acid.<br><br>All animals were euthanized and necropsied at the end of the study. When present, the following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus, and uterus. Sperm morphology was evaluated.<br><br>The following tissues were preserved and those from the control and high-dose groups were examined microscopically by a pathologist: adrenals, bone and marrow (sternum, rib, and femur), brain (3 sections), epididymis, eye and optic nerve, esophagus, Harderian glands, heart and aorta, intestine (cecum, colon, and rectum), intestine (duodenum, jejunum, and ileum), kidneys, lacrimal glands, larynx, liver, lung, lymph nodes (cervical, mesenteric, and those draining treated site), mammary gland, muscle (skeletal from thigh), nerve-peripheral (sciatic), ovaries, pancreas, parathyroids, pituitary, prostate, salivary glands, seminal vesicles, skin (treated area), spinal cord (cervical and thoracic), spleen, stomach (squamous and glandular), testis, thymus, thyroid, tongue, trachea, urinary bladder, uterus (cervix, corpus, horns),vagina, and any gross lesions. |

## Test Results – Repeated-Dose Toxicity

**Concentration (LOAEL/LOAEC/ NOAEL/NOAEC):**

| LOAEL/LOAEC/ NOAEL/NOAEC | Population | Value Description | Value/Lower Concentration | Upper Concentration | Units |
|---|---|---|---|---|---|
| NOAEL | Both sexes | = | 2,000 | | mg/kg /day |
| | | | | | |

**Results Remarks:**

Minimal dermal irritation was reported at the site of dosing of the grease and consisted of slight erythema, flaking of the skin, and, microscopically, hyperplasia of sebaceous glands.

No significant differences related to treatment were reported in body weights, clinical signs, serum chemistry, urinalysis, gross appearance at necropsy, and microscopic examination. No differences were seen in sperm morphology.

Among endpoints for hematology, the number of platelets tended to increase with exposure to the grease, as shown in the following graph. The number of platelets was similarly different in the group treated only with the mineral oil used in the grease.

**Mean Number of Platelets ($10^3$/$mm^3$)**

| | | Grease | | Mineral Oil |
|---|---|---|---|---|
| Sex | 0 mg/kg/day | 500 mg/kg/day | 2000 mg/kg/day | 2000 mg/kg/day |
| Male | 975 | 1045* | 1018 | 1068* |
| Female | 962 | 1000 | 1069 | 1064 |

* Significantly different from controls ($p<0.05$)

Increased absolute or relative weights of liver or kidney were noted in some groups, as shown in the table below.

**Mean Body Weight (BW) and Organ Weights at 13 Weeks in Groups Treated with Generic Calcium Complex Grease**

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Dose (mg/kg) | 0 | 500 | 2000 | 0 | 500 | 2000 |
| BW (g) | 490.2 | 492.7 | 461.1 | 251.9 | 250.4 | 242.4 |
| Kidneys (g) | 3.69 | 3.95 | 3.96 | 2.24 | 2.10 | 2.22 |
| Liver (g) | 14.52 | 17.16* | 16.37* | 9.05 | 8.58 | 9.98* |
| Kidney/BW (%) | 0.75 | 0.80 | 0.86* | 0.89 | 0.84 | 0.92 |
| Liver/BW (%) | 2.96 | 3.48* | 3.56* | 3.59 | 3.43 | 4.12* |

| | |
|---|---|
| | *Statistically different from controls ($p<0.05$)<br><br>These changes were not considered by the study director to be adverse because no confirmatory changes in serum chemistry or microscopic appearance were observed. In addition, similar trends were observed in males treated only with the mineral oil used in the grease (next table), suggesting that the trends may have been related to treatment with that oil.<br><br>**Mean Body Weight (BW) and Organ Weights at 13 Weeks in Groups Treated with Specific Mineral Oil Used in the Grease**<br><br>Male / Female<br>Dose (mg/kg): 0 / 2000 / 0 / 2000<br>BW (g): 490.2 / 452.6* / 251.9 / 240.0<br>Kidneys (g): 3.69 / 3.94 / 2.24 / 2.15<br>Liver (g): 14.52 / 15.24 / 9.05 / 9.03<br>Kidney/BW (%): 0.75 / 0.87* / 0.89 / 0.90<br>Liver/BW (%): 2.96 / 3.37* / 3.59 / 3.77 |
| **Conclusion:** | The NOAEL for Generic Calcium Complex Grease was 2,000 mg/kg/day in a 13-week dermal toxicity study based on a lack of evidence of systemic effects. Slight irritation occurred at the site of application of the grease. Also, the number of circulating platelets tended to increase in the exposed animals, as did weights of liver and kidney. These differences were judged not to represent an adverse effect. |
| **Reliability / Data Quality – Repeated-Dose Toxicity** | |
| **Reliability:** | **1**. Reliable without restriction |
| **Reliability Remarks:** | |
| **Key Study Sponsor Indicator:** | Key study<br>Thirteen-week dermal administration of Generic Calcium Complex Grease to rats. |
| **Reference – Repeated-Dose Toxicity** | |
| **Reference:** | Thirteen-week dermal administration of Generic Calcium Complex Grease to rats. 1988. Mobil Environmental and Health Science Laboratory Final Report 60041. |

# High Production Volume Information System (HPVIS)

## Repeated-Dose Toxicity

| Test Substance – Repeated-Dose Toxicity | |
|---|---|
| **Category Chemical:** | 7620-77-1, 53422-16-5, and 68783-36-8 |
| **Test Substance:** | "Envelope" Grease |
| **Test Substance Purity/Composition and Other Test Substance Comments:** | "Envelope" Grease was a complex lithium grease that contained approximately 7.9% lithium hydroxystearate (CAS No. 7620-77-1), 0.9% lithium salts of C16-C22 fatty acids (CAS No. 68783-36-8), 20.0% performance additives, and the remainder as base oil. Note that the fatty acids used to make the grease thickeners are not pure chemicals themselves, resulting in a range of carbon lengths for the resulting grease thickeners. Also note that this grease was not a commercial formulation; the percent of additives was intentionally doubled for the purpose of testing the toxicity of a grease with exaggerated levels of additives. |
| **Category Chemical Result Type:** | Measured |
| **Method – Repeated-Dose Toxicity** | |
| **Route of Administration:** | Dermal |
| **Type of Exposure:** | Non-occluded |
| **Species:** | Rat |
| **Mammalian Strain:** | Sprague Dawley (Rat/Tac:N(SD)fBR/Taconic, Germantown, NY) |
| **Gender:** | Male and female |
| **Number of Animals per Dose:** | 10 males/10 females |
| **Dose:** | 0, 500, or 2000 mg/kg |
| **Year Study Performed:** | 1995 |
| **Method/Guideline Followed:** | Similar to OECD 411 (Subchronic Dermal Toxicity: 90-Day Study), but with higher upper dose and additional endpoints |
| **GLP:** | Study was conducted in accordance with EPA Good Laboratory Practices. |
| **Exposure Period:** | 13 weeks |
| **Frequency of Treatment:** | 5 days/week |

| | |
|---|---|
| **Post-Exposure Period:** | None |
| **Method / Guideline and Test Condition Remarks:** | Hair was clipped from the dorsal trunk of each animal approximately 24 hours before initial dosing and periodically as necessary during the study. Dosing was performed on weekdays. The grease was delivered by a Tridak grease dispenser and spread evenly over the back with a spatula. Exposure sites were not covered; the animals wore cardboard "Elizabethan" collars to minimize ingestion of the test material. Collars were lined with latex tubing to minimize irritation and were removed on weekends after residual test material was wiped off the back. Controls were handled in the same manner.<br><br>Clinical signs were recorded daily. Individual body weights and dermal irritation were recorded weekly throughout the study. Individual food consumption was measured during the study. Freshly voided urine was analyzed during week 13 for color, clarity, bilirubin, blood, glucose, ketone, protein, pH, specific gravity, and urobilinogen.<br><br>Blood was drawn from fasted animals during weeks 5 and 13 for determination of hematocrit, haemoglobin, and the number of platelets, red blood cells, and white blood cells. MCV, MCH, and MCHC were calculated. White blood cell differentials were performed and morphology of red blood cells was evaluated. Serum was analyzed for alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, glucose, inorganic phosphorus, potassium, sodium, sorbitol dehydrogenase, total protein, triglycerides, urea nitrogen, and uric acid.<br><br>All animals were euthanized and necropsied at the end of the study. When present, the following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus, and uterus.<br><br>The following tissues from the control and high-dose groups were preserved and examined microscopically by a pathologist: adrenals, bone and marrow (sternum), brain (3 sections), epididymis, eye and optic nerve, heart, intestine (colon), intestine (duodenum), kidneys, liver, lung, muscle (skeletal from thigh), nerve-peripheral (sciatic), ovaries, pancreas, salivary gland (submaxillary),skin (2 section from treated area), spleen, stomach (squamous and glandular), testis, thymus, thyroid, urinary bladder, and any gross lesions.<br><br>The left epididymides and testis of males in the control and high-dose group were evaluated for weight of the testicular parenchyma and cauda epididymis, testicular spermatid count, epididymal spermatozoa count, and morphology of spermatozoa.<br><br>Statistical analysis: Quantitative data were analyzed initially by ANOVA and associated F-test, followed by |

Dunnett's Test or Tukey's multiple comparison test with statistical significance in the ANOVA. Data from male reproductive evaluations were analyzed by ANOVA and associated F-test followed by Tukey's test if there was statistical significance with the ANOVA.

**Test Results – Repeated-Dose Toxicity**

**Concentration (LOAEL/LOAEC/ NOAEL/NOAEC):**

| LOAEL/LOAEC/ NOAEL/NOAEC | Population | Value Description | Value/Lower Concentration | Upper Concentration | Units |
|---|---|---|---|---|---|
| NOAEL | Both sexes | < | 500 | | mg/kg /day |

**Results Remarks:**

No abnormal clinical signs related to the test material were noted except for perineal staining in 3 male and 1 female in the high-dose group. Local effects from the collars were observed (neck irritation, chromodacryorrhea, and reddish nasal discharge). Minimal skin irritation was noted at the treatment area. Body weights and food consumption were not affected by treatment. Parameters in urinalysis were not affected by treatment.

In hematology endpoints, minimal, but statistically significant, changes were reported in haemoglobin (Hb) and hematocrit (Hct) as shown in the following table. However, the values for the exposed groups were within the $10^{th}$ and $90^{th}$ percentiles of historical controls, reducing the likelihood that the changes were biologically significant.

**Summary of Selected Hematology Endpoints**

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Dose (mg/kg) | 0 | 500 | 2,000 | 0 | 500 | 2,000 |
| Hb at 5 wk | 16.9 | 16.5 | 16.2* | 16.7 | 17.0 | 16.4 |
| Hb at 13 wk | 17.3 | 16.6 | 16.3* | 16.8 | 16.8 | 16.9* |
| Hct at 5 wk | 48.9 | 47.6 | 46.5* | 47.0 | 47.2 | 45.6 |
| Hct at 13 wk | 51.4 | 49.9 | 49.1 | 49.2 | 48.9 | 47.0 |

*Statistically different from controls ($p<0.05$)

Among endpoints for serum chemistry, statistically significant differences were reported at 13 weeks for aspartate aminotransferase, sorbitol dehydrogenase, and uric acid in males and for sorbitol dehydrogenase,

cholesterol, and blood urea nitrogen in females.

No treatment-related macroscopic changes were noted at necropsy. Absolute and relative liver weights were increased in both sexes. Relative kidney weight was increased in males given 2,000 mg/kg. Spleen weight was increased in females. (See table.) Adrenal weights were not significantly affected by treatment.

**Summary of Mean Body Weight (BW) and Selected Organ Weights at Necropsy**

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Dose (mg/kg) | 0 | 500 | 2,000 | 0 | 500 | 2,000 |
| BW (g) | 423 | 414 | 408 | 254 | 248 | 253 |
| Liver (g) | 13.51 | 14.11 | 16.05* | 7.63 | 8.55 | 9.70* |
| Liver/BW (%) | 3.20 | 3.40 | 3.93* | 3.02 | 3.45* | 3.82* |
| Kidney (g) | 3.36 | 3.24 | 3.50 | 2.02 | 2.05 | 2.07 |
| Kidney/BW (%) | 0.80 | 0.78 | 0.86* | 0.80 | 0.83 | 0.82 |
| Spleen (g) | 0.88 | 0.88 | 0.95 | 0.63 | 0.66 | 0.74* |

*Statistically different from controls ($p<0.05$)

Microscopically, treated skin had an increased incidence and severity of acanthosis and hyperkeratosis of the epidermis and hyperplasia of the sebaceous glands. The increased liver and kidney weights were not accompanied by microscopic changes. However, the increase spleen weight was associated with an increased incidence of hyperplasia of red pulp in the spleen in females receiving 2,000 mg/kg/day, as shown in the following table.

**Incidence of Hyperplasia of Red Pulp**

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Dose (mg/kg) | 0 | 500 | 2,000 | 0 | 500 | 2,000 |
| None | 9 | 8 | 7 | 7 | 7 | 3 |
| Minimal | 1 | 2 | 3 | 3 | 3 | 5 |
| Slight | 0 | 0 | 0 | 0 | 0 | 2 |

Hypertrophy of the follicular epithelial cells of the thyroid was increased in all the treated groups, as shown in the following table.

**Incidence of Hypertrophy of Follicular Epithelium in Thyroid**

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Dose (mg/kg) | 0 | 500 | 2,000 | 0 | 500 | 2,000 |
| None | 8 | 4 | 4 | 10 | 4 | 4 |
| Minimal | 0 | 3 | 1 | 0 | 6 | 3 |
| Slight | 2 | 2 | 5 | 0 | 0 | 3 |
| Moderate | 0 | 1 | 0 | 0 | 0 | 0 |

Among the reproductive endpoints in high-dose males,

| | |
|---|---|
| | testicular parenchyma weight, spermatid number, and percentage of abnormal sperm were lower than in untreated controls. See the following table.<br><br>**Selected Reproductive Parameters in High-Dose Males**<br><br>Dose (mg/kg) \| 0 \| 2,000<br>No. of spermatids ($x10^6$) \| 237 \| 200*<br>Weight of testis (g) \| 1.78 \| 1.67*<br>No. spermatids ($x10^6$)/g testis \| 133 \| 120<br>% abnormal sperm \| 2.9 \| 1.9* |
| **Conclusion:** | A NOAEL of <500 mg/kg/day was reported for Envelope Grease based on slight effects on several organs. Increased kidney weights, increased serum urea nitrogen, and perineal staining observed in the high-dose animals suggested that the kidneys may have been slightly affected by the test material. Increased liver weights, serum cholesterol, and SDH may indicate an effect on the liver. Increased extramedullary hematopoiesis in the spleen and increased spleen weights were probably related to decreased hemoglobin and hematocrit and were thus probably compensatory. Follicular epithelial hyperplasia in the thyroid was also noted. Although testicular endpoints were altered in high-dose males, those effects were slight and were not believed to be biologically significant. |
| **Reliability / Data Quality – Repeated-Dose Toxicity** | |
| **Reliability:** | **1**. Reliable without restriction |
| **Reliability Remarks:** | |
| **Key Study Sponsor Indicator:** | Key study<br>Thirteen-week dermal administration of “Envelope” Grease to rats. |
| **Reference – Repeated-Dose Toxicity** | |
| **Reference:** | Thirteen-week dermal administration of “Envelope” Grease to rats. 1995. Stonybrook Laboratories Inc. Report 66155. |

# High Production Volume Information System (HPVIS)

| Developmental Toxicity/Teratogenicity | |
|---|---|
| **Test Substance** | |
| **Category Chemical: (CAS #)** | 7620-77-1, 53422-16-5, and 68783-36-8 |
| **Test Substance: (CAS #)** | Lithium 12-Hydroxystearate – Generic Grease (SRR 225 A) |
| **Test Substance Purity/Composition and Other Test Substance Comments:** | "Lithium 12-Hydroxystearate – Generic Grease (SRR 225 A)" was a complex lithium grease that contained approximately 8.1% 7620-77-1, 0.9% 68783-36-8, 18.4% performance additives, and the remainder as base oil. Note that the fatty acids used to make the grease thickeners are not pure chemicals themselves, resulting in a range of carbon lengths for the resulting grease thickeners. Also note that this grease was not a commercial formulation; the percent of additives was intentionally doubled for the purpose of testing the toxicity of a grease with exaggerated levels of additives. |
| **Category Chemical Result Type:** | Measured |
| **Method** | |
| **Route of Administration:** | Dermal |
| **Type of Exposure:** | Non-occluded |
| **Species:** | Rat |
| **Mammalian Strain:** | Sprague Dawley (Crl COBS CD(SD)BR) |
| **Gender:** | Female (dosed with test material) and male (used only for mating and not dosed with test material) |
| **Number of Animals per Dose:** | 15 presumed-pregnant females |
| **Dose:** | 0, 500, or 2000 mg/kg/day |
| **Year Study Performed:** | 1989 |
| **Method/Guideline Followed:** | Study was similar to OECD 414 (Prenatal Developmental Toxicity Study). Main differences were that fewer females were used (15/group rather than 20) and the high dose was twice that for an OECD limit dose. |
| **GLP:** | Study was conducted in accordance with EPA Good Laboratory practices. |
| **Exposure Period:** | Gestation days 0 through 19 |

| | |
|---|---|
| **Frequency of Treatment:** | Daily |
| **Post-Exposure Period:** | None |
| **Method/Guideline and Test Condition Remarks:** | Prior to the initiation of dosing with the test material, females were placed with males. Once mating occurred, the individual females were randomly assigned to a treatment group and dosing began for that animal. Lithium grease was applied to the clipped and intact dorsal back of presumed-pregnant females starting on gestation day 0. The grease was dispensed with a Tridak Dispense System and spread evenly with a spatula. The site was not covered; the animals wore "Elizabethan" collars to minimize ingestion of the test material. Collars were lined with latex tubing to minimize irritation. Controls were handled in the same manner. Each female was observed daily for clinical signs. Body weights and food consumption were measured at intervals during gestation.<br><br>Each female was sacrificed on day 20 of gestation. Aortic blood was sampled for analysis of alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, globulin, glucose, iron, inorganic phosphorus, lactate dehydrogenase, potassium, sodium, sorbitol dehydrogenase, total protein, triglycerides, urea nitrogen, and uric acid. Thoracic and abdominal organs were examined grossly. Ovaries and uterus were excised and examined grossly. The number of corpora lutea per ovary and the weight of the gravid uterus were recorded. In the uterus, the number and location of implantations, early and late resorptions, and live and dead fetuses were recorded.<br><br>Each fetus was weighed and grossly examined. Approximately half of the fetuses were used for examination of soft tissues (viscera) using a modification of Wilson's technique. The other half were differentially stained for cartilage and bone, cleared, and examined for skeletal abnormalities.<br><br>Statistical analysis: Data from the maternal biophase, caesarean section, and gross fetal examinations were evaluated by ANOVA followed by group comparisons using Fisher's Exact or Dunnett's Test. Data from skeletal and visceral examination were evaluated by ANOVA followed by group comparisons using Fisher's Exact Test. Data on serum chemistry were evaluated with ANOVA followed by Tukey's multiple comparison test. |

**Test Results**

**Concentration (LOAEL/LOAEC/NOAEL/NOAEC)**

| Type | Population | Value Description | Value/Lower Concentration | Upper Concentration | Units |
|---|---|---|---|---|---|
| NOAEL | Develop | > | 2000 | | mg/kg /day |
| NOEL | Parental females | < | 500 | | mg/kg /day |

**Results Remarks:**

No treatment-related clinical signs were noted during the study except for a dose-related increase in the incidence of dermal irritation at the site of dosing. On day 10, for example, the number of animals with erythema was 0, 3, and 9 in the 0, 500, and 2000 mg/kg/day groups, respectively.

No differences were noted in serum chemistry except for a significant decrease in calcium levels with the high dose (10.4, 10.0, and 9.8 mg/dL for 0, 500, and 2000 mg/kg/day, respectively). The toxicological significance of this change was not known.

Mean body weight in the 2000 mg/kg/day group tended to be lower than the controls throughout the study, as seen in the table; but the mean gains in body weight were not, in general, significantly different.

**Mean maternal body weight (g)**

| Mg/kg/day | 0 | 500 | 2000 |
|---|---|---|---|
| Day 0 | 283.6 | 285.4 | 274.5 |
| Day 3 | 296.8 | 290.4 | 283.1 |
| Day 6 | 313.1 | 310.3 | 294.1* |
| Day 10 | 337.8 | 330.4 | 315.0* |
| Day 13 | 358.5 | 352.6 | 334.1* |
| Day 16 | 383.5 | 376.5 | 358.1* |
| Day 20 | 452.6 | 447.0 | 425.6* |

*Significantly different from control group

When the weight of the gravid uterus is excluded, the net maternal body weight gain for both treated groups was lower than that of the controls. (See table below.) Except for an initial decrease at the start of dosing, food consumption appeared to be unaffected by treatment.

**Mean values of selected weights (g)**

| Mg/kg/day | 0 | 500 | 2000 |
|---|---|---|---|
| Total maternal body weight change, day 0 to 20 | 169 | 162 | 151 |
| Weight of gravid uterus | 82.1 | 89.3 | 83.1 |

| | |
|---|---|
| | <table><tr><td>Maternal weight without gravid uterus</td><td>370.5</td><td>357.7</td><td>342.5*</td></tr><tr><td>Net change in maternal weight from day 0</td><td>86.9</td><td>72.4*</td><td>68.0*</td></tr></table><br>*Significantly different from control group<br><br>Reproductive parameters were unaffected by treatment, including numbers of corpora lutea, implantations, viable fetuses, resorptions, or dams with resorptions. Fetal body weights of either sex were not affected by treatment. No findings related to the test material were noted in the visceral or skeletal examinations. |
| **Conclusion:** | Dermal administration of Lithium Grease to pregnant rats produced moderate skin irritation (erythema) at the site of application and a significant decrease in net maternal body weight gain, a sign of maternal toxicity, in both treated groups. This grease, with its lithium thickeners, did not induce adverse reproductive or developmental effects and is not considered a developmental toxicant under conditions of this screening procedure. |
| **Reliability / Data Quality** | |
| **Reliability:** | **1.**- Reliable without restrictions |
| **Reliability Remarks:** | |
| **Key Study Sponsor Indicator:** | Key study<br>Developmental toxicity study in rats exposed dermally to lithium 12-hydroxystearate – generic grease (SRR 225 A). |
| **Reference** | |
| **Reference:** | Developmental toxicity study in rats exposed dermally to lithium 12-hydroxystearate – generic grease (SRR 225 A).1989. Final report on study 63132 from Mobil Environmental and Health Science laboratory, Princeton, NJ |

# High Production Volume Information System (HPVIS)

## Repeated-Dose Toxicity

| Test Substance – Repeated-Dose Toxicity | |
|---|---|
| **Category Chemical:** | 7620-77-1, 53422-16-5, and 68783-36-8 |
| **Test Substance:** | Mobilgrease HP |
| **Test Substance Purity/Composition and Other Test Substance Comments:** | Mobilgrease HP was a complex lithium grease that contained approximately 7.9% lithium hydroxystearate (CAS No. 7620-77-1), 0.9% lithium salts of C16-C22 fatty acids (CAS No. 68783-36-8), 10.8% performance additives, and the remainder as base oil. Note that the fatty acids used to make the grease thickeners are not pure chemicals themselves, resulting in a range of carbon lengths for the resulting grease thickeners. |
| **Category Chemical Result Type:** | Measured |
| **Method – Repeated-Dose Toxicity** | |
| **Route of Administration:** | Dermal |
| **Type of Exposure:** | Non-occluded |
| **Species:** | Rat |
| **Mammalian Strain:** | Sprague Dawley (Rat/Tac:N(SD)fBR/Taconic, Germantown, NY) |
| **Gender:** | Male and female |
| **Number of Animals per Dose:** | 10 males/10 females |
| **Dose:** | 0, 500, or 2000 mg/kg |
| **Year Study Performed:** | 1987 |
| **Method/Guideline Followed:** | Similar to OECD 411 (Subchronic Dermal Toxicity: 90-Day Study), but with higher upper dose and additional endpoints |
| **GLP:** | Study was conducted in accordance with EPA Good Laboratory Practices. |
| **Exposure Period:** | 13 weeks |
| **Frequency of Treatment:** | 5 days/week |
| **Post-Exposure Period:** | None |

| | |
|---|---|
| **Method / Guideline and Test Condition Remarks:** | Hair was clipped from the dorsal trunk of each animal approximately 24 hours before initial dosing and periodically as necessary during the study. Dosing was performed on weekdays. The grease was delivered by a Tridak grease dispenser and spread evenly over the back with a spatula. Exposure sites were not covered; the animals wore cardboard “Elizabethan” collars to minimize ingestion of the test material. Collars were lined with latex tubing to minimize irritation and were removed on weekends after residual test material was wiped off the back. Controls were handled in the same manner.<br><br>Clinical signs were recorded daily. Individual body weights and dermal irritation were recorded weekly throughout the study. Freshly voided urine was analyzed during weeks 5 and 13 for color, clarity, bilirubin, blood, glucose, ketone, protein, pH, specific gravity, and urobilinogen.<br><br>Blood was drawn from fasted animals during weeks 5 and 13 for determination of hematocrit, haemoglobin, and the number of platelets, red blood cells, and white blood cells. MCV, MCH, and MCHC were calculated. White blood cell differentials were performed. Serum was analyzed for sorbitol dehydrogenase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphate, inorganic phosphorus, urea nitrogen, cholesterol, triglycerides, total protein, albumin, bilirubin, creatinine, glucose, uric acid, potassium, calcium, and sodium.<br><br>All animals were euthanized and necropsied at the end of the study. When present, the following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus, and uterus. The following tissues from the control and high-dose groups were preserved and examined microscopically by a pathologist: adrenals, brain, bone and marrow, eye, heart, large intestine, kidneys, liver, duodenum, lung, ovaries, skeletal muscle, optic nerve, pancreas, sciatic nerve, esophagus, trachea, submaxillary gland, treated skin, spleen, stomach (squamous and glandular), testis, thymus, thyroid, urinary bladder, and any gross lesions. Liver and skin of both sexes and kidneys of males in the low-dose group were similarly examined.<br><br>The left epididymides and testis of males in the control and high-dose group were evaluated for weight of the testicular parenchyma and cauda epididymis, testicular spermatid count, epididymal spermatozoa count, and morphology of spermatozoa.<br><br>Statistical analysis: Quantitative data were analyzed initially by ANOVA and associated F-test, followed by Tukey’s Studentized Range Test or Student-Newman-Keuls multiple comparison test with statistical significance in the ANOVA. |

## Test Results – Repeated-Dose Toxicity

**Concentration (LOAEL/LOAEC/ NOAEL/NOAEC):**

| LOAEL/LOAEC/ NOAEL/NOAEC | Population | Value Description | Value/Lower Concentration | Upper Concentration | Units |
|---|---|---|---|---|---|
| NOAEL | Both sexes | = | 500 | | mg/kg /day |
| NOAEL, reproductive system | Males | = | 2,000 | | mg/kg /day |
| | | | | | |

**Results Remarks:**

No clinical signs related to the test material were noted. Local effects from the collars were observed (neck irritation, chromodacryorrhea, and reddish nasal discharge). Minimal skin irritation was noted at the treatment area. Body weights and parameters in urinalysis were not affected by treatment.

In hematology endpoints, statistically significant changes were reported in haemoglobin (Hb), hematocrit (Hct), and platelets, as shown in the following table. However, the values for the exposed groups were within the $10^{th}$ and $90^{th}$ percentiles of historical controls, reducing the likelihood that the changes were biologically significant.

**Summary of Selected Hematology Endpoints**

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Dose (mg/kg) | 0 | 500 | 2,000 | 0 | 500 | 2,000 |
| Hb | 17.8 | 17.2 | 17.0* | 17.0 | 16.8 | 16.8 |
| Hct | 60.0 | 58.1* | 58.0* | 57.6 | 57.7 | 57.8 |
| Platelet | 1067 | 1038 | 1142* | 1113 | 1085 | 1172 |

*Statistically different from controls ($p<0.05$)

Among endpoints for serum chemistry, statistically significant differences were reported at 13 weeks for creatinine, total protein, phosphorus, and sodium in males and for sorbitol dehydrogenase, phosphorus, and potassium in females. However, values in the treated groups were within the $10^{th}$ and $90^{th}$ percentiles of historical data.

No treatment-related macroscopic changes were noted at necropsy. Absolute and relative liver weights were increased in males at both doses and in females at 2000 mg/kg/day. Relative kidney weight was increased in males given 2,000 mg/kg. (See table.) Adrenal weights were not significantly affected by treatment.

**Summary of Body Weight (BW) and Selected Organ Weights at Necropsy**

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Dose (mg/kg) | 0 | 500 | 2,000 | 0 | 500 | 2,000 |

| | |
|---|---|
| | <table><tr><td>BW (g)</td><td>420.8</td><td>434.5</td><td>401.2</td><td>254.3</td><td>257.3</td><td>247.1</td></tr><tr><td>Liver (g)</td><td>12.50</td><td>15.10*</td><td>14.27*</td><td>7.85</td><td>8.42</td><td>8.90*</td></tr><tr><td>Liver/BW (%)</td><td>2.97</td><td>3.47*</td><td>3.56*</td><td>3.09</td><td>3.28</td><td>3.60*</td></tr><tr><td>Kidney (g)</td><td>3.26</td><td>3.64</td><td>3.48</td><td>2.02</td><td>2.00</td><td>1.98</td></tr><tr><td>Kidney/BW (%)</td><td>0.77</td><td>0.84</td><td>0.87*</td><td>0.80</td><td>0.78</td><td>0.81</td></tr></table><br>*Statistically different from controls ($p<0.05$)<br><br>The only effects noted histologically were epidermal hyperplasia in all dosed groups and slightly increased vacuolation of the adrenal cortex in 4/10 males at 2,000 mg/kg/day. No changes were noted in liver or kidneys that might be related to altered weight of those organs.<br>No differences were noted in any of the reproductive endpoints in males. |
| **Conclusion:** | A NOAEL of 500 mg/kg/day was reported for Mobilgrease HP based on the changes in weight of the liver (without a histological correlate), increased relative kidney weight in males (without a histological correlate), slightly increased vacuolation in adrenal cortex of high-dose males, and marginal changes some endpoints for hematology and serum chemistry. This choice of NOAEL was considered to be “conservative”. |
| **Reliability / Data Quality – Repeated-Dose Toxicity** | |
| **Reliability:** | **1**. Reliable without restriction |
| **Reliability Remarks:** | |
| **Key Study Sponsor Indicator:** | Key study<br>Thirteen-week dermal administration of Mobilgrease HP (RR 207 C-2) to rats. |
| **Reference – Repeated-Dose Toxicity** | |
| **Reference:** | Thirteen-week dermal administration of Mobilgrease HP (RR 207 C-2) to rats. 1992. Mobil Environmental and Health Sciences Laboratory Report 61955. |

# High Production Volume Information System (HPVIS)

| Pharmacokinetics and Metabolism | |
|---|---|
| **Test Substance – Pharmacokinetics and Metabolism** | |
| **Category Chemical:** | 7620-77-1, 53422-16-5, and 68783-36-8 |
| **Test Substance:** | Mobilux EP-2 |
| **Test Substance Purity/Composition and Other Test Substance Comments:** | Mobilux EP-2 was a complex lithium grease that contained approximately 5.6% lithium hydroxystearate (CAS No. 7620-77-1), 0.7% lithium salts of C16-C22 fatty acids (CAS No. 68783-36-8), 7.0% performance additives, and the remainder as base oil. Note that the fatty acids used to make the grease thickeners are not pure chemicals themselves, resulting in a range of carbon lengths for the resulting grease thickeners.<br>The goal of this study was limited to the measurement of *in vivo* dermal penetration of radiolabelled dotriacontane that had been added to the applied grease as a surrogate for the chemical components in the grease. This was not a pharmacokinetic study and no metabolites were measured. |
| **Category Chemical Result Type:** | Measured |
| **Method – Pharmacokinetics and Metabolism** | |
| **Route of Administration:** | Dermal |
| **Type of Exposure:** | Non-occluded |
| **Species:** | Rat |
| **Mammalian Strain:** | Sprague Dawley (Rat/Tac:N(SD)fBR/Taconic, Germantown, NY) |
| **Gender:** | Male and female |
| **Number of Animals per Dose:** | 5 males/5 females |
| **Dose:** | 2000 mg/kg of the grease fortified with $^{14}$C-dotriacontane |
| **Year Study Performed:** | 1986 |
| **Method/Guideline Followed:** | Similar to OECD 417 (Toxicokinetics) |
| **GLP:** | Study was conducted in accordance with EPA Good Laboratory Practices. |
| **Exposure Period:** | 13 weeks for non-labeled Mobilux EP-2 followed by four days for Mobilux EP-2 containing $^{14}$C-dotriacontane |

| | |
|---|---|
| **Frequency of Treatment:** | See description of the method. |
| **Post-Exposure Period:** | None |
| **Method/Guideline and Test Condition Remarks:** | This study was ancillary to a 13-week dermal toxicity study on Mobilux EP-2 (Mobil Environmental and Health Science Laboratory Final Report 52372). Extra groups of untreated controls and animals receiving 2000 mg/kg/day were included in the dosing regimen of that study. During treatment with non-radiolabeled grease, hair was clipped from the dorsal trunk of each animal approximately 24 hours before initial dosing and periodically as necessary during the study. Dosing was performed on weekdays. The grease was delivered by a Tridak grease dispenser and spread evenly over the back with a spatula. Exposure sites were not covered; the animals wore cardboard "Elizabethan" collars to minimize ingestion of the test material. Controls were handled in the same manner except for the actual application of the grease.<br><br>After treatment for 13 weeks with the non-radiolabeled grease, treated animals and untreated controls (5/sex) were dosed dermally once with grease containing $^{14}C$-dotriacontane. Dosing was on the shaved backs of the animals where the non-radiolabeled grease had been applied. A plastic ring with an inside area of 1.3 $cm^2$ was securely attached to the back of each animal with cyanoacrylate and epoxy adhesives. The grease was applied to completely cover the skin within the ring. A wire mesh cover was applied to the ring to prevent the rats from removing the grease. The animals were then fitted with "Elizabethan" collars as an additional measure to prevent ingestion and then placed in individual metabolism cages for daily collection of urine and feces.<br><br>After four days, the animals were sacrificed and the skin removed. Radioactivity in the samples of urine and feces and in the whole body at the time of sacrifice was measured by liquid scintillation counting. |

**Test Results – Pharmacokinetics and Metabolism**

| Dermal Penetration | Population | Value Description | Value/Lower Concentration | Upper Concentration | Units |
|---|---|---|---|---|---|
| Percent of Applied Dose Absorbed | Both sexes | ≤ | 0.9 | | % |
| | | | | | |

<table>
<tr><td>Results Remarks:</td><td>The dermal bioavailability of $^{14}$C-dotriacontane over four days was less than 0.1% of the applied dose among the untreated controls. There was an apparent increase in dermal penetration among the animals previously treated with Mobilux EP-2 for 13 weeks; mean percent of recovered $^{14}$C was 0.9% in males and 0.6% in females.<br>Mean Percent of $^{14}$C-Dotriacontane Recovered (± SD)
<table>
<tr><td></td><td>Urine</td><td>Feces</td><td>Tissues</td><td>Total</td></tr>
<tr><td>Untreated Males</td><td>0.0 ± 0.0</td><td>0.0 ± 0.0</td><td>0.0 ± 0.0</td><td>0.0 ± 0.0</td></tr>
<tr><td>Untreated Females</td><td>0.0 ± 0.0</td><td>0.0 ± 0.0</td><td>0.0 ± 0.0</td><td>0.0 ± 0.0</td></tr>
<tr><td>Males Treated with Mobilux EP-2</td><td>0.0 ± 0.0</td><td>0.0 ± 0.0</td><td>0.9 ± 1.0</td><td>0.9 ± 1.0</td></tr>
<tr><td>Females Treated with Mobilux EP-2</td><td>0.0 ± 0.0</td><td>0.2 ± 0.2</td><td>0.4 ± 0.3</td><td>0.6 ± 0.4</td></tr>
</table>
Given that dotriacontane represented the relatively more soluble components in the oil phase of the grease matrix, the dermal penetration of the physically larger and less mobile grease thickeners would be expected to be significantly much less.</td></tr>
<tr><td>Conclusion:</td><td>Mean bioavailability of $^{14}$C-dotriacontane in dermally applied Mobilux EP-2 grease was less than 0.1% among the untreated controls and $\leq$0.9% among animals previously treated with Mobilux EP-2 for 13 weeks. Given that dotriacontane represented the relatively more soluble components in the oil phase of the grease matrix, the dermal penetration of the physically larger and less mobile grease thickeners would be expected to be significantly much less.</td></tr>
<tr><td colspan="2">Reliability / Data Quality – Pharmacokinetics and Metabolism</td></tr>
<tr><td>Reliability:</td><td>1. Reliable without restriction</td></tr>
<tr><td>Reliability Remarks:</td><td></td></tr>
<tr><td>Key Study Sponsor Indicator:</td><td>Key study<br>Percutaneous absorption of Mobilux EP-2 in the rat.</td></tr>
<tr><td colspan="2">Reference – Pharmacokinetics and Metabolism</td></tr>
<tr><td>Reference:</td><td>Percutaneous absorption of Mobilux EP-2 in the rat. 1988. Mobil Environmental and Health Science Laboratory Final Report 52372A.</td></tr>
</table>

# High Production Volume Information System (HPVIS)

## Repeated-Dose Toxicity

| Test Substance – Repeated-Dose Toxicity | |
|---|---|
| **Category Chemical:** | 7620-77-1, 53422-16-5, and 68783-36-8 |
| **Test Substance:** | Mobilux EP-2 |
| **Test Substance Purity/Composition and Other Test Substance Comments:** | Mobilux EP-2 was a complex lithium grease that contained approximately 5.6% lithium hydroxystearate (CAS No. 7620-77-1), 0.7% lithium salts of C16-C22 fatty acids (CAS No. 68783-36-8), 7.0% performance additives, and the remainder as base oil. Note that the fatty acids used to make the grease thickeners are not pure chemicals themselves, resulting in a range of carbon lengths for the resulting grease thickeners. Also note that the final report from the toxicology lab indicated 9% lithium hydroxystearate, but a more recent calculation of the amount of thickeners yielded the percents given above. |
| **Category Chemical Result Type:** | Measured |
| **Method – Repeated-Dose Toxicity** | |
| **Route of Administration:** | Dermal |
| **Type of Exposure:** | Non-occluded |
| **Species:** | Rat |
| **Mammalian Strain:** | Sprague Dawley (Rat/Tac:N(SD)fBR/Taconic, Germantown, NY) |
| **Gender:** | Male and female |
| **Number of Animals per Dose:** | 10 males/10 females |
| **Dose:** | Applied doses of grease were 0, 300, 1200, or 2000 mg/kg/day. In addition, a fifth group was included that received 1200 mg/kg/day of the specific mineral oil that comprised the majority of the grease formulation. This dose was chosen to approximate the dose of mineral oil in the group given 2000 mg/kg/day of grease. |
| **Year Study Performed:** | 1985-1986 |
| **Method/Guideline Followed:** | Similar to OECD 411 (Subchronic Dermal Toxicity: 90-Day Study), but with higher upper dose and additional endpoints |
| **GLP:** | Study was conducted in accordance with EPA Good Laboratory Practices. |

| | |
|---|---|
| **Exposure Period:** | 13 weeks |
| **Frequency of Treatment:** | 5 days/week |
| **Post-Exposure Period:** | None |
| **Method/Guideline and Test Condition Remarks:** | Hair was clipped from the dorsal trunk of each animal approximately 24 hours before initial dosing and periodically as necessary during the study. Dosing was performed on weekdays. The grease was delivered by a Tridak grease dispenser and spread evenly over the back with a spatula. Mineral oil was applied with a syringe. Exposure sites were not covered; the animals wore cardboard "Elizabethan" collars to minimize ingestion of the test material. Controls were handled in the same manner.<br><br>Clinical signs were recorded daily. Individual body weights and dermal irritation were recorded weekly throughout the study. Freshly voided urine was analyzed during weeks 5, 9, and 13 for color, clarity, bilirubin, blood, glucose, ketone, protein, pH, specific gravity, and urobilinogen.<br><br>Blood was drawn from fasted animals during weeks 5, 9, and 13 for determination of hematocrit, haemoglobin, and the number of platelets, red blood cells, and white blood cells. MCH and MCHC were calculated. White blood cell differentials were performed and morphology of red blood cells was evaluated. Serum was analyzed for alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, globulin, glucose, inorganic phosphorus, iron, potassium, sodium, sorbitol dehydrogenase, total protein, triglycerides, urea nitrogen, and uric acid.<br><br>All animals were euthanized and necropsied at the end of the study. When present, the following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus, thyroid, and uterus.<br><br>The following tissues from the control and high-dose groups were preserved and examined microscopically by a pathologist: adrenals, bone and marrow (sternum), brain (3 sections), eye and optic nerve, heart, intestine (colon), intestine (duodenum), kidneys, liver, lung, ovaries, pancreas, skin (2 section from treated area), spleen, stomach (squamous and glandular), testis, thymus, thyroid, urinary bladder, and any gross lesions.<br><br>The left epididymides of males in the control and high-dose group were evaluated for epididymal spermatozoa count and morphology of spermatozoa.<br><br>Statistical analysis: Quantitative data were analyzed initially by ANOVA, followed by Duncan's multiple range test with statistical significance in the ANOVA. |

## Test Results – Repeated-Dose Toxicity

**Concentration (LOAEL/LOAEC/ NOAEL/NOAEC):**

| LOAEL/LOAEC/ NOAEL/NOAEC | Population | Value Description | Value/Lower Concentration | Upper Concentration | Units |
|---|---|---|---|---|---|
| NOAEL | Both sexes | = | 2,000 | | mg/kg /day |
| | | | | | |

**Results Remarks:**

Minimal dermal irritation was reported at the site of dosing of the grease and consisted of slight erythema and/or flaking of the skin. Microscopically slight hyperplasia, hyperkeratosis of the epidermis, and increased skin thickness were observed in some animals.

No significant differences in treated animals compared to controls were reported in clinical signs, serum chemistry, urinalysis, gross appearance at necropsy, organ weights, and microscopic examination. The only differences that were noted were in hematological endpoints, in which slight, but statistically significant, changes were reported in the number of red blood cells (RBC), hemoglobin (Hb) and hematocrit (Hct) as shown in the following table.

**Summary of Selected Mean Hematology Endpoints in Groups Treated with Grease**

| | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| Dose (mg/kg) | 0 | 300 | 1200 | 2000 | 0 | 300 | 1200 | 2000 |
| Hb at 5 wk | 16.3 | 16.0 | 15.3* | 15.4* | 15.8 | 15.7 | 15.1 | 14.9* |
| Hb at 9 wk | 16.3 | 15.6* | 15.4* | 14.7* | 15.8 | 15.9 | 15.6 | 14.7* |
| Hb at 13 wk | 16.2 | 15.3* | 15.5* | 15.1* | 15.1 | 15.2 | 14.8 | 14.4 |
| | | | | | | | | |
| Hct at 5 wk | 47.5 | 46.6 | 44.8* | 45.4* | 46.6 | 46.3 | 45.1 | 45.3 |
| Hct at 9 wk | 50.5 | 48.8 | 48.2* | 45.9* | 48.6 | 48.6 | 47.1 | 45.4 |
| Hct at 13 wk | 47.1 | 44.9* | 45.2* | 43.7* | 44.3 | 44.9 | 43.3 | 42.4 |

*Statistically different from controls ($p<0.05$)

The differences were slight, the values for the exposed groups were within the ranges of historical controls, and bone marrow was normal. These differences were therefore judged by the study director not to represent toxicity.

| | |
|---|---|
| | In addition, similar decreases were reported in males and females treated only with the mineral oil used in the grease (next table), suggesting that the differences from controls may have been related to treatment with that oil.<br><br>**Summary of Selected Mean Hematology Endpoints in Groups Treated with Specific Mineral Oil Used in the Grease**<br><br>Male / Female<br>Dose (mg/kg): Male 0 / Male 1200 / Female 0 / Female 1200<br>Hb at 5 wk: 16.3 / 15.5* / 15.8 / 14.9*<br>Hb at 9 wk: 16.3 / 15.2* / 15.8 / 15.6<br>Hb at 13 wk: 16.2 / 15.2* / 15.1 / 15.1<br>Hct at 5 wk: 47.5 / 45.6* / 46.6 / 45.0<br>Hct at 9 wk: 50.5 / 47.9* / 48.6 / 46.1*<br>Hct at 13 wk: 47.1 / 44.8* / 44.3 / 44.0<br>*Statistically different from controls ($p<0.05$) |
| **Conclusion:** | The NOAEL for Mobilux EP-2 was 2,000 mg/kg/day in a 13-week dermal toxicity study based on a lack of evidence of systemic effects. Slight irritation occurred at the site of application of the grease. Slight, but statistically significant, changes were reported in the number of red blood cells, hemoglobin and hematocrit. However, the differences were slight, the values for the exposed groups were within the ranges of historical controls, and bone marrow was normal. These differences were therefore judged not to represent an adverse effect. |
| **Reliability / Data Quality – Repeated-Dose Toxicity** | |
| **Reliability:** | **1**. Reliable without restriction |
| **Reliability Remarks:** | |
| **Key Study Sponsor Indicator:** | Key study<br>Thirteen-week dermal administration of Mobilux EP-2 to rats. |
| **Reference – Repeated-Dose Toxicity** | |
| **Reference:** | Thirteen-week dermal administration of Mobilux EP-2 to rats. 1988. Mobil Environmental and Health Science Laboratory Final Report 52372. |