# Robust Summaries

**Melting Point**

| | |
|---|---|
| Type | Melting Point |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-O |

**Method**

| | |
|---|---|
| • Guideline | None |
| • Test Type | Melting Point |
| • GLP | No |
| • Year | Unknown |

**Result**

| | |
|---|---|
| • Melting Point | -95 deg C |
| Remarks Field for Kesults | Handbook data |

**Conclusions**

| | |
|---|---|
| Remarks Field | The melting point is -95 'C |

**Data Quality**

| | |
|---|---|
| • Reliability | Klimisch Code 2. A reliability code of 2 is assigned to data from reference handbooks. |

**References**

1. Lide, D.R. (ed). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press, 1 99 1-l 992.,p. 3-2 18

**Other**

**Boiling Point**

| | |
|---|---|
| **Type** | Boiling Point |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o |

**Method**

| | |
|---|---|
| Guideline | **None** |
| Test Type | Boiling Point |
| GLP | No |
| Year | Unknown |

**Result**

| | |
|---|---|
| Boiling Point | 78 deg C @ 765 mm Hg (1) |
| Remarks Field for Results | Handbook data |

**Conclusions**

| | |
|---|---|
| Remarks Field | Boiling point is 78 deg C @ 765 mm Hg (1) |

**Data Quality**

| | |
|---|---|
| Reliability | Klimisch Code 2. A reliability code of 2 is assigned to data from reference handbooks. |

**References**

1. Lide, D.R. (ed). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press, 199 1-l 992. p. 3-218

**Other**

**Vapor Pressure**

| | |
|---|---|
| **Type** | Vapor Pressure |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o |
| **Method** | |
| • Guideline | None |
| • Test Type | Vapor Pressure |
| • GLP | No |
| • Year | Unknown |
| **Result** | |
| • Vapor Pressure | 70 mm Hg @ 20 Deg C |
| Kemarks Field for Results | Handbook data |
| **Conclusions** | |
| Remarks Field | Vapor pressure is 70 mm Hg at 20 °C |
| **Data Quality** | |
| • Reliability | Klimisch Code 2. A reliability code of 2 is assigned to data from reference handbooks. |
| **References** | Sax, N.I. and R.J. Lewis, Sr. (cds.). Hawley's Condensed Chemical Dictionary. 11 th ed. New York: Van Nostrand Reinhold Co. p 425 (1987). |
| **Other** | |

**Partition Coefficient, Octanol-Water**

| | |
|---|---|
| **Type** | Partition Coefficient, Octanol-Water |
| **'Test Substance** | 1,3-Dioxolane |

**Method**

| | |
|---|---|
| ■ Guideline | Not specified |
| ■ Test Type | Partition Coefficient, Octanol-Water |
| ● GLP | No |
| ● ✡ℳ☺❒ | 1995 |

**Result**

| | |
|---|---|
| ■ Log $k_{o/w}$ | Experimental -0.37' |
| | Calculated by KOWWIN -0.31 [2] |
| Remarks Field for Results | Dioxolane was one of the reference compounds for development of the KOWWIN program (module of EPIWJN). The experimental value is from the literature. The calculated value is the result of the KOWWIN calculation. The experimental value is given priority and the calculated value is supporting. |

**Conclusions**

| | |
|---|---|
| Remarks Field | The log $K_{o/w}$ is approximately -0.35. This material is expected to be relatively water soluble and not bioaccumulate to any significant degree. |

**Data Quality**

| | |
|---|---|
| ■ Reliability | Klimisch Code 2. A reliability code of 2 is generally assigned to literature values not conducted under OECD guidelines or glps. |

**References**


1. Hansch. C., A. Leo and D. Hoekman. Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Professional Reference Book. Wshington, DC: American Chemical Society. 1995.
2. KOWWIN v 1.66, Syracuse Research Corporation, Syracuse, NY (April 2000)


**Other**

## Water Solubility

| | |
|---|---|
| **Type** | Water Solubility |
| **Test Substance** | 1,3-Dioxolane |
| **Method** | |
| • Guideline | None specified |
| • Test Type | Water Solubility |
| . G L P | No |
| • Year | Unknown |
| **Result** | |
| • Solubility | Soluble in water in all proportions. |
| Remarks Field for Results | Handbook data |
| **Conclusions** | |
| Remarks Field | Material is soluble in water in all proportions. |
| **Data Quality** | |
| • Reliability | Klimisch Code 2. A reliability code of 2 is assigned to data from reference handbooks. |
| **References** | 1. Lide, D.R. (ed). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press, 199 1-1992.,p. 3-2 18 |
| **Other** | |

**Photodegradation**

| | |
|---|---|
| **Type** | Photodegradation |
| **Test Substance** | 1 .3-Dioxolanc |
| **Method** | |
| ● Guideline | Estimated Using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) [1] which estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. |
| • Test Type | Photodegradation Estimate |
| ● GLP | No |
| ● Year | **2000** |
| **Results** | |
| ● Result | APOWIN estimated OH rate constant 11.2 x l O-l 2 cm3/molecule-sec |
| Remarks Field for Results | The APOWIN estimate for the reaction rate is based on simple hydrogen abstraction. Similar compounds provide estimates close to measured values for this rate constant. Thus, the method is expected to provide an accurate estimate of the reaction rate constant with hydroxyl radical. Based on the estimated rate constant and using the defaults in APOWIN for atmospheric hydroxyl radical concentration, the estimated half-life is approximately l 1.5 hours. |
| **Conclusions** | |
| Remarks Field | The atmospheric half-life of 1,3-Dioxolane in the atmosphere is estimated to be in the range of 11.5 hours |
| **Data Quality** | |
| ● Reliability | Klimisch Code 2. A reliability code of 2 is assigned a result using an accepted method of estimation. |
| **References** | 1. Syracuse Research Corporation, Syracuse, NY (April 2000) |
| **Other** | |

## Water Stability

**Type** Water Stability

**Test Substance** 1,3-Dioxolane
CAS Number: 646-06-O
Purity 99.98%

**Method**

- Guideline: OECD 111 (1981)
- Test Type: Hydrolysis as a Function of pH
- GLP: Yes
- Year: 2000

Remarks Field for Test Conditions

- Duration: Four days (preliminary test only)
- Analytical Method: Direct injection into GC using flame ionization detector.
- Buffers:

| Target pH | Buffer System | Measured pH at 50' C |
|---|---|---|
| 4.0 | Potassium biphthalate | 3.96 |
| 7.0 | Potassium phosphate | 7.03 |
| 9.0 | Sodium borate | 9.04 |

- Vessels: Amber Teflon-lined screw cap vials
- Replicates: Two at each pH.
- Temperature: 51 ± 0.2° c
- Additional Testing: Not conducted since material showed less than ten percent degradation at 50' in four days.

**Results**

- Nominal Target Concentration: 400 mg/l
- Measured Concentrations

| pH | Measured Concentration (mg/l) | | Percent Degradation |
|---|---|---|---|
| | Initial | Final | At 51° C in 4 days |
| 3.96 | 484 | 574 | 2.1 |
| 7.03 | 345 | 342 | 0.9 |
| 9.04 | 529 | 523 | 1.2 |

- Percent Degradation: Less than 10% at 50' in four days
- Breakdown Products: None

Remarks Field for Results: Data were consistent at all pH values, there may have been slightly more degradation at the lower pH.

**Conclusions**

Remarks Field:

- Dioxolane is stable at pH 4, 7 or 9 for four days at 5 1 ° C under the conditions specified in the OECD 111 Guideline.
- Dioxolane is considered to have a half-life ($t_{1/2}$) at 25 ° C greater than one year at pH 4, 7 and 9.

**Data Quality**

- Reliability: Klimisch Code 1. May be used without restriction.

**Reference**

1,3-Dioxolane CAS No. 646-06-o: Hydrolysis as a Function of pH. Toxikon Corporation, Laboratory Project ID 00J0009f, October 2000.

**Other**

The hydrolysis-modeling program found in EPIWIN contains no valid model for estimating hydrolysis of ethers.

References for supporting studies:

1. HY DROWIN modeling program, version 1.67, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

**Theoretical Distribution (Fugacity)**

**Type** Theoretical Distribution (Fugacity)

**Test Substance** 1,3-Dioxolane

**Method**

- Guideline: Estimated using the MacKay Level III model with standard defaults contained in EPIWIN v 3.05. [1]
- Test Type: Level III Fugacity Model
- GLP: No
- Year: 2000

Remarks Field for Method: Fugacity was calculated using EPIWIN v 3.05 with a Single Level III output based on the Emission values shown below, other parameters used in the model are also given below. The data inputs using the EPIWIN MacKay III model were judged reasonable and adequate for this HPV estimate.

```
Molecular Wt:  74.09
Henry's LC   : 2.45e-005 atm-m3/mole (EPIWIN Henry database)
vapor Press : 104 mm Hg  (Mpbpwin program)
Log Kow      :  -0.3'7  (Kowwin program)
Soil Koc     :  0.1'15  (calc by model)

          Concentration    Half-Life     Emissions
             (percent)        (hr)        (kg/hr)
Air          4.11            23            1000
water        54.1            360           1000
Soil         41.7            360           1000
Sediment     0.0905          1.44e+003     0

          Fugacity     Reaction     Advection    Reaction     Advection
            (atm)       (kg/hr)      (kg/hr)     (percent)    (percent)
Air        1.01e-010     920          306          30.7         10.2
water      6.65e-010     774          402          25.8         13.4
Soil       1.87e-008     597          0            19.9         0
Sediment   5.54e-010     0.324        0.0135       0.0108       0.000449

Persistence Time:  248 hr
Reaction  Time:    324 hr
Advection Time:    1.05e+003 hr
Percent Reacted:   76.4
Percent Advected:  23.6

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin) :
   Air:       23.01
   Water:     360
   Soil   :   760
   Sediment:  1440
     Biowin estimate: 3.018 weeks

Advection Times (hr):
   Air:      100
   water:    1000
   Sediment:  Se+004
```

**Result**

- Distribution
  - o Air **4.1** %
  - 0 Water **54** %
  - 0 Soil **42** %
  - o Sediment 0.1 %

Kemarks Field for Results: This is the currently accepted model for theoretical distribution estimation.

**Conclusions**

Remarks Field: This material is expected to environmentally distribute primarily in water and soil.

**Data Quality**

- Reliability: Klimisch Code 2. A reliability code of 2 is assigned a result using an accepted method of estimation.

**References**

1. Syracuse Research Corporation, Syracuse, NY (April 2000)

**Other**

## Biodegradation

| | |
|---|---|
| **Type** | **Ready Biodegradation** |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o |
| **Method** | |
| ● Guideline | OECD 301D |
| ● Test Type | Keady Biodegradation |
| + GLP | Yes |
| ● Year | 2000 |
| ● Contact Time | 35 Days |
| ● lnnoculum | Municipal Water Treatment Secondary Effluent |
| Remarks for Test Conditions | |
| ◊ Innoculum | ● Fresh domestic water treatment secondary effluent collected about one week prior to test<br>● One ml filtered effluent per liter final concentration.<br>• Not acclimated |
| ◊ Test Material Concentration | ● 3.04 mg/l |
| ◊ Reference Material | • Sodium Benzoate at 3 mg/l<br>● Reference material showed 78% of THOD |
| ◊ Incubation Temperature | ● 22' C in the dark |
| ◊ Sampling Frequency | ● 0, 3, 5, 11, 14, 17, 21, 28 and 35 days<br>● Duplicate bottles sampled |
| ◊ Analytical Method | ● Oxygen consumption |
| ◊ Controls and Blanks | ● Innoculum blank without test material<br>● Positive control using Sodium Benzoate |

**Result**

| | |
|---|---|
| • Degradation Percent after time | 3.7 % Biodegradation in 35 days |
| • Result | Not Readily Biodegradable |
| • Kinetics | Not applicable |
| • Breakdown Products | None determined |
| Remarks Field for Results | Not readily biodegradable |

**Conclusions**

| | |
|---|---|
| Remarks Field | Not readily biodegradable under these conditions |

**Data Quality**

| | |
|---|---|
| • Reliability | Klimisch Code 1. Recent glp study meeting current OECD guideline, may be used without restriction |

**References**

1 ,3-Dioxolane (CAS 646-06-o): Ready Biodegradability: Closed Bottle Test. Toxikon Corporation September 2000, submitted to Ticona and Ferro Corporations

**Other**

This study is supported by an earlier study, sponsored by Celanese, in which Dioxolane was tested for biodegradation using a municipal secondary effluent and measuring oxygen consumption with a manometric respirometer. After 15 days Dioxolane showed 5.2 % of the THOD. [1]

This result is supported by the BIOWIN V4.0 model found in EPIWIN. Two of the three models included predict that Dioxolane will not rapidly biodegrade. [2]

References for Supporting Studies


1. Report to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
2. EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

**Acute Toxicity to Fish**

| | |
|---|---|
| **Type** | Acute Toxicity to Fish |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o<br>Purity 99.98% |
| **Method** | |
| Guideline | OECD 203 (1992) |
| Test Type | Acute Toxicity to Fish |
| GLP | Yes |
| Year | 2000 |
| Species/Strain | Bluegill sunfish ( *Lepomis macrochirus* ) |
| Analytical Monitoring | At the beginning and end of each 24-hour renewal period |
| Exposure Period (unit) | *96* hours |
| Test Details | Static renewal, renewal every 24 hour |
| Statistical Methods | None applied to biology due to lack mortality |

| | | |
|---|---|---|
| Remarks Field for Test Conditions | Fish Size and Age | *Lepomis macrochirus* juveniles, 29 ± 1.9 mm average standard length and 0.57 ± 0.11 g average wet weight. |
| | Test conditions | As specified in OECD 203 |
| | Solvent | None, test material water soluble |
| | Dilution Water Source and Chemistry | Filtered city of Jupiter (Florida) freshwater with an initial hardness and alkalinity of 66 and 28 mg/l as CaC03, respectively and an initial dilution water temperature range of 23.1 to 23.2 °C. Final hardness and alkalinity on day 4 were 74 and 30 mg/l as CaC03, respectively. |

| | |
|---|---|
| ◊ Stock and Test Solutions | A stock solution with target concentration of 10,000 mg/kg was prepared by weighing the test material and dissolving it in dilution water. This stock was diluted to prepare the test solutions each day. Concentration of Dioxolane in both the stock and the test dilutions were determined by gc analysis using a glp validated method. |
| ◊ Vessels and Lighting | The test chambers were 3.5-gallon glass jars (22-cm diameter x 30-cm height) containing 9.0 L of dilution water and providing a water depth of approximately 25 cm. All test chambers were covered throughout the exposure period to reduce evaporation or contamination. The test chambers were positioned in a water bath set to maintain 23 ± 2°C under fluorescent lighting regulated to a photoperiod of 16 hours light and 8 hours darkness. The light intensity ranged between 11.9 and 17.1 microMols per square meter per second |
| ◊ Fish per Vessel and Group | There were IO fish in each vessel, 30 fish (3 vessels) per treatment and control |
| ◊ Dose Selection | Dose selection of 100 mg/l was based on a range-finding study under static conditions with concentrations as high as 1000 mg/l showing no mortality. |
| ◊ Water Chemistry in Control and in One Concentration Where Effects Were Observed | Dissolved oxygen averaged about 8.5 mg/l at the beginning of each renewal period and was found to be between 5.9 and 6.9 mg/l at the end of each 24-hour period. Only one measurement of 48 total was below 6.0 mg/l. The initial pH varied from 7.7 to 8.0 at the beginning of each 24-hour renewal period and ranged from 6.9 to 7.7 at the end of each period (only one measurement of 48 total was below 7.0). The pH and DO were similar in the control and treatment group. |
| ◊ Renewal | Solutions were renewed after 24 hours. The test material was known to be volatile. |
| ◊ Exposure period and Observations | Four days with daily observations |

- Temperature range: 22.0 to 23.2" C as measured at eh beginning and end of each 24-hour renewal. The diurnal range of the water bath temperature was continuously monitored using a minimum/maximum thermometer and recorded daily and ranged from 2 1.6 to 23.4" C over the testing period.

- Analytical Results

| | Measured Concentration (mg/l) † | |
|---|---|---|
| Day | Initial | 24 hours |
| 1 | 108 | 93.8 |
| 2 | 102 | 91.8 |
| 3 | 105 | 72.1 |
| 4 | 99.6 | 90.5 |

Mean Concentrations* over all measurements 95.4 mg/l

Test material below limit of detection in controls

† Composite sample from all three vessels

* Arithmetic mean

**Results**

- Nominal Concentrations: 0,100 mg/l
- Measured Concentrations: 95.4 mg/l (mean over 96 hours of test)
- Uuits: mg./l.

- $LC_{50}$: >95.4 mg/l at 24, 48, 72 and 96 hours.
- $LC_0$: 95.4 mg/l at 24, 48, 72 and 96 hours
- NOEC: 95.4 mg/l at 24, 48, 72 and 96 hours

- Mortality: No fish died during the exposure period
- Sub-lethal Effects: No sub-lethal effects of the test substance were observed.

**Conclusions**

Remarks Field — The 96-hour $LC_{50}$ for the limit test was >95.4 mg/l (based on measured concentrations). The no-observed-effect-concentration (NOEC) for the limit test was 95.4 mg/l.

**Data Quality**

- Reliability — Klimisch Code 1. May be used without restriction.

**Reference**

1,3-Dioxolane CAS No. 646-06-o: Acute Toxicity to Bluegill, Lepomis macrochirus, Under Static-Renewal Test Conditions. Toxikon Corporation, Laboratory Project ID 00J0009g, October 2000.

**Other**

- This study is supported by an earlier study, sponsored by Celanese, in which Sheepshead minnows (Cyprinodon variegates, 5 per group) were exposed to Dioxolanc at concentrations of 7500, 11000, 13000, 15000 and 25000 mg/l. In this study the 48 hour-$LC_{50}$ was reported to be 12000 mg/l. and the 96-hour $LC_{0}$ was 7500 mg/l, the 96 hour-$LC_{50}$ was reported to be 10000 mg/l. and the 96-hour $LC_{0}$ was 7500 mg/l. In this study 5 fish per concentration were exposed and a clear dose-response was established with a 24-hour mortality of 5/5 at 25000 mg/l. Based on the volatility of Dioxolane, the actual value for the $EC_{50}$ in this study is likely somewhat than reported. Nevertheless, this study supports the low order of toxicity found for Dioxolane toward bluegill sunfish 1
- The EPA ECOSAR Modeling Program found in EPIWIN, estimates the 96-hour $LC_{50}$ for fish to bc 8 150 mg/l.2

References for supporting studies


1. Report to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
2. ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

**Acute Toxicity to Aquatic Invertebrates**

| | |
|---|---|
| **Type** | Acute Toxicity to Aquatic Invertebrates |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o<br>Purity 99.98% |
| Method | |
| • Guideline | OECD 202 (1984) |
| • Test Type | Daphnia, acute immobilization |
| • G L P | Yes |
| • Year | 2000 |
| • Analytical Procedures | Determination of test material concentration at 0, 24 and 48 hours |
| • Species/Strain | *Daphn ia magna* |
| • Test Details | Static renewal |
| • Statistical Methods | None necessary due to lack of greater than 50% mortality |

| | | |
|---|---|---|
| Remarks Field for Test Conditions | • Age at study initiation | Less than 24 hours |
| | • Test conditions | As specified in OECD 202. |
| | • Solvent | Moderately hard fresh water |
| | • Vessel | Crystallizing dishes 10 cm diameter by 5 cm deep. Vessels were kept covered with glass plate during test to reduce loss of test material. Dishes contained 200 ml of solution |
| | • Daphnids per group | Twenty |
| | • Daphnids per vessel | Five |
| | • Exposure period | 48 hours |
| | • Temperature range | 19.2 to 20.0” C |
| | • Observation times | 24 and 48 hours |
| | • Solution pH range | 7.6 to 7.8 at all concentrations at both 0 and 48 hours |

- Dissolved oxygen: Above 8.8 mg/l for all solutions at 0 and 48 hours
- Renewal: Solutions were renewed after 24 hours
- Analytical Results

| Target Conc | Measured Concentration (mg/l) Initial | 24 hours | 24 hours Renewed | 48 Hours | Mean Conc* |
|---|---|---|---|---|---|
| 250 | 244 | 179 | 240 | 190 | 213 |
| 500 | 485 | 315 | 495 | 348 | 411 |
| 1000 | 982 | 546 | 990 | 570 | 772 |

* Arithmetic mean

- Water Parameters:
  - Hardness of 68 mg/l as calcium carbonate
  - Alkalinity of 26 mg/l as calcium carbonate
  - Specific conductivity of 527 microSiemens

**Results**

- Nominal Concentrations: 0, 250,500 and 1000 mg/l
- Measured Concentrations: 213,411 and 772 mg/l (mean over 48 hours of test)

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- Units: mg./l
- $EC_{50}$: >764 at 24 hours; > 772 at 48 hours
- $EC_0$:
  - 24 hours: 764 mg/l
  - 48 hours: Not determined

Remarks Field for Immobilization Results

| Concentration | Cumulative Number Immobilized 24 hours | 48 hours |
|---|---|---|
| 0 | 0 | 0 |
| 213 | 0 | 8120 |
| 411 | 0 | 6/20 |
| 772 | 0 | 9/20 |

**Conclusions**

Remarks Field

- The $EC_{50}$ (24 hour) was >764 mg/l and the $EC_0$ (24 hour) was 764 mg/l based on the mean measured concentrations. The $EC_{50}$ (48 hour) was >772 mg/l and the and $EC_0$ was not found based on the mean measured concentrations
- The volatility of the test substance makes it difficult to accurately determine the exposure concentrations. Based on the lack of mortality and the lack of a typical dose-response curve, it appears that the stress of the renewal conditions may have contributed to the immobilization. It is clear that the test material has a low order of toxicity to daphnids. Because of this result and the difficulty in maintaining concentration levels, further studies were not conducted.
- A preliminary range-finding test was conducted as part of this study. In this test, ten daphnids were exposed for 48 hours to nominal concentrations of 0, 0.1, 1 .0, 10.0, 100 or 1000 mg/l Dioxolane in water. Under these conditions, no immobilization was observed.

**Data Quality**

- Reliability

Klimisch Code I. May be used without restriction.

**Reference**

1,3-Dioxolane CAS No. 646-06-o: Acute Toxicity to the Water Flea, *Daphnia magna*, Under Static-Renewal Test Conditions. Toxikon Corporation, Laboratory Project ID 00J0009c October 2000.

**Other**

- This study is supported by an earlier study, sponsored by Celanese, in which Dioxolane was tested at 1000, 5000, 6500, 8000, 9000, 10000 and 12500 mg/l. In this study the 24-hour EC50 was reported to be 7650 mg/l and the 48-hour EC50 was reported to be 6950 mg/l. [1] In this study 20 daphnids were exposed and a clear dose-response relationship was obtained. Based on the volatility of Dioxolane, the actual value for the EC50 in this study is likely lower than reported. Nevertheless, this study supports the low order of toxicity found for Dioxolane toward daphnids.
- The EPA ECOSAR Modeling Program found in EPIWIN, estimates the 48-hour $LC_{50}$ for daphnia to be 7400 mg/l. [2]

References for supporting studies


1. Keport to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
2. ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

**Toxicity to Aquatic Plants**

| | |
|---|---|
| **Type** | Toxicity to Aquatic Plants |
| None | 1,3-Dioxolane<br>CAS Number: 646-06-O<br>Purity 99.98% |

**Method**

- Guideline: OECD 201
- Test Type: Algae Growth Inhibition
- GLP: Yes
- ✡♏♋❒: 2000
- Species/Strain: *Selenastrum capricornutum* The culture originated from an inoculum received from the Carolina Biological Supply Company (Burlington, NC) and has been maintained in the laboratory since December 3, 1999.
- Element Basis: Number of cells per ml. And area under the growth curve
- Exposure Period: 72 hours
- Analytical Monitoring: Yes
- Statistical Methods:
  - $EC_{50}$ values were calculated based on both biomass growth (comparison of area under the growth curves), the $E_bC_{50}$, and on the average specific growth rate, the $E_rC_{50}$. $EC_{50}$ values and their 95 percent confidence limits were estimated by a computer program (U.S. EPA, 1994) for calculating EC values by probit analysis.
  - In addition to the $EC_{50}$ values, a no-observed-effect concentration (NOEC) was calculated by analysis of variance (ANOVA) with statistical differences between cell density means determined by Dunnett's procedure (U.S.EPA, 1988). Statistical diffcrcnccs were determined at a probability level of 0.05.
  - Inhibition calculations are based upon a comparison of the areas under the growth curves and are reported using the symbol $E_bC_{50}$. The 24, 48 and 72-hour $E_bC_{50}$ values and their 95 percent confidence limits were calculated.

Remarks Field for Test Conditions

- Test Temperature Range: The temperature ranged from 24.4 to 26.8" C.
- Growth Medium Chemistry: The base water for the test medium was deionized water. The base water was enhanced with reagent-grade nutrients as described in ASTM (1994). The pH of the test medium was adjusted to 7.5 ± 0.1 prior to the addition of the test substance.

  [American Society for Testing and Materials (ASTM). 1990. Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae. ASTM Designation El 218-90.]
- Dilution Water Source: Deionized water from the Town of Jupiter Florida, supplemented as above.
- Exposure Vessel: Sterile 250-mL glass Erlenmeyer flasks covered with gas exchange caps containing 100 ml of algal medium.
- Stock Solutions Prepared: Approximately 1 .0182 g of the chemical was brought to volume in a 100 ml volumetic flask with deionized water to prepare a stock concentration of 10,200 mg/l. The following amounts of stock (1.9, 3.75, 7.5, 15 and 30 ml) were used to make the test concentrations by mixing with 298.1, 296, 292.5, 285, and 270 ml of freshwater algal media individually.
- Light Level and Quality: Lighting was continuous fluorescent lighting and intensity was measured daily at the surface of the test solutions during the 72-hour exposure period and ranged from 84 to 138 $\mu E/m^2/s$ as measured by a LI-COR, Inc. Model LI-189 light meter equipped with a 2π quantum sensor.
- Test Design
  - Replicates: three replicates for each test concentration. Six replicates were used for the dilution water control.
  - Concentrations were determined by gc using a glp validated method.
    - Target: 0, 62.5, 125, 250, 500 and 1000 mg/l
    - Mean measured Control (<3 1 .0), 36.9, 8 1 .0, 163, 280 and 877 mg/l.

- Analytical Determination of Test Material Concentrations

| Measured Concentrations mg/l | | | | |
|---|---|---|---|---|
| Nominal Conc | Day-1 | Day-3 | Mean | Percent nominal |
| Control | ND | ND | — | --- |
| **62.5** | **60.6** | 13.1 | **36.9** | **59** |
| **125** | **124** | **37.9** | XI | **64.8** |
| 250 | 262 | 64.7 | 163 | 65.2 |
| 500 | 520 | 39.3 | 280 | 56 |
| 1000 | 1027 | 726 | 877 | 87.7 |

- Method of calculating mean: Arithmetic based on composite samples of each replicate for each concentration at study initiation and study termination
- Exposure period: 72 hours
- Cell Counts: Algal growth was measured by direct cell count using a 0. 1 mm deep hemacytometer under a compound microscope. Algal counts were conducted on day one and approximately every 24 hours thereafter. Morphological observations were also conducted on the test treatment using a compound microscope to detect abnormal cell morphology and coloration as compared to the control replicates.

**Results**

- Nominal Concentrations: 0, 62.5, 125, 250, 500 and 1000 mg/l
- Measured Concentrations: (<31.0) , 36.9, 81.0, 163,280 and 877 mg/l
- Units: mg./l.
- $EC_{50}$: The $E_bC_{50}$ and $E_rC_{50}$ (O-72 hours) were >877 mg /l.
- NOEC: **877** mg/l (72-hour)

Remarks Field for Results

- Biological Observations

After 72 hours of exposure to 1,3-Dioxolane, the percentage cell growth inhibition (based on area under the growth curve) compared to the control was 19% at the mean measured concentration of 877 mg/l. The growth curves of both the control and the test solution exhibited a pattern of exponential growth during the 72-hour growth period. Observations of cell morphology dctcctcd no changes in exposed cells as compared to cells in the control media. There was no significant statistical difference between the algal growth of the control and the test solutions

- Daily Cell Counts From Each Replicate

These are presented to substantiate that there was no unusual variation between replicates associated with the possible selective volatilization of the test material from individual flasks.

| Measured Conc (mg/l) | Replicate | Cell Numbers (x $10^4$)/ml 24.7hrs | 48 25 hrs | 72 77hrs |
|---|---|---|---|---|
| | B | 2.1 | 36 | 378 |
| Control | C | 1.1 | 33 | 280 |
| | D | 0.9 | 43 | 358 |
| | E | 1.8 | 28 | 224 |
| | F | 1.9 | 26 | 289 |
| | A | 1.3 | 13 | 318 |
| 36.9 | C | 22t.3 | 23 16 | 2 4 |
| | AB | 1.6t.9 | 1 | 2 |
| 81 | | | | |
| | AC | | 48 30 | 3 4 |
| 163 | B | | | |
| | C | 0.9 18 | 30 56 | 3 9 |
| | A | 1.7 | 29 | 324 |
| 280 | B | 2.0 | 24 | 278 |
| | C | 1.3 | 40 | 291 |
| | A | 2.1 | 33 | 229 |
| 877 | B | 3.3 | 37 | 287 |
| | C | 2.1 | 34 | 211 |

- Mean Cell Density at Each Concentration at Each Time Point

| Measured Conc (mg/l) | Mean Cell Numbers (x $10^4$)/ml (s.d.) 24 hours | 48 hours | 72 hours |
|---|---|---|---|
| Control | 1.6 (0.475) | 32 (6.91) | 323 (69.4) |
| 36.9 | 1.6 (0.520) | 17 (5.13) | -273 (50.8) |
| 81 | 1.5 (0.513) | 34 (14.0) | 320 (19.2) |
| 163 | 1.0 (0.709) | 39 (15.0) | 276 (77.3) |
| 280 | 1.7 (0.351) | 31 (8.18) | 298 (23.7) |
| 877 | 2.5 (0.693) | 35 (2.08) | 242 (39.7) |

- Percent Inhibition

| Measured Conc (mg/l) | Percent Inhibition 0-24hrs | 24-48 hrs | 48-72 hrs |
|---|---|---|---|
| 36.9 | 0 | -47 | -21 |
| 81 | -17 | 6 | 0 |
| 163 | -100 | 18 | -9 |
| 280 | 17 | -2 | -7 |
| 877 | 150 | 15 | -19 |

**Conclusions**

Remarks Field

The $E_bC_{50}$ and $E_rC_{50}$ (O-72 hours) were >877 mg /I (based on measured concentrations). The 72-hour no-observable-effect concentration (NOEC) was 877 mg/l.

The test material was somewhat volatile; however, sufficient Dioxolane remained in the culture flasks (especially at the highest concentration tested) to provide a valid estimate of the growth inhibition potential of the test material to green algae.

**Data Quality**

- Reliability

Klimisch Code 1. Reliable without restriction. Study was conducted in accord with current OECD guideline under glp conditions. Analytical measurements verified exposure concentrations.

**References**

1,3-Dioxolane: Toxicity to The Freshwater Green Alga, *Selenastrum capricornutum*, Under Static Test Conditions. Toxikon Laboratories, Jupiter FL, Project ID 00J0009b, 27 September 2000, submitted to and sponsored by Ticona Corporation and Ferro Corporation.

**Other**

This study is supported by an earlier study, sponsored by Celanese, in which Trioxane was tested for growth inhibition of *Selenastrum capricornutum*. In this study, algae growth was measured out to 14 days of exposure at levels of 1000, 5000 or 10000 mg/l with counts recorded on days 3, 6, 10 and 14. Significant inhibition was seen only at 5000 mg/l and above 1000 mg/l was determined to be the NOEC. Graphically, the 96-hour $EC_{50}$ can be determined to be in the range of 4000 mg/l; however, loss of test material may affect this estimate'

The EPA ECOSAR Modeling Program found in EPIWIN, estimates the 96-hour $EC_{50}$ for green algae to bc 4075 mg/l. [2]

References for supporting studies


1. Report to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
2. ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

**Acute Oral Toxicity**

| | |
|---|---|
| **Type** | Acute Oral Toxicity |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-O<br>Commercial Grade |

**Method**

| | |
|---|---|
| • Guideline | Based on methods described in 16 CFR 1500.3 (c) Consumer Products Safety Commission: Federal Hazardous Substance Act |
| • GLP | No |
| • Year | 1980 |
| • Species | Rat |
| • Strain | Albino Rats (Sprague-Dawley CD®) |
| • Route of administration | Oral Gavage |
| • Doses | 2500, 3500, 5000, 7 100, 10000 mg/kg |
| • sex | Males and Females |
| • Number of Animals/group | Five |
| • Vehicle | None, administered neat |

| | | |
|---|---|---|
| Remarks Field for Test Conditions | • Age at Study Initiation | Unknown, Young Adults At study initiation males weighed 265 to 289 grams and females weighed 233 to 245 grams |
| | • Volume administered | 1.3 to 9.2 ml/kg body weigh |
| | • Post-dose observation period | 14 Days |

**Results**

- $LD_{50}$ — LD-50 = 5.2 g/kg with 95% confidence limits of 4.3 to 6.1 g/kg
- Number of deaths at each dose level

| Dose Level mg/kg | Mortality, males | Mortality, females |
|---|---|---|
| 2,500 | 0/5 | 015 |
| 3,500 | 2/5 | 0/5 |
| 5,000 | 1/5 | 215 |
| 7,100 | 5/5 | 5/5 |
| 10,000 | 5/5 | 5/5 |

Remarks Field for Kesults

- Time of death

| Dose mg/kg | Males (d= day) | Females |
|---|---|---|
| 3500 | d-l, d-l | none |
| 5000 | 4 hours | d-l, d-l |
| 7100 | 2 hours, d-l, d-l, d-2, d-2 | 1 hour, 4 hours, d-1, d-l, d-l |
| 10,000 | 2 hours, 4 hours, 6 hours, 6 hours, d-l | 1 hour, 2 hours, 2-hours, 4 hours, 4-hours |

- Clinical Signs

| Dose mg/kg | Clinical Signs |
|---|---|
| 2,500 | • Ataxia, 1 female from 2 to 4 hours after dosing.<br>• Respiratory rate decrease, 3 animals, 2 to 4 hours<br>• Motor activity decrease, all animals 1 to 4 hours. |
| 3,500 | • Ataxia, 2 females 2 or 4 hours after dosing.<br>• Fine tremors, 1 male, 4-hours after dosing only<br>• Respiratory rate decrease, 5 animals, 2 to 4 hours<br>• Motor activity decrease, 9/10 animals starting at I hour, decreasing to 2 animals at 24 hours.<br>• Piloerection, 3 animals, 2 hours to day-2<br>• Prostration, 3 animals, from 2 to 4 hours. |
| 5,000 | • Ataxia, I male, only at 2 4 hours after dosing.<br>• Fine tremors, 1 female 1- 4 hours after dosing<br>• Respiratory rate decrease, 5 animals, 2 to 4 hours<br>• Motor activity decrease, 9 animals starting at 1 hour, decreasing to 4 animals at 24 hours.<br>• Prostration, 5 animals total, various times from 1 to 24 hours.<br>• Hypothermia, 2 animals at 4 or 24 hours after dosing |
| 7,100 | • Respiratory rate decrease, 8 animals total, various 1 to 24 hours after dosing<br>• Motor activity decrease, 1 animal 1-2 hours after dosing.<br>• Prostration, 8 animals total, various times from 1 to 24 hours.<br>• Hypothermia, 4 animals at 4 or 24 hours after dosing |
| 10,000 | • Respiratory rate decrease, 6 animals total, various 1 to 4 hours after dosing<br>• Labored breathing, 2 animals at 1 hour after dosing only<br>• Prostration, 9 animals total, various times from 1 to 4 hours.<br>• Hypothermia, 1 animal at 2 hours after dosing |

- Necropsy Findings: Necropsy only conducted on animals dying less than 24 hours after dosing and limited to determining if gavage was the cause of death.
- Target Organs: No potential target organs were identified in report, CNS may have been affected by solvent narcosis.
- Sex Differences: Effects on males and females were similar.

**Conclusions**

Remarks Field: The oral $LD_{50}$ in the rat was determined to be 5,200 mg/kg. Solvent induced narcosis may have been the cause of deaths occurring on day-l.

Study documentation is good. Results are consistent with other data for the material, including literature studies.

**Data Quality**

- Reliability: Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

**References**

Acute Oral Toxicity Study in Rats, Bio/dynamics Inc., Project No. 6004-79 (06/05/80), Sponsored by The Celanese Corporation, New York, NY.

**Other**

This study is supported by a study in the literature that reports the acute oral $LD_{50}$ in the rat to be 5,800 mg/kg [1]. This study is also supported by a limit test, conducted by Hoechst, that reported the $LD_{50}$ to be greater than 2000 mg/kg [2]

References for supporting studies


1. Czajkowska, T, Krysiak, B and Popiânska, E. Experimental studies of toxic effects of 1,3,5-trioxane and 1,3-dioxolane. 1. Acute toxic effect. Med Pr: Vol 38, 1987, P184-90.
2. 1,3-Dioxolan - Profung der akuten oralen Toxizitatan der Wistar Ratte. Hoechst AC Pharma Research Toxicology Report No. 87.1441, September 1987.

**Acute Inhalation Toxicity**

| | |
|---|---|
| **Type** | Acute Inhalation Toxicity |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-O<br>Commercial Grade |
| **Method** | |
| ■ Guideline | None specified |
| ● GLP | No |
| ■ Year | 1980 |
| ■ Species | Rat |
| ● Strain | Charles River CD@ |
| ■ Route of administration | Whole-body inhalation as vapor |
| . Doses | 0, 37.9, 60.6, 67.9, 8X.4 and 201.9 mg/l (nominal concentrations) |
| + Sex | Males and Females |
| ● Exposure Period | Four hours |
| . Number of Animals/group | Five animals of each sex per dose level |
| ● Vehicle | Air |

| | | |
|---|---|---|
| Remarks Field for Test Conditions | ◊ Age at Study Initiation | Unknown, Young Adults At study initiation males weighed 204 to 295 grams and females weighed 211 to 273 grams |
| | ◊ Doses | Initial dose was 201.9 mg/l (nominal) which caused 100% mortality. Second group was 37.9 mg/l, which caused 0% mortality. Additional groups were added until enough data were accumulated to calculate an accurate $LC_{50}$. |
| | ◊ Post-dose observation period | 14 Days |

**Results**

| | |
|---|---|
| ● $LC_{50}$ | 68.4 mg/l (95% confidence interval of 61 .0 – 766. mg/l) |

- Number of deaths at each dose level

| Concentration (mg/l) | Number of deaths | | |
|---|---|---|---|
| | Males | Females | Total |
| 0 | 0 | 0 | 0 |
| 37.9 | 0 | 0 | 0 |
| 60.6 | 0 | 1 | 1 |
| 67.9 | 4 | 1 | 5 |
| 88.4 | 4 | 5 | 9 |
| 201.9 | 5 | 5 | 10 |

Remarks Field for Results

- Time of death

| Conc. (mg/l) | Males and Females (d=day, h=hour) |
|---|---|
| 60.6 | 611 |
| 67.9 | 5h, 8h, dl, dl, dl |
| 88.4 | 411,411, 511, 511, 6h, 711, dl, dl, dl |
| 201.9 | All ten within 2 hours |

Clinical Signs

37.9 mg/l

| Sign | Onset | Duration | # Animals |
|---|---|---|---|
| Lacrimation | 30 min | 1 day | Most |
| Labored breathing | 2 hours | 3 hours | Most |
| Prostrate | 3 hours | 3 hours | All |
| No response to auditory stimuli | 3 hours | 1 hour | Most |
| Loss of muscle tone coordination | 45 min | < 24 hours | Most |

Clinical Signs

60.6

| Sign | Onset | Duration | # Animals |
|---|---|---|---|
| Lacrimation | 30 min | 2 days | Most |
| Labored breathing | 2 hours | 6 hours | Some |
| Rapid breathing | 3 hours | 3 hours | All |
| Reduced activity | 30 min | < 24 hours | All |
| Prostrate | 2 hours | 3 hours | All |
| No response to auditory stimuli | 2 hours | 3 hours | All |
| Loss of muscle tone coordination | 1 hour | 2 hours | Most |

| | Sign | Onset | Duration | # Animals |
|---|---|---|---|---|
| Clinical Signs | Lacrimation | 15 min | 2 days | Most |
| | Labored breathing | 2 hours | 6 hours | Half |
| 67.9 mg/l | Rapid breathing | 3 hours | <24 hours | All |
| | Reduced activity | 15 min | < 24 hours | All |
| | Prostrate | 2 hours | 3 hours | All |
| | No response to auditory stimuli | 45 min | 3 hours | All |
| | Loss of muscle tone coordination | 45 min | 30 min | Most |

| | Sign | Onset | Duration | # Animals |
|---|---|---|---|---|
| Clinical Signs | Lacrimation | 15 min | < 24 hours | Most |
| | Labored breathing | 4 hours | < 24 hours | Many |
| 88.4 mg/l | Rapid breathing | 2 hours | 3 hours | All |
| | Reduced activity | 15 min | < 24 hours | All |
| | Prostrate | 2 hours | 4 hours | All |
| | No response to auditory stimuli | 60 min | 4 hours | All |
| | Loss of muscle tone coordination | 45 min | 2 hours | Most |

| | Sign | Onset | Duration | # Animals |
|---|---|---|---|---|
| | Lacrimation | 15 min | < 2 hours * | All |
| Clinical Signs | Labored breathing | 45 min | < 2 hours * | All |
| 201.9 mg/l | Salivation | 14 min | < 2 hours * | All |
| | Reduced activity | 15 min | < 2 hours * | All |
| | No response to auditory stimuli | 45 min | < 2 hours * | All |
| | Dead | < 2 hours | | All |

* Animals died after 2 hours of exposure

| Body Weights Group | | Day Mean Body Weights (grams) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 4 | 7 | 14 |
| Control | Males | 282 | 285 | 285 | 297 | 314 | 345 |
| | Females | 227 | 225 | 228 | 231 | 236 | 244 |
| 37.9 mg/l | Males | 285 | 264 | 263 | 276 | 290 | 318 |
| | Females | 255 | 240 | 244 | 244 | 255 | 262 |
| 60.6 mg/l | Males | 259 | 232 | 242 | 254 | 268 | 303 |
| | Females | 220 | 203 | 209 | 214 | 222 | 226 |
| 67.9 mg/l | Males | 248 | 231 | 224 | 242 | 264 | 311 |
| | Females | 223 | 200 | 208 | 216 | 228 | 241 |

Necropsy Findings

| Group | Finding | Number |
|---|---|---|
| Controls | Mottled lungs | 8/10 |
| 37.9 mg/l | Mottled lungs | 2/10 |
| 60.6 mg/l | Lung, red foci | 2/10 |
| | Mottled lungs | 1/10 |
| 67.9 | Mottled lungs | 6/10 |
| | Liver purple | 2/10 |
| 88.4 | Mottled lungs | 10/10 |
| | Liver discolored | 10/10 |
| | Spleen dark red | 1/10 |
| 201.9 mg/l | Mottled lungs | 10/10 |
| | Lungs blood filled | 3/10 |
| | Liver discolored | 6/10 |

Other

Necropsy Gndings also included gastrointestinal gas distention in animals dying prior to sacrifice. Bladders distended with fluid were also observed in these early deaths.

**Conclusions**

Remarks Field

An $LC_{50}$ of 68.4 mg/l was determined using a series of concentrations. Solvent induced narcosis appeared as a common effect and may have been the cause of death at the high concentration. Lung and liver abnormalities were common and severity was dose related.

**Data Quality**

. Reliability

Klimisch Code 2 Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

**References**

An acute inhalation toxicity study of C-121 [1,3-Dioxolane] in the rat. Project No. 79-7304, Bio/dynamics Inc., July 11, 1980.

**Other**

This study is supported by a literature study that reported an $LC_{50}$ of 118 mg/l in the rabbit (exposure time not given), 87 mg/l/ (4 hours) in the male rat and 166 mg/l (4 hours) in the guinea pig. [1]

References for supporting data

1. Czajkowska, T, Krysiak, B and Popiânska, E. Experimental studies of toxic effects of 1,3,5-trioxane and 1,3-dioxolane. I. Acute toxic effect. Mcd Pr; Vol 38, 1987, P184-90.

**Repeated Dose Toxicity, Two-week Gavage**

| | |
|---|---|
| **Type** | Repeated Dose Toxicity, Two-week Inhalation |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o<br>Purity listed as 99.9% |
| **Method** | |
| Guideline | ◊ Toxic Substances Control Act Test Guidelines - 798.4900<br>◊ Meets OECD 412 Guideline except for lack of clinical chemistry determinations |
| GLP | Yes |
| Year | 1991 |
| Species | Rat |
| Strain | Crl:CD®(SD)BR, Charles River |
| Route of administration | Gavage in corn oil. |
| Duration of Test | Fifteen days |
| Doses | Water control, vehicle control, 75, 250, 750 and 2000 mg/kg/day |
| **Sex** | Rats of each sex. |
| Frequency of l'rcatment | Daily for fourteen consecutive days. |
| Number of Animals/group | Ten of each sex |
| Control Group and Treatment | Water treated controls and corn oil treated controls. (Ten animals of each sex per group) |
| Post-Exposure Observation Period | Overnight |

- Statistical Methods
  - All hematology data were statistically compared to both control groups using a one-way Analysis of Variance Test and then Dunnett's Test, to determine the statistical significance of the individual groups in comparison with each of the control groups
  - All body weight, feed consumption and organ weight data were statistically compared against both control groups using the following statistical tests: Analysis of Variance, Dunnett's Test, Bartlett's Test of homogeneity, Kruskal-Wallis Test and Dunn's Test.

Remarks Field for Test Conditions

| | |
|---|---|
| Age at study initiation | Males: approximately nine weeks<br>Females: approximately nine weeks |
| Number animals of each sex per dose | Ten of each sex per concentration |
| Satellite groups | None |
| Housing | Individual wire-bottom cages |
| Clinical observations performed and frequency | Mortality twice daily and gross signs daily about 30 minutes after dosing. Body weights daily. Feed consumption weekly. |
| Terminal observations | ▪ Blood samples were evaluated for the following parameters: red blood cell count, hematocrit, hemoglobin, mean corpuscular volume, white blood cell count, white blood cell differential count, red blood indices for mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, reticulocyte counts (absolute), red blood cell morphological assessment and platelet counts (absolute).<br>▪ Postmortem examination included gross lesions of the thoracic, abdominal and pelvic viscera. organ weights were recorded for the liver, kidneys, adrenals, spleen, pancreas, lungs, pituitary, thymus, as well as the reproductive organs (male rats - testis, epididymides and accessory sex glands; female rats - ovaries and uterus) |

- Histopathology
  - Histopathology was performed on all tissues showing gross changes, liver, kidneys, adrenals, spleen, pancreas, lungs, pituitary, thymus, as well as the reproductive organs (male rats - testis, epididymides and accessory sex glands; female rats - ovaries and uterus). Initially, tissues form both controls and the high-dose were examined. Because this preliminary examination of tissues from both control groups and the 2000 mg/kg/day dosage group revealed potential histopathology in the kidney, liver, thymus (male and female rats) and testes (male rats), these tissues were also examined in the remaining dosage groups.
- Differences from OECD Guideline
  - This study is in accord with the current OECD 412 guideline for 14 or 28-day inhalation studies with the exception that clinical chemistry parameters were not determined. The current study uses twice the number of animals required by the OECD guideline and one additional dose level.

**Results**

- NOAEL
  - Males: 75 mg/kg/day
  - Females: 75 mg/kg/day
- LOEAL
  - Males: 250 mg/kg/day
  - Females: 250 mg/kg/day
- Mortality
  - Males: Three high dosage group male rats were found dead on days 4, 9 and 14 of the study. Deaths were preceded by clinical observations, and two of these rats had severe weight losses. Nccropsy of one rat revealed gastric erosions. No other deaths occurred during the conduct of the study.
  - Females: Four 2000 mg/kg/day dosage group female rats died. These deaths occurred on days 3, 8, 9 and IO of the study and were preceded by clinical observations. These rats had moderate, although sometimes transient, weight losses occur, and necropsy of one rat revealed reddened areas in the stomach. Two additional high dosage group deaths occurred on days 10 and 13 of the study: these deaths were related to intubation accidents. No other deaths occurred during the conduct of the study.

| Dose (mg/kg/day) | Toxic Response (statistical comparisons noted are against the corn oil control) |
|---|---|
| 75 | ■ Males: No significant effects<br>■ Females: No significant effects |
| 250 | ▪ Males<br>o Slight reduction in platelets ($p>.05$)<br>o Reduction in body weight gain<br>o Reduced feed consumption<br>▪ Females<br>o Reduction in lymphocyte count ($p \leq .01$) |
| 750 | ▪ Males<br>o Reduction in platelets ($p \leq .01$)<br>o Reduction in body weight gain<br>o Reduced feed consumption<br>o Liver and lung relative weights increased<br>o Reduced relative thymus weight<br>▪ Females<br>o Excess salivation in some females.<br>o Reduction in lymphocyte ($p \leq .05$) and reticulocyte count ($p \leq .01$)<br>o Liver relative weight increased<br>o Spleen and thymus relative weights decreased |

2000

- Males
  - Clinical observations after dosing included hypotonia, excess salivation, ataxia, decreased motor activity, rales, impaired and lost righting reflex, labored breathing, red penile exudate and urine-stained abdominal fur
  - Reduced feed consumption
  - Reduction in platelets ($p \leq .01$)
  - Overall loss in body weight.
  - Liver, lung and kidney relative weights increased
  - Prostate, spleen and thymus weights decreased
  - Histopathological findings in liver (6/10), kidney (4/10), thymus (6/10) and testis (2/10).
- Females
  - Clinical observations after dosing included excess salivation (750 and 2000 mg/kg/day), hypotonia, ataxia, decreased motor activity, impaired and lost righting reflex, gasping, bradypnea, oral exudate, rales, lacrimation and urine-stained abdominal fur
  - Reduction in lymphocyte count ($p \leq .01$)
  - Reduction in platelet count ($p \leq .01$)
  - Reduction in body weight gain
  - Liver weight marginally increased
  - Kidney relative weight increased
  - Spleen weight marginally decreased
  - Thymus weight decreased
  - Pancreas relative weight deceased
  - Histopathological findings in liver (5/10), kidney (3/10), thymus (5/9) and spleen (2/10)

Remarks Field for Body Weights Gains Results

Mean Body Weight Gains (grams)

| Dose (mg/kg) | Males | | Females | |
|---|---|---|---|---|
| | Day 1-8 | Day 8-15 | Day 1-8 | Day 8-15 |
| Water only | 29.5 | 33.8 | 11.0 | 12.6 |
| Corn Oil | 28.5 | 38.5 | 8.6 | 11.4 |
| 75 | 27.5 | 37.4 | 11.5 | 9.5 |
| 250 | 26.7 | 32.6 | 5.2 | 10.8 |
| 750 | 20.5 | 31.0 | 4.2 | 16.0 |
| 2000 | -29.0 | 9.3 | -10.1 | 11.0 |

- Clinical Signs
  - Males: Clinical observations after dosing included hypotonia, excess salivation, ataxia, decreased motor activity, rales, impaired and lost righting reflex, labored breathing, red penile exudate and urine-stained abdominal fur.
  - Females: Clinical observations attributable to the 750 and 2000 mg/kg/day dosages of the test substance included excess salivation (750 and 2000 mg/kg/day), hypotonia, ataxia, decreased motor activity, impaired and lost righting reflex, gasping, bradypnea, oral exudate, rales, lacrimation and urine-stained abdominal fur (2000 mg/kg/day).
- Hematology
  - Group designations are used to describe the hematology results, groups are: Group I, water control; Group II, corn oil control; Group III, 75 mg/kg/day; Group IV, 250 mg/kg/day; Group V, 750 mg/kg/day; Group VI, 2000 mg/kg/day.
  - Among male animals, statistically significant differences were seen only in the platelet counts. Groups IV, V, and VI differed significantly from group 1 at the 0.0 1 level. Groups V and VI differed significantly from group 11 at the 0.01 level, but the difference between groups II and IV was not statistically significant

- Hematology
  - Among female animals, statistically significant differences were seen in the total leukocyte count, lymphocyte count, platelet count, nucleated red blood cells, and reticulocyte count. In the total leukocyte count, groups IV, V, and VI differed significantly from group I at the 0.05 level; none of the treated groups differed significantly from group II. With respect to lymphocytes, groups IV, V, and VI differed significantly from group 1 at 0.01; group IV and VI differed significantly from group II at 0.01 while group V differed significantly from group II at 0.05. No significant differences occurred among groups with respect to neutrophils or minor cell types indicating that the differences in total leukocyte count was a function of differences in lymphocytes. No corresponding effect was evident in males. Nucleated red blood cells were significantly different in groups 111, V, and VI from group I at 0.05 but did not differ significantly from group 11. Reticulocytes were significantly higher in group VI than in group I at 0.05, and lower in group IV than in group 11 at 0.01. No other differences among groups were significant with respect to reticulocytes. It appears that the differences in nucleated red blood cells and reticulocytes, cells of the same lineage differing only with respect to maturity, are a function of random variation rather than a treatment effect, particularly because differences from controls occur in both directions. In female animals platelets in group VI were significantly lower than in group 1 and group 11 at the 0.01 level.
  - From the above data, it appears that a decrease in circulating platelets was a treatment effect as it occurred at a statistically significant level in both males and females. In males, rats in groups IV, V, and VI had significantly lower platelet counts than group I and groups V and VI had significantly lower platelet counts than group II. In females group VI animals had significantly lower platelet counts than groups 1 and 11. Among the other groups there was a non-significant dose-related trend.

- Hematology Comments

Treatment related hematologic changes observed in this study were a decrease in platelets, seen in both sexes, and a decrease in lymphocytes (also reflected in total leukocytes) seen in females only. No hematologic treatment effects were observed in the rats given 75 mg/kg/day of the test compound.

- Organ Weights: Treatment related organ weight changes are given above under toxic effects. Organ weigh changes were not remarkable although some reached statistical significance.
- Necropsy findings: Examination of each animal during necropsy did not indicate any exposure-related gross pathologic changes
- Histopathology: No treatment-related microscopic changes were observed in any of the male and female rats given 75, 250, or 750 mg/kg/day of 1,3-Dioxolane.

Treatment-related microscopic changes were observed in the liver, kidney, thymus, and testis of rats given 2000 mg/kg/day of 1,3Dioxolane. The treatment -related changes consisted of ccntrilobular. hepatocellular hypertrophy and midzonal hepatocellular vacuolation (male and female rats); thymic atrophy (male and female rats); renal cortical tubular basophilia and dilatation and accumulations of birefringent intratubular crystals (male rats); subacute renal pyelitis (male and female rats), and multifocal testicular degeneration. Incidence values are given above.

Conclusions

Remarks Field

Gavage administration of Dioxolane to rats of each sex was associated with hematologic effects, body weight gain reductions and organ weight changes at doses of 750 and 2000 mg/kg/day. Thc 250-mg/kg/day dose level was associated with decreases in platelets in male rats and lymphocyte counts in female rats.

The authors of the laboratory report elected to call 250 mg/kg/day a NOEL in the female rat. The writer of this summary is not in agreement with that conclusion and has selected 75 mg/kg/day as the appropriate NOEL.

Data Quality

- Reliability

Klimisch Code 1. May bc used without restriction.

References

Repeated Dose Oral Toxicity Study of 1,3-Dioxolane Administered Via Gavage to Crl:CD®(SD)BR Rats. Argus Research Laboratories, Inc. 905 Sheehy Drive Horsham, Pennsylvania 19044, Laboratory Project ID 508-002P.

Other

- This study is supported by a two-week inhalation study of Dioxolane conducted by BioDynamics for Celanese Corporation. In this study Charles River CD rats (5/sex/group) were exposed for six hours per day, five days per week, for two weeks at concentrations of 0,984 or 3280 ppm. All animals survived the duration of the study. No treatment related observations were recorded. Body weights, organ weights, clinical chemistry parameters, and macropathology were unremarkable in treated animals. Depressed leukocyte values in male and female exposed animals were reported.'
- This study is supported by a 12-day inhalation study of Dioxolane conducted by Dow Chemical. The primary effect of Dioxolane inhalation exposure to rats was a reduction in white blood cells counts at 23 19 or 5 132 ppm. Body weight gain was affected at the 5 132-ppm exposure level. There was no corresponding myeloid toxicity or inflammatory foci [2].

References for supporting studies


1. A two-week inhalation toxicity study of C-121 (1,3-Dioxolane) in the rat. Bio/Dynamics Inc Project No. 80-7429, June 22, 198 1.
2. 1,3-Dioxolane: A Two-Week Vapor Inhalation Toxicology Study in Fischer 344 Rats. Dow Chemical Company 1989 Study ID K-010634-005

**Repeated Dose Toxicity, Four-week Drinking Water**

| | |
|---|---|
| **Type** | Repeated Dose Toxicity, Four-week Oral |
| Test Substance | 1,3-Dioxolane<br>CAS Number: 646-06-o |
| **Method** | |
| Guideline | None |
| GLP | No |
| Year | 1977 |
| Species | Rat, mouse, golden Syrian hamster. |
| Strain | Charles River strain |
| Route of administration | Drinking water |
| Duration of Test | Four weeks |
| Doses | 0, 0.5, 1 .O and 2.0% in drinking water |
| **Sex** | Male and Female |
| Exposure Period | Twenty-four hours per day |
| Frequency of Treatment | Continuous, seven days a week. |
| Number of Animals/group | Five of each sex |
| Control Group and Treatment | Five animals of each sex exposed only to water |
| Post-Exposure Observation Period | None |
| Statistical Methods | Body weight data analyzed by ANOVA followed by Tukey's or Scheffe's test of multiple comparison. |

| | | |
|---|---|---|
| Remarks Field for Test Conditions | Age at study initiation | Not specified |
| | Number animals of each sex per dose | Five of each sex per concentration |
| | Satellite groups | None |

- Housing: Individual
- Clinical observations performed and frequency:
  - Mortality daily.
  - Body weights weekly.
  - Water consumption twice weekly.
- Terminal observations: Body weights
- Test Substance: Prepared weekly in tap water.

**Results**

- NOAEL
  - Rats
    - Males: 0.5%
    - Females: 0.5%
  - Mice
    - Males: 0.5%
    - Females: 0.5%
  - Hamsters
    - Males: 1.0%
    - Females: 1.0%
- LOEAL
  - Rats
    - Males: 1.0%
    - Females 1.0%
  - Mice
    - Males: 1.0%
    - Females 1.0%
  - Hamsters
    - Males: 2.0%
    - Females 2.0%

- Mortality: All animals survived the duration of the study.

- Toxic Responses

| Dose | | Toxic Responses | |
|---|---|---|---|
| 0.5% | | Reduced water consumption for female rats only. | |
| 1.0% | Rats | Males | Reduced water consumption<br>Reduction in body weight gain †* |
| | | Females | Reduced water consumption<br>Slight reduction in body weight gain |

| | | | |
|---|---|---|---|
| | . Mice. | Males | Slight reduction in body weight gain |
| | | Females | Reduced water consumption<br>Slight reduction in body weight gain |
| | ▪ Hamsters | Males | None |
| | | Females | None |
| | . Rats | Males | Reduced water consumption<br>Reduction in body weight gain †† |
| 2.0% | | Females | Reduced water consumption<br>Reduction in body weight gain † |
| | ▪ Mice | Males | Slight reduction in body weight gain |
| | | Females | Reduced water consumption<br>Slight reduction in body weight gain |
| | ▪ Hamsters | Males | Slight reduction in body weight gain |
| | | Females | Slight reduction in body weight gain |

† = p ≤ 0.05 †† = p≤ 0.01

* Although absolute body weights were not significantly reduced, body weight gain was.

Remarks Field for Results

◊ Body Weights

**Mean Body Weights (n=5)**

| Drinking Water Level (percent) | Rats Body Weight on Week (grams) | | | | |
|---|---|---|---|---|---|
| Males | 0 | 1 | 2 | 3 | 4 |
| 0 | 210 | 271 | 331 | 376 | 391 |
| 0.5 | 209 | 264 | 314 | 351 | 364 |
| 1.0 | 210 | 256 | 298 | 332 | 334 |
| 2.0 | 209 | 237 | 273-j. | 304-y | 309-1-r |
| Females | | | | | |
| 0 | 156 | 182 | 209 | 231 | 238 |
| 0.5 | 157 | 178 | 203 | 221 | 227 |
| 1.0 | 156 | 182 | 196 | 208 | 215 |
| 2.0 | 157 | 164 | 182 | 198† | 193† |

† = p≤ 0.05 †† = p≤ 0.01

- Body Weights

| Drinking Water Level (percent) | Mice Body Weight on Week (grams) | | | | |
|---|---|---|---|---|---|
| Males | 0 | 1 | 2 | 3 | 4 |
| 0 | 27 | 33 | 34 | 37 | 38 |
| 0.5 | 28 | 32 | 34 | 36 | 37 |
| 1.0 | 28 | 32 | 35 | 36 | 37 |
| 2.0 | 28 | 30 | 32 | 33 | 34 |
| Females | | | | | |
| | 23 | 27 | 28 | 29 | 30 |
| 0.5 | 23 | 26 | 28 | 28 | 28 |
| 1.0 | 23 | 26 | 27 | 27 | 29 |
| 2.0 | 23 | 24 | 25 | 26 | 27 |

- Body Weights

| Drinking Water Level (percent) | Hamsters Body Weight on Week (grams) | | | | |
|---|---|---|---|---|---|
| Males | 0 | 1 | 2 | 3 | 4 |
| 0 | 69 | 80 | 93 | 99 | 104 |
| 0.5 | 69 | 84 | 96 | 104 | 107 |
| 1.0 | 70 | 77 | 90 | 99 | 105 |
| 2.0 | 69 | 77 | 85 | 92 | 95 |
| Females | | | | | |
| 0 | 64 | 71 | 80 | 88 | 93 |
| 0.5 | 63 | 74 | 86 | 94 | 100 |
| 1.0 | 64 | 74 | 83 | 90 | 98 |
| 2.0 | 64 | 72 | 79 | 83 | 86 |

- Clinical Signs

None recorded

**Conclusions**

Remarks Field

- Rats of each sex showed reduced body weigh gains at the two highest dose levels. Body weight gains of high-dose mice and hamsters also appear to be affected but did not achieve statistical significance. The authors of the report only noted the statistically significant changes in body weight gains for rats (males 1.0 and 2.0 %, females 2.0%). 1 lamsters appear to bc less sensitive to the toxic effects of dioxolane than rats and mice and rats appear to be the most sensitive based solely on body weigh gains.
- The dosing for the male rats at 1% drinking water with water consumption at 30 ml per day calculates to approximately 1000 mg/kg/day Dioxolane. This is the approximate dose level were body weight changes are observed in other studies.

**Data Quality**

Reliability: Klimisch Code 2. Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

**References**

Four-week pilot study with Dioxolane in drinking water of albino rats, albino mice and golden Syrian hamsters. Industrial BIO-Test Laboratories, Inc., Project No. 8560-10579, July 12, 1977.

Other

This study is supported by a 14-day corn-oil gavage study in rats of each sexing which hematologic effects, body weight gain reductions and organ weight changes were reported at doses of 750 and 2000 mg/kg/day. The 250-mg/kg/day dose did not affect body weigh gain.

References for supporting studies

Repeated Dose Oral Toxicity Study of 1,3-Dioxolane Administered Via Gavage to Crl:CD®(SD)BR Rats. Argus Research Laboratories, Inc. 905 Sheehy Drive Horsham, Pennsylvania 19044, Laboratory Project ID 508-002P.

**Repeated Dose Toxicity, Two-week Inhalation**

| | |
|---|---|
| **Type** | Repeated Dose Toxicity, Two-week lnhalation |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-O<br>Purity 99.99 % by analysis |
| **Method** | |
| • Guideline | This study was conducted to meet the requirements of the United States Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards in 40 CFR part 792; TSCA Health Effects Test Guideline in 40 CFR 798.250; and OECD ISBN 92-64-l 2367-9 Paris 1982. Study also meets OECD 412 Guideline. |
| • GLP | Yes |
| • Year | 1989 |
| • Species | Rat |
| • **Strain** | Fischer 344<br>Charles River, Kingston NY |
| • Route of administration | Whole body inhalation of vapor |
| • Duration of Test | Twelve days |
| • Doses | 0, 5 16, 23 19 or 5 132 (Mean, measured concentrations) |
| • Sex | Male and Female |
| • Exposure Period | Six hours per day |
| • Frequency of Treatment | ◊ Five days a week<br>◊ Nine treatments |
| • Number of Animals/group | Five of each sex |
| • Control Group and Treatment | Five animals of each sex exposed only to air under the same chamber conditions |
| • Post-Exposure Observation Period | Overnight |

| | | |
|---|---|---|
| Statistical Methods | Descriptive statistics (mean and standard deviation), were used to report chamber concentrations, temperature, and relative humidity. | |
| | Remaining parameters were first tested for equality of variance using Bartlett's test. If the results for Bartlett's test rejected the equality of variances, the parameter was flagged for careful evaluation of results. All parameters were then subjected to appropriate parametric analysis as described below. In-life body weights, hematologic (excluding differential WBC) and clinical chemistry parameters, terminal body weights, and organ weights (absolute and relative, except testis) were evaluated using a two-way analysis of variance (ANOVA) with the factors of sex and dose. Results for absolute and relative testis weights were analyzed using a one-way ANOVA. If significant dose effects were determined in the one-way ANOVA, then separate doses were compared to controls using Dunnett's test. For those parameters examined by a two-way ANOVA, examination was made first for a significant sex-dose interaction. If this existed, a one-way ANOVA was done separately for each sex. If no sex-dose interaction was identified, and a dose effect was identified, or if in the subsequent ANOVAs separated by sex a dose effect was identified, the separate ANOVAs were used for each exposure group with control. To control for multiple comparisons with control, a Bonferroni correction was used. | |
| Remarks Field for Test Conditions | Age at study initiation | Males: eight weeks<br>Females: eight weeks |
| | Number animals of each sex per dose | Five of each sex per concentration |
| | Satellite groups | None |
| | Housing | Not specified in report |
| | Clinical observations performed and frequency | Mortality and gross signs daily prior to exposure.<br>Body weights on days 1, 3, 5, 8, and 11.<br>Behavior pattern and nervous system activity were assessed by specific observations for lethargy, tremors, convulsions, salivation, lacrimation, diarrhea, and other sign of altered nervous system function. |

- Terminal observations
  - Blood taken for hematology (red cell count, hemoglobin, hematocrit, total white blood cell count, platelets, differential leukocyte counts and morphology of WBCs, RBCs and platelets) and clinical chemistry (urea nitrogen, alanine aminotransferase activity, aspartate aminotransferase activity, alkaline phosphatase activity, glucose, total protein, albumin, globulin, total bilirubin, cholesterol, triglycerides, phosphorus and calcium)
  - Complete gross postmortem examination.
  - Organ weights for brain, heart, lungs, liver, kidneys, and testis were recorded for all animals.
- Histopathology
  - The following tissues were examined from all control and high-exposure animals only. Adrenals, aorta, bone, bone marrow, brain (cerebrum, brainstem, cerebellum), cecum, cervix, coagulating glands, colon, duodenum, epididymides, esophagus, eyes, gross lesions, heart, ileum, jejunum, kidneys, lacrimal/harderian glands, larynx, liver, lungs, mammary gland, mediastinal lymph node, mediastinal tissues, mesenteric lymph node, mesenteric tissues, nasal tissues, oral tissues, ovaries, oviducts, pancreas, parathyroid glands, peripheral nerve, pituitary, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid-gland, tongue, trachea, urinary bladder, uterus, vagina
  - Microscopic examination of tissues from rats in lower 1,3-dioxolane exposure groups was not conducted since no target organs were identified in the high-exposure group.
- Differences from OECD Guideline

This study is in accord with the current OECD 4 12 guideline for 14 or 28-day inhalation studies. There arc slight differences in the suggested parameters for clinical chemistry and in the duration of the study (12-days) but these are not considered scientifically significant.

**Results**

- NOAEL
  - Males: 516 ppm
  - Females: 516 ppm
- LOEAL
  - Males: 2319 ppm
  - Females: 23 19 ppm
- Mortality

All animals survived the duration of the study.

- Toxic Responses

| Dose | Response |
|---|---|
| 5 16 ppm | · No significant effects |
| 2319 ppm | ▪ Reduction in white-blood cell counts for males and females.<br>. Decrease in spleen weight in females<br>. Increased relative liver weight in females |
| 5132 ppm | . Reduction in white-blood cell counts for males and females.<br>. Deceased body weight gains for males (11%) and for females (4%)<br>▪ Decreased alertness and slight incoordination at the end of each exposure lasting 45 to 85 minutes.<br>. Other changes detailed below were not considered toxicologically significant. |

Remarks Field for Results

- Body Weights

| | Body Weight on Day (grams) | | | | |
|---|---|---|---|---|---|
| **Males** | 1 | 3 | 5 | 8 | 11 |
| Control | 136 | 146 | 154 | 166 | 184 |
| 516 ppm | 135 | 146 | 150 | 168 | 176 |
| 2319 ppm | 134 | 142 | 150 | 165 | 175 |
| 5132 ppm | 135 | 134 | 139 | 156 | 164 |
| **Females** | | | | | |
| Control | 112 | 117 | 118 | 124 | 129 |
| 516 ppm | 113 | 118 | 120 | 129 | 132 |
| 2319 ppm | 114 | 118 | 119 | 128 | 131 |
| 5132 ppm | 112 | 110 | 111 | 12: | 124 |

- Clinical Signs

Animals exposed to 5 132 ppm dioxolane appeared to have decreased alertness and incoordination at the end of each exposure lasting 45 to 85 minutes.

- Hematology WBC

| | Mean White Blood Cells ($x10^3$) | |
|---|---|---|
| Conc (ppm) | Males | Females |
| Control | 9.5±0.9 | 7.0±2.1 |
| 516 ppm | 9.1±0.8 | 7.0±1.7 |
| 2319 ppm | 6.9±0.9 | 5.2io.7 |
| 5 132 ppm | 5.811.4 | 4.6±1.2 |

- **Hematology Comments**
  - The mean RBC, HGB, HCT and PLAT values of males and females exposed to 23 19 and 5 132 ppm were statistically increased when compared to mean control values. These slight increases were exposure-related but were not considered toxicologically significant. Historical control hematologic data from previous 2-week inhalation studies conducted in this laboratory with Fischer 344 rats revealed that each of the identified differences were within the historical normal range except PLAT values for the 23 19 and 5 132 ppm, females and the 5 132 ppm males.
  - Mean WBC counts of males and females from the two highest exposure groups were statistically decreased from control values in an apparent concentration-dependent manner. Examination of mean WBC differential data indicated increased neutrophil:lymphocyte ratios of male and female rats in these two exposure groups. The WBC counts in this study were within the range of historical controls for 29 two-week inhalation studies in this laboratory.
  - There was no demonstrable microscopic evidence of inflammatory foci in tissues or decreased cellularity of lymphoid organs and bone marrow.
- **Clinical Chemistry** — Clinical chemistry analyses indicated statistically decreased mean values for AP in the 23 19 and 5 132 males, and the 5 132-ppm females when compared to respective control mcans. These slightly decreased values were not considered toxicologically significant.
- **Organ Weights** — The relative weights of brain, heart, kidneys, liver, lungs, and testes of male rats exposed to 5132 ppm were statistically increased from those of male controls. Similarly, relative weights of heart, liver, and lungs from high exposure group females were increased statistically from the control females. All of the relative organ weight changes were secondary to decreased terminal body weights.
- **Necropsy f'indings** — Examination of each animal during necropsy did not indicate any exposure-related gross pathologic changes
- **Histopathology** — The few microscopic changes seen during the histopathologic examination were considered to be spontaneous events unrelated to exposure. There were no microscopic changes in the brains of high-exposure group rats to account for the transitory clinical signs observed after each exposure..

**Conclusions**

Remarks Field — The primary effect of dioxolane inhalation exposure to rats was a reduction in white blood cells counts at 23 19 or 5 132 ppm. Body weight gain was affected at the 5 132-ppm exposure level. There was no corresponding myeloid toxicity or inflammatory foci.

**Data Quality**

- Reliability — Klimisch Code 1. May bc used without restriction.

References — 1,3-Dioxolane: A two-week Vapor Inhalation Toxicology Study in Fischer 344 Rats. Dow Chemical Company 1989 Study ID K-010634-005

**Other** — This study is supported by a two-week inhalation study of Dioxolane conducted by BioDynamics for Celanese Corporation. In this study Charles River CD rats (5/sex/group) were exposed for six hours per day, five days per week, for two weeks at concentrations of 0, 984 or 3280 ppm. All animals survived the duration of the study. No treatment related observations were recorded. Body weights, organ weights, clinical chemistry parameters, and macropathology were unremarkable in treated animals. Depressed leukocyte values in male and female exposed animals were reported. [1]

References for supporting studies — A two-week inhalation toxicity study of C-121 (1,3-Dioxolane) in the rat. Bio/Dynamics Inc Project No. 80-7429, June 22, 1981.

**Repeated Dose Toxicity, 13-Week Inhalation**

| | |
|---|---|
| **Type** | Repeated Dose Toxicity, 13-Week Inhalation |
| Test Substance | 1,3-Dioxolane<br>CAS Number: 646-06-O<br>Purity >99.78 % by analysis |
| **Method** | |
| Guideline | This study was conducted to meet the requirements of the United States Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards in 40 CFR part 792; TSCA Health Effects Test Guideline in 40 CFR 798.250; and OECD ISBN 92-64-12367-9 Paris 1982. |
| G L P | Yes |
| Y e a r | 1990 |
| Species | Rat |
| Strain | Fischer 344<br>Charles River, Kingston NY |
| Route of administration | Whole body inhalation of vapor |
| Duration of Test | Thirteen weeks plus eight week recovery study |
| Doses | 0, 298, 1000 or 30 10 (Mean, measured concentrations) |
| ♦ | Male and Female |
| Exposure Period | Six hours per day |
| Frequency o f Treatment | Five days a week<br>Thirteen weeks |
| Number of Animals/group | Ten of each sex |
| Control Group and Treatment | Ten animals of each sex exposed only to air under the same chamber conditions |
| Post-Exposure Observation Period | 24 Hours for main groups; eight weeks for satellite recovery groups. |

- Statistical Methods
  - Descriptive statistics (mean and standard deviation), were used to report chamber concentrations, temperature, and relative humidity.
  - Body weights, absolute and relative organ weights, clinical chemistry, bone marrow myeloid/erythroid ratios and appropriate hematology data for animals to be terminated after 13 weeks were evaluated by Bartlett's test for equality of variances. Clinical chemistry, bone, marrow myeloid/erythroid ratios and appropriate hematology data for satellite animals were evaluated the same way.
  - WBC differential counts are normally analyzed, in this laboratory, with descriptive statistics. Because effects on the white cell was anticipated based on a two-week study, absolute lymphocyte numbers, absolute neutrophil numbers and myeloid to erythroid cell ratios were evaluated in the following statistical evaluation. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or non-parametric **analysis** of variance (ANOVA), followed respectively by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons.
  - Statistical outliers were to be identified by a sequential test and excluded from analysis only for documented scientifically sound reasons that were unrelated to exposure.

Remarks Field for Test Conditions

| | |
|---|---|
| Age at study initiation | Males: seven weeks<br>Females: seven weeks |
| Number animals of each sex per dose | Ten of each sex per concentration |
| Satellite groups | Ten of each sex per dose level and control were exposed for thirteen weeks. Blood samples were taken at four and thirteen weeks of exposure and **again at** four and eight weeks into the recovery period. Satellite rats were necropsied at the end of the tight-week recovery period. |
| Housing | Not specified in report |
| Clinical observations performed and frequency | Mortality and gross signs daily<br>Body weights: weekly<br>Functional observation battery during the sixth and last week of exposure for main group animals consisting of observations for any unusual conditions with respect to: pupil size, respiration, movement, skin and haircoat, salivation, lacrimation, urine staining, fecal staining, locomotor behavior and responsiveness to touch, noise and tail pinch.<br>Animals were evaluated for alertness and activity at the beginning and end of each exposure to evaluate sedative effects |

- Terminal observations
  - Blood taken for hematology (packed cell volume, hemoglobin, erythrocyte count, total leukocytes platelets, differential leukocyte counts and RBC morphology) and clinical chemistry (urea nitrogen, creatinine, alanine aminotransferase activity, aspartate aminotransferase activity, alkaline phosphatase activity, glucose, total protein, albumin, globulin, total bilirubin, cholesterol, triglycerides, phosphorus, calcium, sodium, potassium and chloride)
  - Urine samples obtained for urinalysis (bilirubin, glucose, ketones, blood, pH, protein and urobilinogen, specific gravity and microscopic examination of sediment)
  - Complete gross postmortem examination.
  - Organ weights for brain, heart, lungs, liver, kidneys, adrenals, thymus, spleen and testes were recorded for each rat.
- Histopathology
  - The following tissues were examined from all control and high-exposure animals only. Adrenals, aorta, bone, bone marrow, brain (cerebrum, brainstem, cerebellum), cecum, cervix, coagulating glands, duodenwn, epididymides, esophagus, eyes, gross lesions, heart, ileum, jejunum, kidneys, lacrimal/harderian glands, larynx, liver, lungs, mammary gland, mediastinal lymph node, mediastinal tissues, mesenteric lymph node, mesenteric tissues, nasal tissues, oral tissues, ovaries, oviducts, pancreas, parathyroid glands, peripheral nerve, pituitary, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid-gland, tongue, trachea, urinary bladder, uterus, vagina
  - Microscopic examination of tissues from rats in lower 1,3-dioxolane exposure groups was limited to liver, brain, kidneys, lungs, spleen, bone marrow and gross lesions.

**Results**

- NOAEL
  - Males: 1000 ppm
  - Females: 298 ppm
- LOEAL
  - Males: 3010 ppm
  - Females: 1000 ppm
- Mortality

All animals survived the duration of the study.

- Toxic Responses

| Dose | Response |
|---|---|
| 298 ppm | ■ No significant effects |
| 1000 ppm | ■ Reduction in white-blood cell counts for females.<br>■ Decrease in spleen weight in females<br>■ Increased relative liver weight in females |
| 3010 ppm | ■ Reduction in white-blood cell counts for males and females.<br>■ Mild changes in liver of males.<br>■ Deceased spleen weights in males and females<br>■ Decreased alertness at the end of each exposure<br>■ Decreased urine specific gravity |

Remarks Field for Results

- Body Weights

| | Exposure Period Day (n=20) | | | Recovery Period Day (n=lO) | | |
|---|---|---|---|---|---|---|
| **Males** | Day-l | 46 | 86 | 96 | 116 | 152 |
| Control | 150 | 253 | 299 | 294 | 325 | 323 |
| 298 ppm | 150 | 253 | 300 | 294 | 319 | 314 |
| 1000 ppm | 147 | 247 | 298 | 287 | 315 | 311 |
| 3010 ppm | 147 | 257 | 306 | 303 | 332 | 329 |
| **Females** | | | | | | |
| Control | 109 | 164 | 182 | 186 | 192 | 187 |
| 298 ppm | 108 | 165 | 183 | 180 | 191 | 186 |
| 1000 ppm | 108 | 161 | 182 | 183 | 196 | 190 |
| 3010 ppm | 106 | 158 | 179 | 173 | 189 | 188 |

- Clinical Signs
  - Some exposed male and female rats had notable clinical observations including: red swollen eyes, perineal soiling, diarrhea and darkened crusty material around the eyes and/or nares. One male rat exposed to 1000 ppm had persistent redness, swelling and/or crusty material around one eye. This condition, however, was resolved by study termination. The orbital sinus blood sampling procedure may have resulted in some of the observations of crusty material around the eyes. Because of a lack of a dose response relationship and persistence in any of the observations, none were considered to be exposure-related or toxicologically significant
  - Animals exposed to 3000-ppm dioxolane appeared to have decreased alertness and responsiveness at the end of each exposure. The animals appeared to be fully recovered by the time they were removed from the exposure chamber (approximately 30 minutes after cessation of test material exposure),

- Functional Observation Battery

Functional Observational Battery exams were conducted at 6 and 13 weeks. Isolated observations of perineal soiling, decreased activity (one observation) and chromorhinorrhea were noted. These were not thought to be exposure-related due to the low incidence and lack of a dose response.

- Hematology
- W B C

| Conc (ppm) | Mean White Blood Cells (x10 $^3$) Week of Study | | | |
|---|---|---|---|---|
| **Males** | 4-week | 13-week | Recov-4 | Recov-8 |
| Control | 10.7±2.1 | 9.1±1.3 | 10.2±1.0 | 10.0±1.2 |
| 298 | 9.4±2.1 | 9.6±1.4 | 8.2±0.8* | 9.1±1.1 |
| 1000 | 8.2±0.8* | 8.6±1.0 | 8.7±1.5* | 8.4±0.5* |
| 3010 | 5.5±1.0* | 6.0±1.0* | 8.9±0.9* | 8.6±1.2* |
| **Females** | | | | |
| Control | 7.3±1.4 | 9.2±0.9 | 7.2±1.3 | 6.4±1.1 |
| 298 | 8.7±1.6* | 9.1±1.1 | 6.4±1.1 | 6.7±0.8 |
| 1000 | 6.2±0.8 | 7.8±0.5* | 6.1±0.9 | 6.0±0.6 |
| 3010 | 4.9±0.9* | 5.1±0.6* | 6.0±1.0* | 5.8±0.6 |

- Hematology Comments
  - Although exposure decreased the WBC of rats the magnitude of the decrease at any given time interval was never below the lower limits of historical controls.
  - The percent eosinophils in 3010 ppm exposed rats was increased relative to controls after 13 weeks of exposure
  - Small increases in mean platelet counts occurred during the exposure period in some of the 1000 and 3010 ppm groups. There was a single observation of increased platelets in females exposed to 300 ppm at 4 weeks, however, there was no apparent exposure-response relationship and no difference at 13-weeks. The minimal statistical platelet elevations in the 1000 and 3000-ppm groups were within available historical control data; hence these differences were considered to be of minor biological significance.
  - Sporadic statistically significant variances in red blood cells and hematocrit were in the range of historical controls and did not show concentration-response relationships and are considered of no toxicological significance
- Clinical Chemistry

  A number of'statistically identified differences in various clinical chemistry parameters in exposed male or female rats were identified when compared to their respective controls; however, there was no clear relationship to exposure. Since these parameters were generally within historical control ranges or were not associated with corresponding gross or microscopic effects, most of these clinical chemistry changes were not considered to be diagnostic of a target organ effect. Increased ALT in 3000 ppm exposed rats at 4 weeks, and, in females only at 13 weeks, could be related to the small increases in liver weight. Histological changes were also noted in livers of 3010 ppm exposed males.
- Urinalysis

  Male and female rats exposed to 3010 ppm for 13 weeks (main study and satellite groups) had statistically decreased urine specific gravity. Kcpeat urinalyses revealed similar results. The specific gravity alteration was not associated with a morphologic indication ofnephrotoxicity. It may represent a physiologic response to repeated exposure to dioxolanc vapors.

- Organ Weights
  - Spleen Weights: The mean absolute spleen weight of female rats exposed to 3000 ppm was statistically decreased (27% reduction as compared with control), as were the mean relative spleen weights of males exposed to 3000 ppm (8%) and females exposed to 1000 (11 %) and 3000 ppm. The toxicological significance of this observation is uncertain because there were no morphologic alterations observed in the spleen from any animal in the study. It is possible that the decreased spleen weights are associated with reduced WBC counts.
  - Liver Weights: The mean absolute and relative liver weighs of male and female rats exposed to 3000 ppm dioxolane were statistically increased when compared to control values, as was the mean relative liver weight of females exposed to 1000 ppm. Male rats had minimal microscopic liver changes while females had no morphologic liver alterations. The liver weight increases may be an adaptive physiologic response following repeated exposure.
  - Other Organs: Sporadic statistically significant variances in other organs were observed but did not show concentration-response relationships or morphological evidence of effect and are considered of no toxicological significance
- Necropsy findings: Gross postmortem examinations revealed no differences among groups which were considered to represent an effect of exposure to the test substance

- Histopathology
  - Except for liver changes in high-dose male rats, there were no pathologic findings of significance. In male rats exposed to 3000 ppm dioxolanc, hepatocytes in centrilobular regions of lobules were slightly larger and had more cytoplasmic eosinophilia than controls (incidence 1 0/10).
  - Microscopic examination of bone from exposed and control rats following 13 weeks of exposure indicated that myeloid and erythroid cells were approximately similar in number and, had normal morphology and apparently normal maturation sequences. In addition, there was no cytotoxicity of the microenvironment at the light microscopic level. Therefore, the decreases in WBC counts **were** not associated with observable histopathologic changes **in** bone marrow.
  - The ratio was lower in male and female rats exposed to 3010 ppm for 13 weeks relative to controls; however, the myeloid/erythroid ratio of this exposure group was similar to that of respective controls after 8 weeks post exposure.

**Conclusions**

Kcmarks Field

The primary effect of dioxolane inhalation exposure to rats was a reduction in white blood cells counts at 1000 or 3000 ppm. Kats appeared healthy, body weight gain was not affected and there was no corresponding myeloid toxicity although there was a slight reduction in myeloid cells of the bone marrow at 3000 ppm. Exposure related pathologic effects were limited to slight enlargement of centrilobular hepatocytes of males at 3000 ppm. There was no indication of testicular toxicity based on careful light microscopic examination.

**Data Quality**

- Reliability

Klimisch Code 1. May be used without restriction,

**References**

1,3-Dioxolane: 13-Week Vapor Inhalation Toxicology Study in Fischer 344 Rats. Dow Chemical Company 1990

Other

This study is supported by a 12-month inhalation study in the literature where a concentrations of 2500 mg/m $^3$ was found not to produce dominant lethal effects or significant signs of toxicity manifest by gross observations or mortality [1]

References for supporting studies

[1] Baraânski B; Stetkiewicz J; Czajkowska T; Sitarek K; Szymczak W Mutagenic and gonadotoxic properties of trioxane and dioxolane Med Pr; Vol 35: P245-55 (1984)

* = $p < 0.05$

**Reverse Mutation Assay - *S. typhimurium***

**Type** Reverse mutation assay - S. typhimurium

**Test Substance** 1,3-Dioxolane
CAS Number: 646-06-O

**Method**

- Guideline: None specified
- GLP: No
- System of Testing: Bacterial
- Year: 1980
- Species/Strain: S. *typhimurium:* TA98, TA100, TA1535, TA 1537, TA1538
- Metabolic activation:
  - Tested with and without
    - o Rat liver S-9
    - o Aroclor 1245 induced
    - 0 0.5 ml S-9 per 100 ml agar plate
- Concentrations tested:
  - TA98 0, 0.005, 0.010, 0.10, 1, 5, 10, 25, 50 µl/plate
  - TA100 0, 0.005, 0.010, 0.10, 1, 5, 10, 25, 50 µl/plate
  - TA1535 0, 0.005, 0.010, 0.10, 1, 5, 10 µl/plate
  - TA1537 0, 0.005, 0.010, 0.10, 1, 5, 10 µl/plate
  - TAl538 0, 0.005, 0.010, 0.10, 1, 5, 10 µl/plate
  - All tested with and without metabolic activation
- Statistical Methods:

Evaluation criteria were used as follows:

- Strains TA-1535, TA-1537 and TA-1538: If the solvent control value is within the normal range, a test material that produces a positive dose response over three concentrations with the highest increase equal to three times the solvent control value will be considered to be mutagenic.
- Strains TA-98 and TA-100: If the solvent control value is within the normal range, a test material that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-98 and TA-100 will be considered to be mutagenic.

Remarks Field for Test Conditions

- One replicate
- Positive Controls
  - 0 Without activation
    - Sodium azide: TA1 00, TA 1535
    - 9-Aminoacridine: TA1537
    - 2-Nitrofluorene: TA98, TA1538
  - 0 With activation
    - 2-Anthramine: all strains
- Solvent: none
- Repeat study: TA-1535 was repeated with and without activation because of low solvent values observed in the initial assay.
- Evaluation Criteria: Dose-response related increase in revertants specific for historical rates in each strain

**Results**

- Result: No mutagenic activity under activation or non-activation conditions.
- Cytotoxic Conccntration: No significant cytotoxicity at any concentration
- Genotoxic Effects:
  - Not genotoxic under non-activation conditions
  - Not genotoxic under activation conditions.

Remarks Field for Results

- o Material was soluble in water

**Conclusions**

Remarks Field

- o Not genotoxic under non-activation conditions
- o Not genotoxic under activation conditions.

**Data Quality**

- Reliability: Klimisch Code 2. Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint. Study was conducted under glp's. Conditions of the assay concerning time of incubation may vary somewhat from current OECD guideline.

**References**

Mutagenicity Evaluation of C-121 (1,3-Dioxolane) in the Ames Salmonella/Microsome Plate Test, Litton Bionetics Study 20988, 2/29/1980 submitted to Celanese Chemical Company.

**Other**

This study is supported by other bacterial genotoxicity studies specifically:

- A negative single-plate 5-strain Salmonella reverse mutations assay with and without metabolic activation by Goodyear [1]
- A negative 5-strain Salmonella reverse mutations assay with and without metabolic activation by Hilltop conducted for Ferro under glp conditions. [2]
- Published negative 5-strain Salmonella reverse mutations assay with and without metabolic activation.'
- A combined S. typhimurium and Saccharomyces cerevisiae mutation evaluation conducted by Litton Bionetics for Dow Chemical Company [4].

References for Supporting Studies


1. Mutagenicity Evaluation of 1,3-Dioxolane, Goodyear Tire and Rubber Company. Laboratory report #79-55, 29 November 1979.
2. The Salmonella/Microsomal Assay for Bacterial Mutagenic Activity of Unstabilized 1,3-Dioxolane. Hilltop Research Project No 83-0212-21 10 March, 1983.
3. Kowalski Z, Spiechowicz E, Barahnski B. Absence of mutagenicity of trioxane and dioxolane in Salmonella typhimurium. Mutat Res 136: 169-71 (1984).
4. Mutagenicity Evaluation of Compound D8 (1,3-Dioxolane). Litton Bionetics, l/20/1975 submitted to Dow Chemical Company.

**Mutagenic Assay - *S. typhimurium* and *Saccharomyces cerevisiae***

| | |
|---|---|
| **Type** | Mutagenic assay - *S. typhimurium* and *Saccharomyces cerevisiue* |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o |

**Method**

- Guideline: None specified
- GLP: No
- System of Testing: Bacterial
- **Year**: 1975
- Species/Strain: *S. typhimurium* TA1535, TA 1537, TA1538
  *Saccharomyces cerevisiae* strain D4
- Metabolic activation
  - Tested with and without induced rat, mouse and monkey homogenates of liver, lung and testis. Animals were induced with a "mixture of polychlorinated biphenyls".
- Concentrations tested
  - *Saccharomyces cerevisiue :*
    - 2.0 and 5.0% in suspension
  - Salmonella:
    - 0.75 and 1.50% for suspension incubations
    - 1.5% for plate tests.
  - All tested **with** and without metabolic activation
- Statistical Methods: None specified

- Remarks Field for Test Conditions
  - Duplicate plates, one replicate
  - Salmonella were tested with rat tissues in the suspension test only. Mouse and monkey tissues were used in the plate incorporation technique only.
  - Salmonella and yeast were tested at high and low dose levels using non-activation conditions and activation conditions with rat liver, rat lung and rat testis homogenates.
  - Salmonella were tested under activation conditions with mouse liver, mouse lung and mouse testis homogenates only at the high dose level.
  - Salmonella were tested under activation conditions with monkey liver, monkey lung and monkey testes homogenates only at the high dose level.
  - Yeast were tested only with rat tissue activation and not mouse or monkey.
  - Positive Controls
    - 0 Without activation
      - Ethylmethane sulfonate
      - 2-Nitrofluorene
      - Quinacrine mustard
    - 0 With activation
      - 2-Acetylfluorene
      - Dimethyhritrosamine
  - Solvent: none (except for positive controls)
  - Evaluation Criteria: Dose-response related increase in revertants specific for historical rates in each strain.

**Results**

- Result — No mutagenic activity under activation or non-activation conditions.
- Cytotoxic Concentration — Fifty percent bacteria survival when tested at 3.0% in the preliminary cytotoxicity test, yeast showed no cytotoxicity at 5% (highest concentration tested).
- Genotoxic Effects
  - Not genotoxic under non-activation conditions
  - Not genotoxic under activation conditions.

Remarks Field for Results

- With the organ homogenates, the positive controls gave significant genotoxic activity only with the liver and testis preparation for the rat, the liver for the mouse and the liver for the monkey.
- Under non-activation conditions, both bacteria and yeast responded as expected to the positive controls.

**Conclusions**

Remarks Field

- Not genotoxic under non-activation conditions
- Not genotoxic under activation conditions.
- The contribution of the lung and testis homogenates to the genotoxic evaluation of the test substance is questionable since the positive controls did not show significant response except for the rat testis with strain TA-1537
- The main value of this study is the negative response of the yeast as other more robust bacterial genotoxicity tests have been conducted using this test material.

**Data Quality**

- Reliability

Klimisch Code 2. Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint although the study was not conducted under glp's.

**References**

Mutagenicity Evaluation of Compound D8 (1,3-Dioxolane). Litton Bionetics, 1/20/1975 submitted to Dow Chemical Company.

**Other**

The Salmonella portion of this study is supported by other Salmonella tests providing clear negative results. The key study is listed below.

Kcferences for Supporting Studies

Mutagenicity Evaluation of C- 12 1 (1,3-Dioxolane) in the Ames Salmonella/Microsome Plate Test, Litton Bionetics Study 20988, 2/29/1980 submitted to Celanesc Chemical Company

## In *Vitro* Mammalian Chromosome Aberration Test

**Type** *In Vitro* Mammalian Chromosome Aberration Test

**Test Substance** 1,3-Dioxolane
CAS Number: 646-06-O

**Method**

- Guideline: None specified
- GLP: Yes
- System of Testing: Non-bacterial
- Year: 1985
- Species/Strain: Chinese Hamster Ovary (CHO-WBL), Origin S. Wolff s laboratory UCSF, Litton Bionetics clone, and average cell cycle time 12-14 hours .
- Metabolic activation: Tested with and without
  - o Rat liver S-9
  - o Aroclor 1245 induced
  - 0 15 microliters per 10 ml culture
- Concentrations tested: 0, 2.0, 3.0, 4.0, 5.0 mg/ml for both non-activation and activation conditions.
- Statistical Methods: Fishers Exact Test for comparisons of treated cultures with pooled controls.

Remarks Field for Test Conditions

- Range-finding test to determine cytotoxicity or cell cycle delay used the following conditions:
  - Single culture per dose levels of 0, 1.67 or 5.0 mg/ml for both non-activation and activation conditions. Level of 0.50 was added under activation conditions.
  - Under non-activation conditions there was a 2-hour exposure to test substance followed by 23 hours of incubation prior to addition of colcemid and 2.5 hours of incubation prior to collection.
  - Under activation conditions the same time intervals were employed and fetal-calf serum was omitted.
- The aberration test used the following conditions:
  - Duplicate cultures at each dose level of 0, 2.0, 3.0, 4.0, 5.0 mg/ml for **both** non-activation and activation conditions
  - Solvent, McCoy's 5a culture media.
  - Under non-activation conditions, there was a 7.5-hour exposure followed by 2.5-hr incubation in the presence of colcemid.
  - Under non-activation conditions there was a 2-hour exposure to test material followed by a wash and an 8-hour incubation in the presence of colcemid.
  - 100 Metaphases from each culture were scored for a total of 200 metaphases per dose level.
  - Negative control, McCoy's 5a
  - Positive controls
    - Without activation – Mitomycin C
    - With activation – Cyclophosphamide

Results

- Result: No increase in the number of aberrations was found at any concentration of test material. Positive controls demonstrated the sensitivity of the test system.
- Cytotoxic Concentration: Cytotoxicity was not observed up to and including the highest concentration tested of 5 mg/ml which is the highest concentration recommended under the current OECD 473 guideline.
- Genotoxic Effects
  - Not genotoxic under non-activation conditions
  - Not genotoxic under activation conditions.

Remarks Field for Results

- Material was soluble in water
- The number of aberrations per cell, the percent cells with aberrations, and the percent cells with more than one aberration were all similar to appropriate controls under both activation and non-activation conditions.
- Positive controls produced clear significant increases in aberrations as measured by aberrations per cell, the percent cells with aberrations and the percent cells with more than one aberration.

**Conclusions**

Remarks Field

- Not genotoxic under non-activation conditions
- Not genotoxic under activation conditions.
- Study was well conducted, although the incubation times and exact procedures vary somewhat from the current OECD guideline the consistently low level ofaberrations observed at all dose levels both with and without activation and the clear responses of the positive controls strongly support lack of genotoxic activity in this assay.

**Data Quality**

- Reliability

Klimisch Code 2. Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint. Study was conducted under glp's. Conditions of the assay concerning time of incubation vary somewhat from current OECD guideline.

**References**

Mutagenicity Evaluation of 1,3-Dioxolane in an In Vitro Cytogenic Assay Measuring Chromosome Aberration Frequencies In Chinese Hamster Ovary (CHO) Cells. Submitted to Ferro Corporation. Litton Bionetics, Inc, Kensington Maryland. LB1 Project No. 20990, February 1985

**Mouse Lymphoma Forward Mutation Assay**

| | |
|---|---|
| **Type** | Mouse lymphoma forward mutation assay |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-O |

**Method**

| | |
|---|---|
| Guideline | None specified |
| GLP | No |
| System of Testing | Non-bacterial |
| Year | 1985 |
| Species/Strain | Mouse lymphoma L5 178Y TK+/- |
| Metabolic activation | Tested with and without<br>- Rat liver S-9<br>- Aroclor 1245 induced<br>Final concentration of S-9 in cell suspensions was five percent. |
| Concentrations tested | 0, 750, 1500, 2500, 3000, 4000, 50000 nl/ml for both non-activation and activation conditions. |
| Statistical Methods | Simple ratio criteria |
| Remarks Field for Test Conditions | - Initial trial under activation conditions discarded because the positive control mutation frequencies were unacceptable.<br>- Duplica tep l ets for counting mutant colonies<br>- Solvent, water<br>- Negative control, water<br>- Positive controls<br>  - Without activation – EMS (2 levels)<br>  . With activation – MCA (2 levels) |

**Results**

- Result

No dose-dependent increase in the number of mutants in the absence or presence of metabolic activation was observed. Dioxolane did not induce significant increases in the mutation frequency at the TK locus in L5 178Y TK+/- cells. . Positive controls demonstrated the sensitivity of the test system.

- Cytotoxic Concentration

Low dose-dependent cytotoxicity observed under non-activation conditions percent relative growths ranges form 85 % to 61% . Under activation conditions, cytotoxicity was dose-dependent but low, with percent relative growths ranging from 95% to 78%.

- Genotoxic Effects

- Not genotoxic under non-activation conditions
- Not gcnotoxic under activation conditions.

Remarks Field for Results

- Material was soluble in water
- Under non-activation conditions, the mutant frequency ranged from 12.8 to 22.5 x $10^{-6}$ without any discernable dose dependency.
- Under activation conditions, the mutant frequency ranged from 26.5 to 43.9.5 x $10^{-6}$ without any discernable dose dependency
- The minimum mutation frequency required for a positive response under non-activation conditions based on the control mutation frequencies in this assay was 38 X $10^{-6}$.
- The minimum mutation frequency required for a positive response under activation conditions, based on the control mutation frequencies in this assay, was 46 X $10^{-6}$.
- The discarded initial activation assay had positive control values that were considered unacceptably low, although clearly positive. In this discarded test, the mutation frequency for the test substance was also low and similar to that found in the repeated test. The discarded thet supports the final result.

**Conclusions**

Remarks Field

- Not genotoxic under non-activation conditions
- Not genotoxic under activation conditions.
- Study was well conducted, although there was no glp certification the study appears to have been conducted using a glp-quality protocol in a glp compliant laboratory.

**Data Quality**

- Keliability

Klimisch Code 2. Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

**References**

Mutagenicity Evaluation of 1,3-Dioxolane in the Mouse Lymphoma Forward Mutation Assay. Submitted to Fcrro Corporation. Litton Bionetics, Inc, Kensington Maryland. LBI Project No. 20989, April 1985

**Other**

## Mammalian Cell Transformation Assay, in *vitro*

| | |
|---|---|
| **Type** | Mammalian Cell Transformation Assay, *in vitro* |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o |

Method

| | |
|---|---|
| ■ Guideline | None specified |
| ■ GLP | No |
| • Year | 1981 |
| ■ Cell Type | C3H 10T-1/2 clone 8 |
| • Concentrations tested | 0, 1, 10, 20, 50, 100,500, 1000, 2000, 5000, 10000, 20000 and 40000 µg/ml |
| ■ Statistical Methods | None used |

Remarks Field for Test Conditions

- Dose selection based on initial cytotoxicity testing using plating efficiency as a metric.
- A set of replicates was exposed in T-25 flasks and a set of replicates was exposed in flat 60 mm culture dishes.
- Incubation of plates carried out in sealed Mason jars to prevent volatilization
- T-25 flasks were sealed to prevent loss of test material.
- Twenty-four hour incubation time with test substance
- Cells washed after 24-hour incubation with test substance
- Cells fed with fresh media 24 hours after exposure was complete and every three days until the 14-day point then weekly (with 10%FCS supplementation) for the duration of the 38 day culture period.
- At the 14-day interval, four to six T-25 flasks at each concentration and three to five dishes were stained and examined.
- At the 38-day interval, two to four T-25 flasks at each concentration and three or four dishes were stained and examined.
- High-concentration levels (>2000 µg/ml) of T-25 flasks at the 38-day interval were contaminated with a fungus in the initial assay and were run a second time about 2 months later. In this repeat assay, six T-25 flasks were incubated as before for 38 days at each concentration level from 1 to 20,000 µg/ml, stained and examined. The data varied from the initial assay in that there was a much higher transformation rate in the positive controls (BP 5 or 6 times higher) and the transformation rate for the test substance was much higher at the repeated concentration levels. The negative control still showed no transformed colonies.
- Positive control: Benzpyrene @ 2.5, 1.0 or 0.5 µg/ml
- Colonies examined macroscopically and microscopically.
- 300 cells plated per flask or dish

- Differences from Guideline: The study is similar to the Draft OECD Guideline " *In Vitro* Syrian Hamster Embryo Cell Transformation Assay". The major differences from this guideline are the type of cells used and the incubation times employed. It was similar or superior in other respects including number of dose levels employed.

**Results**

- Result
  - Keport authors conclude that Dioxolane is a "weak positive" in this cell-transformation assay.
  - The data suggest transforming activity but there are issues of concern with the data. The poor reproducibility, the lack of clear dose-concentration relationships, the test material sometimes giving results similar to positive controls and at other times no response, and the lack of high-concentration toxicity data make it difficult to conclude that this assay is positive.
  - A more conservative interpretation of the data is "Not Interpretable"

- Cytotoxic Concentration — Cytotoxicity observed above 5,000 μg/ml. There was no valid measure of cytotoxicity given for the 38-day examinations

- Genotoxic Effects — Not interpretable

Remarks Field for Kcsults

- The majority of transformed foci were classified as "type II" rather than "type III" This is defined as follows:

  - Type 1. Foci composed of monolayer cells that are more densely packed than the background cells. This type is not considered malignant and is not scored.

  - Type 11. Foci show massive piling up into virtually opaque multilayers. The cells are only moderately polar, thus criss-crossing is not pronounced. Fifty percent of Type 11 foci have been shown to be malignantly transformed.

  - Type III. Foci are composed of highly polar, Gbroblastic, mu ltilayered, criss-crossed arrays of densely stained cells. Eighty-five percent of Type III foci have been shown to be malignantly transformed.

| Conc (μg/ml) | 14-Day T-25 | Foci/plate Dishes | 38-Day T-25 | Foci/plate Dishes | Repeat 38-day T-25 |
|---|---|---|---|---|---|
| 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0 |
| 1 | 0.0 | 0.0 | 0.0 | 0.0 | 2.8 |
| 10 | 0.0 | 0.0 | 0.0 | 0.0 | 2.8 |
| 20 | 0.0 | 0.0 | 0.0 | 0.0 | 3.7 |
| 50 | 0.0 | 0.0 | 0.0 | 0.0 | 3.7 |
| 100 | 0.0 | 0.0 | 1.0 | 0.75 | 4.7 |
| 500 | 0.0 | 0.0 | 0.3 | 1.33 | 4.0 |
| 1,000 | 0.0 | 0.0 | 1.5 | 1.25 | 5.0 |
| 2,000 | 0.6 | - | C | | - |
| 5.000 | 0.0 | 0.0 † | C | 2.0 | 4.66 |
| 10,000 | | | C | 2.25 | 1.2 |
| 20,000 | 0.5† | 0.0† | C | 0.25 | 0 |
| 40,000 | 0.0†† | 0.0†† | - | - | |
| BP 0.5 | 0.3 | 0.75 | 0.5 | 6 | 3.0 |
| BP 1.0 | 1.0 | 1.0 | 1.25 | 12 | 6.0 |
| BP 2.5 | | | - | | 7.0 |

- = Not run, † = Cytotoxic, , †† = Highly Cytotoxic
C = contaminated, BP = Benzpryene Positive Control

**Conclusions**

| | |
|---|---|
| Remarks Field | The report authors concluded that these results represent a weak positive; however, the reviewer (who prepared this summary) disagrees with this interpretation and judges the study not interpretable with regard to genotoxic activity. |

**Data Quality**

| | |
|---|---|
| • Reliability | Klimisch Code 3. Reliable with severe restrictions, study design, conduct and reporting are considered reliable to address the endpoint but the data are not clearly supportive of the author's conclusions. |

**References**

An Assay of Cell Transformation and Cytotoxicity in C3H 10T 1/2 Clonal Cell Line for the Test Chemical C-121 (1,3-Dioxolane). University of Minnesota Environmental Pathology Laboratory, Authors Garry and Nelson, 27 August 1981.

**Other**

This study is supported by a study using BALB/C-3T3 cell cultures. This study, however, reported Dioxolanc to give a clear negative response in cell transformation.

| | |
|---|---|
| Reference for supporting study | Evaluation of 1,3-Dioxolane in the In Vitro Transformation of BALB/C-3T3 Cells Assay. Litton Bionetics, Inc, Project 20992, January 1085. Submitted to Ferro Corporation |

## Mammalian Cell Transformation Assay, *in vitro*

**Type** Mammalian Cell Transformation Assay, in *vitro*

**Test Substance** 1,3-Dioxolane
CAS Number: 646-06-o

**Method**

- Guideline: None specified
- GLP: Not specified (report was reviewed by the quality assurance unit but did not specifically state that the test was conducted under glp)
- Year: 1985
- Cell Type:
  - BALB/C-3T3
  - Subclone 14 of Clone 1- 13 from Takeo Kakunaga
- Concentrations tested: 0, 1000, 4000, 8000, 12000 and 15000 nl/ml
- Statistical Methods: Bailey's modification of Student's t-test was applied to calculate the statistical significance of the positive control and test material transforming activity.

Remarks Field for Test Conditions

- Positive control: 2.0 micrograms/ml MCA
- Seventy-two hour treatment time followed by washing and continued incubation for four weeks with re-feeding twice a week.
- Treatments carried out in sealed 25 $cm^2$ flasks to prevent volatilization.
- Doses based on preliminary cytotoxicity range-finding test.
- 30,000 cells plated per culture vessel
- 18 Cultures per dose level (lower n in Results table due to contaminated flasks)
- Simultaneous cytotoxicity test conducted under same conditions as transformation assay to verify the toxicity of test material to cells.

| **Preliminary Cytotoxicity** Concentration (nl/ml) | Average Number of Colonies per Flask | Kelative cell Survival (%) |
|---|---|---|
| 0 | 94.3 | 100.0 |
| 1000 | 92.0 | 97.6 |
| 2000 | 86.3 | 91.6 |
| 4000 | 74.0 | 78.5 |
| 8000 | 55.0 | 58.3 |
| 16000 | 4.0 | 4.2 |
| Simultaneous Cytotoxicity Test | | |
| 0 | 59.7 | 100.0 |
| 1000 | 51.0 | 85.4 |
| 4000 | 46.3 | 77.6 |
| 8000 | 29.7 | 49.7 |
| 12000 | | 14.0 |
| 15000 | 0.0 | 0.0 |

- Differences from Guideline: The study is similar to the draft OECD Guideline " In *Vitro* Syrian Hamster Embryo Cell Transformation Assay". The major differences from this guideline are the type of cells used and the incubation times employed. It was similar or superior in other respects including number of dose levels, replicates, cytotoxicity testing and MTD selection. The current study protocol was shown to be sensitive by the response of the positive controls.

Results

- Result: No increase in the number of transformed colonies or transformed foci was noted at any concentration of test material. Positive controls demonstrated the sensitivity of the test system
- Cytotoxic Concentration: Severe cytotoxicity observed above 12000 micrograms/ml, moderate dose-dependent cytotoxicity observed at 1000 microgram per ml and above. Data in table above
- Genotoxic Effects: Not genotoxic under these conditions

Remarks Field for Results

The test material was water soluble and precipitation was not recorded.

**Results**

| Concentration (nl/ml) | Transforming Activity $Log_{10}(x+1)$ (mean±sem) | (n) | Foci/Culture average |
|---|---|---|---|
| 0 | 0.050±.027 | (18) | 0.12 |
| 1000 | 0.050±.027 | (18) | 0.12 |
| 4000 | 0.0671.030 | (18) | 0.17 |
| 8000 | 0.050±.027 | (18) | 0.12 |
| 12000 | 0.067±.030 | (18) | 0.17 |
| 15000 | 0.075±.034 | (16) | 0.19 |
| MCA (2µg/ml) | 0.602±.039 | (18) | 3.00** |

** $p<0.01$; MCA = 3 Methylcholanthrene

**Conclusions**

Remarks Field

No genotoxic activity. The transforming activities of all five doses of Dioxolane were comparable to the spontaneous background average rate of 0.12 foci/culture vessel and there was no evidence of a dose-related response with the test material. This range of test material treatments corresponded to 0 to 85% survival in the simultaneous colony survival assay. Although the survival assay, using only 200 cells: suggested there would be no surviving cells with test material treatments at 15000 nl/ml, some cells survived in the transformation assay where 30,000 cells were plated per culture vessel. Therefore, concentrations of 1,3-dioxolane from 1,000 to 15,000 nl/ml were evaluated as being nontransforming to Balb/C-3T3 cells.

**Data Quality**

- Reliability

Klimisch Code 2. Reliable with restrictions, study design conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

**References**

Evaluation of 1,3-Dioxolane in the In Vitro Transformation of BALB/C-3T3 Cells Assay. Litton Bionetics, Inc, Project 20992, January 1985. Submitted to Ferro Corporation

**Other**

This study is supported by a previous study using C3H 10T-1/2 cell cultures. This study reported Dioxolane to give a weak positive response in cell transformation but is considered uninterruptible by the writer of the robust summary.

Rcferencc for Supporting Study

An Assay of Cell Transformation and Cytotoxicity in C3H 1 OT 1/2 Clonal Cell Line for the Test Chemical C-121 (1,3-Dioxolane). University of Minnesota Environmental Pathology Laboratory, Authors Carry and Nelson, 27 August 1981

**Mouse Micronucleus Assay**

| | |
|---|---|
| **Type** | Mouse micronucleus assay |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o |
| **Method** | |
| • Guideline | None specified, Meets or exceeds OECD 474 guideline |
| • GLP | Yes |
| • Year | 1989 |
| • Species/Strain | Mice/ICR Harlan Sprague Dawley |
| • Sex | Mice of each sex |
| • Route of Administration | IP Injection in Corn Oil |
| • Doses | 0,525, 1050 and 2100 mg/kg |
| • Exposure Period | 24, 48 and 72 hours |
| • Statistical Methods | The incidence of micronucleated polychromatic cells was compared to controls with a significant increase relative to the negative control set at the p ≤0.05 level using Kastenbaum-Bowman Tables* |

Remarks Field for Test Conditions

- Age at Study Initiation: Six to eight weeks
- Number of animals per dose: Five of each sex at each dose-time sacrificewere planned, more animals were dosed due to mortality. High-dose mortality was unexpectedly high and a supplemental group of 15 animals of each sex was dosed at 2 100 mg/kg. Of these supplemental animals only four of each sex survived to the scheduled sacrifice at 72 hours. There were insufficient surviving high-dose animals to sacrifice five per dose at the high dose. The resulting dosing and sacrifice of animals is shown below:

| | Number of Animals Sacrificed at Each Time | | | | | |
|---|---|---|---|---|---|---|
| Dose | 24 hours | | 48 hours | | 72 hours | |
| (mg/kg) | M | F | M | F | M | F |
| 0 | 5 | 5 | 5 | 5 | 5 | 5 |
| 525 | 5 | 5 | 5 | 5 | 5 | 5 |
| 1050 | 5 | 5 | 5 | 5 | 5 | 5 |
| 2100 | 5 | 5 | 3 | 5 | 4 | 4 |

- **Control Groups and Treatment**
  - Negative Control: Corn Oil
  - Positive Control: Triethylenemelamine in water at 0.25 mg/kg
- **Clinical Observations**
  - All low and mid-dose animals appeared normal. High-dose animals showed several clinical observations indicating toxicity including prostration, lethargy, irregular breathing, and ruffled fur.
- **Criteria for Evaluating Results**
  - Negative control must not exceed an incidence of 5/1000 micronucleated polychromatic cells.
  - A positive result is indicated if the incidence of micronucleated polychromatic cells is significantly increased relative to the negative control ($p \leq 0.05$, Kastenbaum-Bowman Tables)
  - 1000 polychromatic erythrocytes scored for each animal.
- **Criteria For Selection of MTD**
  - The high dose was set as 80% of the $LD_{50}$ (7 day). A preliminary toxicity study was conducted with results shown below. The $LD_{50}$ (7 day) was calculated by probit analysis to be approximately 2603 mg/kg.

| Prclim Tox Study | Seven Day Mortality | |
|---|---|---|
| IP Dose (mg/kg) | Males | Females |
| Contol | 0/5 | 0/5 |
| 1576 | 0/5 | 0/5 |
| 2048 | 1/5 | 0/5 |
| 2663 | 5/5 | 3/5 |
| 3462 | 5/5 | 5/5 |
| 4500 | 4/5 | 5/5 |

- **Differences from Current OECD Guideline**

This study meets all requirements of the current OECD 474 guideline. The study used more dose levels and time points than indicated in the guideline. As there was a previous positive study in the literature the study design was especially robust.

**Results**

- Effect on PCE/NCE ratio by dose level by sex.

PCE/Total Erythrocytes

| Dose | 24 Hours | | 48 Hours | | 72 Hours | |
|---|---|---|---|---|---|---|
| (mg/kg) | M | F | M | F | M | F |
| 0 | 0.59 | 0.59 | 0.62 | 0.62 | 0.62 | 0.66 |
| 525 | 0.53 | 0.57 | 0.62 | 0.64 | 0.54 | 0.65 |
| 1050 | 0.53 | 0.60 | 0.58 | 0.67 | 0.43 | 0.54 |
| 2100 | 0.31 | 0.44 | 0.26 | 0.41 | 0.37 | 0.35 |
| TEM | 0.57 | 0.57 | | | | |

- Genotoxic Effects: Negative

- NOAEL: 1050 mg/kg for bone marrow toxicity as measured by the PCE/NCE ratio

Remarks Field for Results

- Induction of Micronucleated Cells

Micronucleated Cells/ 1000 PCE

| Dose | 24 Hours | | 48 Hours | | 72 Hours | |
|---|---|---|---|---|---|---|
| (mg/kg) | M | F | M | F | M | F |
| 0 | 0.2 | 0.2 | 0.2 | 0 | 0.2 | 0 |
| 525 | 0.2 | 0.2 | 0.2 | 0 | 0.4 | 0.6 |
| 1050 | 0.2 | 0.2 | 0 | 0.4 | 0 | 0 |
| 2100 | 0.6 | 0 | 0.3 | 0.4 | 0 | 0 |
| TEM | 18.4 | 20.8 | - | - | - | - |

- Mortality
  - All low and mid-dose mice survived to sacrifice.
  - Mortality at high dose was males 23/35 and females 21/35

**Conclusions**

Remarks Field

- There was no induction of micronucleated polychromatic cells associated with administration of Dioxolane.
- There was significant induction of micronucleated polychromatic cells associated with administration of the positive control.
- Dioxolane showed no genotoxicity in this assay under these conditions.
- The highest dose of dioxolane was toxic to the animals including the bone marrow.

**Data Quality**

- Reliability: Klimisch Code 1. Reliable without restriction.

**References**

Micronucleus Cytogenetic Assay in Mice. Test article C-121 [1,3-Dioxolane]. Microbiological Associates Inc., Study No. 1'9034.122, December 28, 1989

* Kastenbaum, M and K Bowman. Tables for determining the statistical significance of mutation frequcncics. Mutation Res. 9:527-549 (1970)

**Other**

An earlier study in the literature reported Dioxolane was positive in the micronucleus assay. In this report mice were given dioxolane ip in two doses at 24 hr intervals. Dioxolane at doses from 1500 mg/kg to 6000 mg/kg caused a significant increase of micronucleated polychromatic erythrocytes in bone marrow of mice as compared to the negative control value.

Reference for Supporting Study

Przybojewska B, Dziubautowska E, Kowalski Z. Genotoxic effects of dioxolane and trioxanc in mice evaluated by the micronucleus test. Toxicol Lett 21:349-52 (1984)

**Dominant Lethal, Oral Dosing**

| | |
|---|---|
| **'Type** | Dominant Lethal, Oral Dosing |
| **Test Substance** | Dioxolane<br>CAS Number: 646-06-o |
| **Method** | |
| • Guideline | None specified but basically in accord with OECD 478 |
| • GLP | **N o** |
| • Year | 1984 |
| • Species | Rat |
| • Strain | Albino, Wistar |
| • Route of administration | Oral gavage |
| • Doses | 0,580, 1160 mg/kg |
| • Sex | Male |
| • Number of Animals/group | 10 |
| • Vehicle | Water |

| Remarks Field for Test Conditions | | |
|---|---|---|
| | • Age at Study Initiation | 3 ½ to 4 months; weight 300 to 320 g |
| | • Doses | 0,580 and I 160 mg/kg/day |
| | • Dosing | Males only |
| | • Dosing Schedule | Five days a week |
| | • Dosing Duration | Eight weeks |
| | • Mating Interval | Weekly |
| | • Mating Ratio | 2: 1 females:males |
| | • Variations from OECD 478 Protocol Guideline | Guideline suggests that the number of males should be sufficient that 30-50 pregnant rats be evaluated at each time interval. In this study, 13 to 20 pregnant females were evaluated at each time; however, more time periods than typical were evaluated in this study. Current guideline suggests three dose levels; only two were used in this study. |

**Results**

- Result: No evidence of dominant lethal effect

- Dominant Lethal Rate

| Dose | Week (Dominant Lethals per Female) 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 0 mgikg | 0.84 | 0.83 | 1.45 | 1.29 | 0.89 | 1.11 | 0.93 | 0.67 |
| 580 mg/kg | 0.73 | 1.31 | 0.94 | 0.89 | 0.75 | 0.78 | 1.06 | 1.29 |
| 1160 mg/kg | 0.94 | 0.64 | 0.88 | 1.23 | 0.56 | 0.46 | 0.47 | 0.67 |

- Number of deaths at each dose level

| Dose | Mortality |
|---|---|
| 0 mg/kg | 0/10 |
| 5 80 mg/kg | 1/10 |
| 1160 mg/kg | 1/10 |

Remarks Field for Results

- Time of death: 5 80 mgikg group one animal during fifth week; 1160 mg/kg group one animal during third week
- Clinical Signs: Behavior and appearance did not differ to any significant degree from controls
- Bodv Weights:

| Dose (mg/kg) | Body Weight gain (percent control gain) |
|---|---|
| 580 | 47% |
| 1160 | 22% |

- Organ Weights: Absolute and relative weights of liver, kidney and spermatic vesicle were increased at both dose levels.
- Necropsy and Microscopic Findings: Necropsy finding arc not discussed. Histopathology was performed on thc testes. Necrosis of the seminiferous epithelium was reported in 1/1 0 controls, number unspecified in the 580 mg/kg group and 3/9 high dose males.

- Target Organs: Testis considered target by histopathology but no change observed in fertility. Other organ weights were altered but there is no histopathology for confirmation. No changes in testes weight resulted from dosing.

- Criteria for Dominant Lethal Effect: Investigators used early resorptions as a measure of dominant lethal effect; however, neither implants per female, live fetuses per female nor preimplantation loss per female was aff'cted by treatment.

**Conclusions**

Remarks Field: Study documentation is good for a published article. The study appears to have been well conducted and is similar to current OECD guideline. Based on reduction in body weigh gains, organ weight changes and histopathology all dose levels produced clear signs of toxicity indicating the study is valid regarding use of a minimally toxic dose as the high-dose level. There was no evidence of a dominant lethal effect.

**Data Quality**

- Reliability: Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards. Study design and reporting meets current EPA/OECD guidelines with minor exceptions.

**References**

Baranski, B; Stetkiewicz, J. Evaluation of Mutagenic and Gonadotoxic Properties of Trioxane and Dioxolane. Medycyna Pracy:35 245-255 (1984)

**Other**

**Dominant Lethal, Inhalation**

| | |
|---|---|
| **Type** | Dominant Lethal, Inhalation |
| **Test Substance** | Dioxolane<br>CAS Number: 646-06-o |
| **Method** | |
| • Guideline | None specified, conducted basically in accord with OECD 478 |
| • GLP | No |
| • Year | 1984 |
| • Species | Rat |
| • Strain | Albino, Wistar |
| • Route of administration | Whole body inhalation |
| • Doses | 0, 2500 mg/$m^3$ |
| • Sex | Male |
| • Number of Animals/group | 14 |
| • Vehicle | Air |
| • Duration of Dosing | 12 Months |
| • Statistical Methods | Kruskal Wallis Test followed by non-parametric tests for groups with and without normal distribution. |

| | | |
|---|---|---|
| Remarks Field for Test Conditions | ○ Age at Study Initiation | 3 ½ to 4 months; weight 300 to 320 g |
| | ○ Doses | 0 and 2500 mg/$m^3$ |
| | ○ Dosing | Males only |
| | ○ Dosing Schedule | Five days a week, five hours a day. |
| | ○ Dosing Duration | 12 Months |
| | ○ Mating Interval | Once at end of study for one week |
| | ○ Mating Ratio | 2: 1 females:males |

- Variations from OECD 478 Protocol Guideline: Guideline suggests that the number of males should be sufficient that 30-50 pregnant rats be evaluated at each time interval. In this study, 18 to 20 pregnant females were evaluated. Current guideline suggests three dose levels; only one was used in this study.
- Other: Concentration of test material measured by chromatography using a published procedure.

**Results**

- Result: No evidence of dominant lethal effect

- Dominant Lethal Rate

| | Effect (per female) | | | | | |
|---|---|---|---|---|---|---|
| Dose | Number Pregnant | Live Fetuses | Dead Implants | Number Implants | Corpora Lutea | Preimplantation loss |
| 0 mg/m3 | 20 | 11.4 | 0.6 | 12.0 | 13.7 | 1.8 |
| 2500 mg/m3 | 18 | 11.1 | 0.17 | 11.3 | 13.6 | 2.3 |

- Number of deaths at each dose level

| Dose | Mortality |
|---|---|
| 0 mg/m$^3$ | 0/14 |
| 2500 mg/m3 | 0114 |

Remarks Field for Kesults

- Time of death: No deaths
- Clinical Signs: Not reported
- Body Weights: Not reported
- Organ Weights: Not reported

| | |
|---|---|
| ◊ Necropsy and Microscopic Findings | Not clearly reported. It was stated that for the "rats exposed to Dioxolane the frequency of necrosis of the spermicidal epithelium was much higher than for the control group rats"; but the incidence or other details was not given. Leydig cell lesions were also mentioned but it was also stated that the treated and control groups were not different regarding this lesion. |
| ◊ Target Organs | None reported. |
| ◊ Criteria for Dominant Lethal Effect | Investigators used early resorptions as a measure of dominant lethal effect; however, neither implants per female, live fetuses per female nor preimplantation loss per female was affected by treatment. |

**Conclusions**

| | |
|---|---|
| Remarks Field | Study documentation is good for a published article. The study appears to have been well conducted and the protocol is similar to the current OECD guideline. Based on reduction in body weigh gains, organ weight changes and histopathology, all dose levels produced clear signs of toxicity indicating the study is valid regarding use of a maximum tolerated dose. There was no evidence of a dominant lethal effect. |

**Data Quality**

| | |
|---|---|
| • Reliability | Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards. Study design and reporting meets current EPA/OECD guidelines with minor exceptions. |

**References**

Baranski, B; Stetkiewicz, J. Evaluation of Mutagenic and Gonadotoxic Properties of Trioxane and Dioxolanc. Medycyna Pracy:35 245-255 (1984)

**Other**

One **Generation Reproduction Study (Drinking Water, low dose)**

| | |
|---|---|
| **Type** | One Generation reproduction Study |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o |
| **Method** | |
| • Guideline | None, generally follows OECD 415 |
| • GLP | No |
| • Year | 1976 |
| • Species | Rat |
| • Strain | Charles River strain albino rats (Charles River Wilmington Mass) |
| • Route of administration | Oral, drinking water |
| • Doses | 0.01, 0.03 or 0.1 % in drinking water |
| • Sex | Males and females |
| • Control Group and Treatment | Received water without test substance |
| • Frequency of Treatment | Seven days a week |
| • Duration of Test | 21 Weeks |
| • Premating Exposure Period for Males | 90 Days |
| • Premating Exposure Period for Females | None for F 1 a generation |
| • Statistical Methods | Not described other than that a 95% confidence interval was used to evaluate statistical significance. |
| • Number of Animals/group | 10 |
| • Vehicle | Water |

| | | |
|---|---|---|
| Remarks Field for Test Conditions | Age at Study Initiation | Not specified |
| | Dosing | Dosed water was available seven days a week and 24 hours a day. |
| | Test Material Preparation | Test material was prepared weekly and added to the water bottle. Bottles were changed weekly. |
| | Dosing Duration | Dosing started 90 days prior to mating for males. Females were dosed starting with the mating period and throughout gestation and lactation. |
| | Mating Interval | Mating continued for 15 days or until conception was confirmed. |
| | Examinations | Females were examined daily for evidence of copulation. |
| | Mating Ratio | Two females to one male |
| | Culling | Litters of more than 10 pups were reduced to that number by random sacrifice on the fourth day of the lactation period. |
| | Weaning | Litters were weaned 21 days postpartum |
| | Variations from current OECD 415 Protocol Guideline | Significant variations include:<br>- Number of pregnant females was about 10 rather than about 20.<br>- Females were treated from the beginning of the mating period rather than from two weeks prior to mating.<br>- Necropsies and microscopic evaluations were not conducted on animals. |
| | Comments | The test material is volatile and significant loss occurs from open systems. Experience indicates that it is stable in solution in closed systems. The weekly preparation and changing of water bottles likely resulted in a small loss of test material concentration as the week progressed. It is unlikclv that this affects the results. |

**Results**

- Result
  - No treatment-related effects were noted among the indices used to evaluate reproductive performance.
  - No significantly significant differences were noted between the control and test group with regard to the number of pups delivered stillborn or viable or cannibalized and viable at lactation days 1, 4, 12, or 21.
  - No treatment-related effects were noted among the progeny survival indices.
  - No treatment-related effects in body weight were noted for the Fla progeny at lactation days 0, 1, 4, 12 or 21.
  - No treatment-related body weight differences were noted for the female parental rats.

- NOAEL: 0.1% in drinking water for parental and F 1 a generations

Remarks Field for Results

- Mortality: One gravid high-dose female died during the Fl a gestation period. Necropsy examination revealed no gross abnormalities

- Parental Data Fla Litter

Indices for Parental Parameters Fl a Litter (percent)

| Dose | Female Mating | Female Fecundity | Male Fertility | Female Fertility | Parturition |
|---|---|---|---|---|---|
| 0 | 76.9 | 100 | 100 | 100 | 100 |
| 0.01 | 60.0 | 100 | 100 | 90 | 88.9 |
| 0.03 | 47.1 | 87.5 | x7.5 | 70 | 85.7 |
| 0.10 | X4.6 | 90.0 | 100 | 100 | 90.0 |

- Parental Body Weights: Parental body weight gains were unaffected by treatment with the test article.

- 1'rogeny Data Fla Litter

| Dose | Survival Indices* | | | # Pups Weaned | | 2 1 -day mean Body Weights (g) | |
|---|---|---|---|---|---|---|---|
| | Live Birth Index | 4-Day | 2 1 -Day | Male | Female | Male | Female |
| 0 | 100 | 94.9 | 96.7 | 46 | 42 | 42 | 40 |
| 0.01 | 97.6 | 97.5 | 82.1 | 35 | 39 | 20 | 39 |
| 0.03 | 96.1 | 90.5 | 80.9 | 26 | 44 | 29 | 42 |
| 0.10 | 100 | 81.9 | 94.7 | 41 | 40 | 30 | 40 |

- Fla Exam
  - All delivered pups appeared outwardly normal. An examination of each pup for external abnormalities was conducted at weaning and all pups were judged free of abnormalities.
  - The significantly reduced mean body weights of 21-day male pups in the 0.01 dose group is considered spurious due to lack of dose response correlation.
- Clinical Signs: None noted

**Conclusions**

Remarks Field

Adverse effects recorded were limited to a significant but likely spurious difference between low-dose treated and control animals in decreased male pup body weight (low-dose group on lactation day 21). No significant differences were observed between treated and control animals in the following: maternal mortality, mating and fertility indices, male and female fertility, incidence of parturition, gross abnormalities in pups, number of pups delivered stillborn or viable, or cannibalized (lactation days 1, 4, 12 or 21), survival indices, female pup body weight, and female parental body weight. It is concluded that 0.1% in drinking water represents a LOAEL for parental and Fla generations.

**Data Quality**

- Reliability

Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

**References**

One-Generation Reproduction Study with 1,3-Dioxolane in Drinking Water in Albino Rats Results of the FO Generation. Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries March 26 1976.

**Other**

These results are supported by an inhalation study at 125 ppm in which no significant effects on parental or pup parameters were observed. [1,2]

References for supporting studies

1. Single Generation Reproduction Study with the Vapors of Dioxolane in Albino Rats Results of the FO and Fla Generations, Status Report. IBT No. 663-05562 Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries March 1, 1976.

2. Single Generation Reproduction Study with the Vapors of Dioxolane in Albino Rats Results of the FO and Fl b Generations, Status Report. IBT No. 663-05562 Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries April 13, 1976.

Definitions

- Female Mating Index = 1 00(number copulations '/number estrus cycles required)
- Female Fecundity Index = 1 OO(number pregnancies/number copulations)
- Male Fertility Index = 100(number sires/number males mated)
- Female Fertility Index = 100(number of pregnancies/number of females mated)
- Parturition Index = 1 00(number of parturitions/number pregnancies)
- Survival Index, 4-day = 1 00(number pups viable at lactation day 4/number of viable pups born)
- Survival Index, 21 -day = 1 00(number pups viable at lactation day 2 l/number of viable pups at lactation day 4)

i) Only one copulation counted per estrus cycle.

**One Generation Reproduction Study (Drinking Water, high dose)**

| | |
|---|---|
| **Type** | One Generation reproduction Study with F1 a and F1 b litters |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o |
| **Method** | |
| • Guideline | None, generally follows OECD 41.5 |
| • GLP | No |
| • Year | 1975 |
| • Species | Rat |
| • Strain | Charles River strain albino rats (Charles River Wilmington Mass) |
| • Route of administration | Oral, drinking water |
| • Doses | 0.5 or 1 .O % in drinking water |
| • Sex | Males and females |
| • Control Group and Treatment | Received water without test substance |
| • Frequency of Treatment | Seven days a week |
| • Duration of Test | 25 Weeks |
| • Premating Exposure Period for Males | 90 Days for males used to produce F 1 a pups, males used to produce F1 b pups were untreated. |
| • Premating Exposure Period for Females | None for Fla generation, dams for Flb generation were treated for approx 67 days prior to mating. This started with the first mating period and throughout gestation, lactation and a ten-day rest period after weaning of Fla litter before mating a second time. |
| • Statistical Methods | Not described other than that a 95% confidence interval was used to evaluate statistical significance. |
| • Number of Animals/group | 10 |
| • Vehicle | Water |

| | |
|---|---|
| Age at Study Initiation | Not specified |
| Dosing | Dosed water was available seven days a week and 24 hours a day. |
| Test Material Preparation | Test material was prepared weekly and added to the water bottle. Bottles were changed weekly. |
| Dosing Duration | Dosing started 90 days prior to mating for males. Females were dosed starting with the mating period and throughout gestation, lactation and a 1 O-day rest period after weaning of Fl a litter before mating a second time. |
| Mating Interval | Mating continued for 15 days or until conception was confirmed. |
| Examinations | Females were examined daily for evidence of copulation. |
| Mating Ratio | Two females to one male |
| Culling | Litters of more than 10 pups were reduced to that number by random sacrifice on the fourth day of the lactation period. |
| Weaning | Litters were weaned 21 days postpartum |
| Production of F1 b Generation Pups | Due to the severe effects on offspring and possible males in the F1a generation, treated dams were rested for ten days after F 1 a pups were weaned, and mated with unexposed proven breeders. Females were exposed to test substance during the ten-day interval after weaning F 1a pups. Neither dams nor males were exposed after the F1 b mating interval started. |
| Sacrifice, Gross Pathology and Organ Weights | Following weaning of the Flb litter, all surviving female rats were necropsied. Early deaths were also necropsied. Weights of adrenal glands, gonads, pituitary glands and uterus were recorded at necropsy. Fixed tissues (adrenals, ovaries, pituitary glands, uterus and all apparent lesions) were sectioned and stained with H&E for microscopic examination. |
| Variations from current OECD 415 Protocol Guideline | Significant variations include:<br>○ Number of pregnant females was about 10 rather than about 20.<br>○ Females were treated from the beginning of the mating period rather than from two weeks prior to mating in the Fla mating. |

- Comments

  The test material is volatile and significant loss occurs from open systems. Experience indicates that it is stable in solution in closed systems. The weekly preparation and changing of water bottles likely resulted in a small loss of test material concentration as the week progressed. It is unlikely that this affects the results especially in light of the significant effects produced by treatment.

**Results**

- Result

  - Mating with males pretreated 90 days with dioxolane to produce the Fla litter:
    - Treated groups copulated less frequently than controls,
    - Fewer pregnant treated animals delivered that than controls.
    - One high-dose male failed to impregnate either female he was paired with and was considered sterile
    - There was a decrease in the number of pups delivered by the high-dose group.
    - Both treated groups showed an increase in the number of stillborn pups.
    - There was reduced survival in progeny from treated animals as compared to controls. Especially the high-dose 24-hour survival, which was zero percent. Reduced survival indices were noted for the 0.5% group at each time interval examined.
    - Dam's body weight was significantly less than control's for the high-dose group on postpartum days one and four.
  - Mating with proven breeders to produce the F1b litter
    - There was a decrease in the fecundity index and in the parturition index in the test groups.
    - There was a lower female fertility index for the exposed groups.
    - Gross necropsy and histopathology of treated dams was unremarkable, although was a tendency for chances in the ovaries.
    - Male fertility (unexposed males) was 100%
    - Mating index was unaffected
    - All pups appeared normal and no significant differences were noted with respect to number of pups born, stillborn, cannibalized, born viable or at lactation days 1, 4, 12, and 21. Pup body weight was unaffected.

- NOAEL — Not found, effects at all concentrations tested for FO and Fl generations
- LOAEL
  - FO (P) Generation: 0.50%
  - Fla Generation: 0.50%
  - Flb Generation: Not applicable since no concurrent exposure

Remarks Field for Results

- Mortality — One high-dose male died during the last week of the 90-day treatment period, necropsy revealed the cause of death due to respiratory problems not related to test article exposure.

- Parental Data Fla Litter

| Dose (%) | Indices for Parental Parameters Fla Litter (percent) | | | | |
|---|---|---|---|---|---|
| | Female Mating | Female Fecundity | Male Fertility | Female Fertility | Parturition |
| 0 | 81.8 | 100 | 100 | 90.0 | 88.9 |
| 0.5 | 40.9 | 88.9 | 100 | 80.0 | 50.0 |
| 1.0 | 35.3 | 100 | 75 | 75.0 | 50.0 |

Number pregnant: control=9; 0.5% = 8, 1 .O% = 6 (one high-dose male was found to be sterile)

- Parental Body Weights Dams Fla

| Dose | Mean weights of dams on day indicated | | | | | | |
|---|---|---|---|---|---|---|---|
| | G-15 | Part | PP-1 | PP-4 | PP-12 | PP-21 | PP-28 |
| 0 | 375 | 369 | 364 | 366 | 345 | 352 | 359 |
| 0.5 | 398 | 362 | 344 | 367 | 377 | 371 | 365 |
| 1.0 | 359 | 331 | 324" | 316" | 338 | 338 | 334 |

G- 15 = gestation day 1.5, Part= day of parturition, PP=postpartum day
a = significant at $p < 0.05$

- Progeny Data Fla Litter

| Dose | Number of pups that were | | | |
|---|---|---|---|---|
| | Delivered | Stillborn | Cannibalized | Viable |
| 0 | 55 | 2 | 0 | 53 |
| 0.5 | 45 | 6 | 0 | 39 |
| I .0 | 14 | 7 | 1 | 6 |

- Fla data

| Dose (%) | Survival Indices* | | | # Pups Weaned | | 2 l-day mean Body Weights (g) | |
|---|---|---|---|---|---|---|---|
| | Live Birth Index | 4-Day | 21-Day | Male | Female | Male | Female |
| 0 | 96.4 | 92.5 | 70.2 | 15 | 18 | 47 | 47 |
| 0.5 | X6.7 | 61.5 | 41.7 | 8 | 2 | 42 | 44 |
| 1.0 | 42.9 | 0.0 | 0.0 | 0 | 0 | - | - |

- Fla Exam: All pups delivered appeared outwardly normal. An examination of each pup for external abnormalities was conducted at weaning and all pups were judged free of abnormalities.

- Clinical Signs: None noted

- Mortality During the F 1 b Mating and Gestation: One high-dose female died post-mating. Necropsy showed non-gravid female with pyometra.

- Flb Litter Data, Parental Data

Indices for Parental Parameters Flb Litter (percent)

| Dose (%) | Female Mating | Female Fecundity | Male Fertility | Female Fertility | Parturition |
|---|---|---|---|---|---|
| 0 | 64.3 | 88.9 | 100 | 80 | 100 |
| 0.5 | 64.3 | 66.7 | 80 | 60 | 83.3 |
| 1.0 | 66.7 | 50.0 | 75 | 50 | 75 |

- D a m Body Weights: Body weights of dams were unaffected by prior treatment with dioxolane during the mating, parturition and lactation phase of the Flb litter production

- F l b Litter Data, Pup Data

Number of pups that were: [total (mean per dam)]

| Dose | Delivered | Stillborn | Cannibalized | Viable |
|---|---|---|---|---|
| 0 | 78 (9.8) | 2(0.3) | 0(0.0) | 76(9.5) |
| 0 5 | 43(8.9) | 2(0.3) | 0(0.0) | 41(8.2) |
| 1.0 | 24(8.0) | 0(0.0) | 0(0.0) | 24(8.0) |

- Flb Lilter Data, Pup Data

| Dose (%) | Live Birth Index | Survival Indices* 4-Day | Survival Indices* 21-Day | # Pups Weaned Male | # Pups Weaned Female | 21-day mean Body Weights (g) Male | 21-day mean Body Weights (g) Female |
|---|---|---|---|---|---|---|---|
| 0 | 97.4 | 92.1 | 86.9 | 30 | 23 | 45 | 44 |
| 0.5 | 95.3 | 92.7 | 69.4 | 10 | 15 | 57 | 51 |
| 1 .0 | 100 | 91.7 | 72.7 | 9 | 9 | 59 | 44 |

- Flb Pup Exam: All pups delivered appeared outwardly normal. An examination of each pup for external abnormalities was conducted at weaning and all pups were judged free of abnormalities.

- Clinical Signs: None noted
- Gross Pathology: No significant differences were noted between control and test group parental females upon gross pathologic examination.
- Dam Organ Weights:

| | Organ weights, absolute in grams, relative in g/100g bw | | | | | |
|---|---|---|---|---|---|---|
| | Adrenals | | Ovaries | | Uterus | |
| Dose | abs | rel | abs | rel | abs | rel |
| 0 | 0.082 | 0.0208 | 0.072 | 0.0179 | 0.713 | 0.1803 |
| 0.5 | 0.076 | 0.0184 | 0.075 | 0.0184 | 0.797 | 0.1909 |
| 1.0 | 0.071 | 0.0172 | 0.065 | 0.0156 | 0.686 | 0.1658 |

  Adrenals, ovaries and uterus weights showed a tendency to reduced weight with increasing dose. None were statistically different from control but the (n) was relatively small. Other organs showed no tendency to decreased weight with increasing dose ofdioxolane.

- Histopathology of Dams
  - All weighed organs were examined microscopically. No histopathological alterations attributable to the effects of the test material were observed. In treated rats, the hilus of the ovary was unusually prominent and the amount of ovarian parenchyma appeared deficient.
  - Although not considered significant by the examining pathologist, the incidence of ovarian focal "leutinization of stroma" was 0/10, 1/10 and 3/S for control, low dose and high dose animals respectively.

**Conclusions**

Remarks Field

Drinking water exposure of rats at 0.5 or 1.0% produced clear adverse effects on reproduction. In the fla mating, it could not be determined if effects were attributable primarily to exposure of males, females or both. Survival of high-dose pups was especially affected. In the second exposure using untreated proven-male breeders, the effects were less severe suggesting that there was a male contribution to the reproductive **toxicity.** This conclusion is clouded, however, since the females were not exposed to test article during mating, gestation or lactation during the production of this second litter. It is clear that the females were still affected and therefore the female was an affected sex regarding reproductive toxicity of dioxolane.

**Data Quality**

- Reliability: Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

**References**

One-Generation Reproduction Study with Dioxolane in Albino Rats, Results of the FO Generation. lndustrial Bio-Test Laboratories, Inc. Submitted to PPG Industries July 2 1, 1975.

One-Generation Reproduction Study with Dioxolane in Drinking Water of Albino Rats Results of the FO Generation Females and Proven Breeder Males. Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries February 16, 1976.

**Other**

The perinatal mortality is supported by a the study of prenatal and postnatal development published by Sitarek et al. in which an increased perinatal death rate was reported after dosing dioxolane at 1150 mg/kg/day, every other day, from day 2-20 of gestation.

References for supporting studies

Sitarek K, Baranski B, Berlinska B. The effect of maternal exposure to dioxolane on prenatal and postnatal development in rats. Pol J Occup Med Environ Health 5: 159-66 (1992).

Definitions

- Female Mating Index = 100(number copulations '/number estrus cycles required)
- Female Fecundity Index = 1 00(number pregnancies/number copulations)
- Male Fertility Index = 1 00( number sires/number males mated)
- Female Fertility Index = 100(number of pregnancies/number of females mated)
- Parturition Index = 100(number of parturitions/number pregnancies)
- Survival Index, 4-day = 100(number pups viable at lactation day 4/number of viable pups born)
- Survival Index, 21-day = 100(number pups viable at lactation day 21/number of viable pups at lactation day 4)

i) Only one copulation counted per estrus cycle.

**One Generation Reproduction Study, Inhalation**

| | |
|---|---|
| **Type** | One Generation reproduction Study with Fla and Flb litters |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-o |
| **Method** | |
| Guideline | None, generally follows OECD 415 |
| GLP | No |
| Year | 1975 |
| Species | Rat |
| Strain | Charles River strain albino rats (Charles River Wilmington Mass) |
| Route of administration | Inhalation, whole body exposure |
| Doses | 125 ppm |
| Sex | Males and females |
| Control Group and Treatment | Untreated controls, under same chamber housing conditions, same number of animals per group |
| Frequency of Treatment | Five days a week (seven hour a day exposure) |
| Duration of Test | 24 Weeks |
| Premating Exposure Period for Males | 90 Days for males used to produce F 1 a pups, 120 days for males (different males) used to produce Flb pups. |
| Premating Exposure Period for Females | None for Fla generation, dams for Flb generation were treated for approx 35 days during the F1 a mating and gestation, then remained unexposed for approximately 35 days prior to mating and the second exposure period. |
| Statistical Methods | Not described other than that a 95% confidence interval was used to evaluate statistical significance. |
| Number of Animals/group | 10 |
| Vehicle | Air |

- Age at Study Initiation: Not specified
- Dosing: Inhalation, only water was supplied during exposure. Exposures were conducted in the same chamber being used for an ongoing chronic study of Dioxolane at the laboratory.
- Test Material Preparation and Measurement: The inhalation chambers were supplied with vapors of dioxolane generated by bubbling nitrogen gas through test material in a gas-washing bottle. These vapors were diluted with filtered-conditioned air to achieve the desired concentration. Vapor concentration was measured by gas chromatography hourly during the first two weeks of the study then twice daily for the remainder of the study.
- Dosing Duration
  - P males for F1 a generation were treated starting 90 days prior to mating. These males were sacrificed after mating.
  - P males for Flb generation were selected from the population in an ongoing chronic study and had been treated for 120 days prior to mating.
  - Dams for the F la litter production were exposed starting with the 15-day mating period and through gestation. Exposure was stopped a day or two before parturition.
  - Dams for the F Lb litter production were exposed about 35 days previously during F1 a litter production. They were then not exposed during delivery and lactation. After weaning, exposure was conducted again during the mating and gestation period of the F1 b litter production. Exposure was stopped a day or two before delivery
- Mating Interval: Mating continued for 15 days.
- Mating Ratio: Two females to one male
- Culling: Litters of more than ten pups were reduced to that number by random sacrifice on the fourth day of the lactation period.
- Weaning: Litters were weaned 21 days postpartum
- Production of Fl b Generation Pups: After weaning the Fllitters of pups, the dams were mated with different males selected from an ongoing chronic study.

- Sacrifice, Gross Pathology and Organ Weights: Males used to produce the F1 a generation were sacrificed after mating and necropsied. Organ weights were recorded for brain, gonads, heart, kidneys, liver, lungs and spleen. A list of 31 tissues were removed and fixed for sectioning and staining including testes, seminal vesicles, prostate and pituitary.

- Variations from current OECD 4 15 Protocol Guideline: Significant variations include :
  - Number of pregnant females was about 10 rather than about 20.
  - Females were treated from the beginning of the mating period rather than from two weeks prior to mating in the Fla mating.
  - Only one dose group utilized.

**Results**

- Result: Treatment did not significantly affect any measured reproductive parameter. There was a tendency toward an overall reduction in the number of pups born and weaned in both the F1 a and F1 b litters of the dosed group but this was not statistically significant.

- NOAEL: Authors conclusions:
  - FO (P) Generation 125 ppm
  - Fla Generation 125 ppm
  - Flb Generation 125 ppm
- LOAEL: Not stated in the report, apparently > 12.5 ppm

Remarks Field for Results

- Mortality During the F1 a Mating and Gestation: No mortality observed

- Parental Data Fla Litter

| | Indices for Parental Parameters, Fla Litter (percent) | | | | |
|---|---|---|---|---|---|
| Dose | Female Mating | Female Fecundity | Male Fertility | Female Fertility | Parturition |
| 0 | 57.9 | 54.5 | 100 | 60.0 | 100.0 |
| 125 | 64.7 | 54.5 | 80 | 60.0 | 100.0 |

Number pregnant: control=6/10 125 ppm = 6/10

- Parental Body Weights Dams Fla

| Dose (ppm) | Mean weights of dams on day indicated | | | | | | |
|---|---|---|---|---|---|---|---|
| | G-15 | Part | PP-1 | PP-4 | PP-12 | PP-21 | PP-28 |
| 0 | 409 | 366 | 361 | 359 | 379 | 373 | 348 |
| 125 | 377 | 348 | 343 | 346 | 354 | 359 | 339 |

G-l 5 = gestation day 15, Part= day of parturition, PP=postpartum day

- Progeny Data Fla Litter

| Dose (ppm) | Number of pups that were | | | |
|---|---|---|---|---|
| | Delivered | Still-born | Cannibalized | Viable |
| 0 | 65 (10.8)" | 2 | 0 | 63 (10.5) |
| 125ppm | 43 (7.2) | 1 | 0 | 42 (7.0) |

a = mean per litter in parentheses

- Fla data

| Fla | | Survival Indices* | | # Pups Weaned | | 2 1 -day mean Body Weights (g) | |
|---|---|---|---|---|---|---|---|
| Dose | Live Birth Index | 4-Day | 2 l-Day | Male | Female | Male | Female |
| 0 | 96.9 | 93.7 | 98.1 | ‘3 | 28 | 49 | 46 |
| 125 | 97.7 | 97.6 | 97.4 | 23 | 14 | 49 | 50 |

- Fla Exam

All pups delivered appeared outwardly normal. An examination of each pup for external abnormalities was conducted at weaning and all pups were judged free of abnormalities.

- Clinical Signs

None noted

- Organ weights P-males

Males used to produce the Fla litter showed no difference in absolute or relative organ weights for brain, gonads, heart, kidneys, liver, or spleen. Absolute lung weighs were reduced by 8 percent in treated males and the relative lung weighs were reduced by 11 percent (statistically significant $p<.05$).

- Histo-pathology

Microscopic examination of tissues from the males used to produce the F1 a litter was unremarkable. The organ weight changes in lungs did not have a histopathological correlate. No adverse effects on reproductive tissues were noted.

- Mortality During the Flb Mating and Gestation

No mortality observed.

- F l b Litter Data, Parental Data

| Indices for Parental Parameters F 1 b Litter (percent) | | | | | |
|---|---|---|---|---|---|
| Dose (ppm) | Female Mating | Female Fecundity | Male Fertility | Female Fertility | Parturition |
| 0 | 92.9 | 69.2 | 100 | 90 | 100 |
| 125 | 50.0 | 60.0 | 80 | 60 | 100 |

Number pregnant: control = 9/10, 125 ppm = 6110

- D a m Body Weights

Body weights of dams were unaffected by prior treatment with dioxolane during the mating, parturition and lactation phase of the Flb litter production

- Flb Litter Data, Pup Data

| | Number of pups that were: [total (mean per dam)] | | | |
|---|---|---|---|---|
| Dose (ppm) | Delivered | Still-born | Cannibalized | Viable |
| 0 | 91(10.1) | 8(0.9) | 0(0.0) | 83(9.2) |
| 125 | 64(10.7) | 2(0.3) | 0(0.0) | 62( 10.3) |

- F l b Litter Data, Pup Data

| | | Survival Indices" | | # Pups Weaned | | 2 1-day mean Body Weights (g) | |
|---|---|---|---|---|---|---|---|
| Dose (ppm) | Live Birth Index | 4-Day | 2 l-Day | Male | Female | Male | Female |
| 0 | 91.2 | 91.6 | 67.7 | 29 | 17 | 50 | 43 |
| 125 | 96.9 | 91.9 | 81.1 | 20 | 23 | 46 | 4.5 |

- Flb Pup Exam

All pups delivered appeared outwardly normal with the exception of a control female pup that had microphthalmia of the right eye. An examination of each pup for external abnormalities was conducted at weaning and all pups were judged free of abnormalities.

- Clinical Signs

None noted

- Other: The report contains no indication that the pups or dams were necropsied at sacrifice, it is assumed they were sacrificed without necropsy.

- Comment: Although treatment did not significantly affect any measured reproductive parameter, there was a tendency toward an overall reduction in the number of pups born and weaned in both the Fla and Flb litters of the dosed group. The lack of statistical significance may partly be a function of the low numbers of animals employed in the study. The possibility that the 125 ppm exposure represents a LOAEL for reproduction in the F 1 a and F 1 b Litters cannot be excluded.

**Conclusions**

Remarks Field: Treatment of male and female albino rats with Dioxolane by inhalation at 125 ppm did not significantly affect any measured reproductive parameter. The tendency toward an overall reduction in the number of pups born and weaned in both the F1 a and F 1 b litters of the dosed group suggests that the statistical significance could have been reached with a larger(n). Although 125 ppm was considered to be NOAEL by the authors of the study, the possibility cannot be excluded that 125 ppm represents a LOAEL for reproduction under these conditions.

**Data Quality**

- Reliability: Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

**References**

This study report was split into two documents by the testing laboratory. As it was a unified experiment with the same dams, both were combined for preparing this robust summary. The two reports are listed below.

- Single Generation Reproduction Study with the Vapors of Dioxolane in Albino Rats Results of the FO and F la Generations, Status Report. IBT No. 663-05562 Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries March 1, 1976.

- Single Generation Reproduction Study with the Vapors of Dioxolane in Albino Rats Results of the FO and F1 b Generations, Status Report. IBT No. 663-05562 Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries April 13, 1976.

**Other**

These results are supported by two oral studies from the same laboratory in four reports (reports were split by litter).

References for supporting studies


1. Industrial BIO-TEST Laboratories, Inc.; One-Generation Reproduction Study With Dioxolane in Drinking Water in Albino Rats, Results of the FO Generation. (1975), EPA Document No. 878213523, Fiche No. OTS0205848.
2. Industrial BIO-TEST Laboratories, Inc.; One-Generation Reproduction Study With Dioxolanc in Drinking Water in Albino Rats, Results of the FO Generation and Proven Breeder Males. (1975), EPA Document No. 878213524, Fiche No. OTS0205848
3. Industrial BIO-TEST Laboratories, Inc.; One-Generation Reproduction Study With Dioxolane in Drinking Water in Albino Rats, Results of the FO Generation. (1975), EPA Document No. 8782 13525, Fiche No. OTS0205848
4. Industrial BIO-TEST Laboratories, Inc.; Single Generation Reproduction Study With the Vapors of Dioxolane in Albino Rats, Results of the FO and F1 a Generations, Status Report. (1976), EPA Document No. 878213527, Fiche No. OTS0205848


Definitions

- Female Mating Index = 100(number copulations '/number estt-us cycles required)
- Female Fecundity Index = 1 00(number pregnancies/number copulations)
- Male Fertility Index = 1 00(number sires/number males mated)
- Female Fertility Index = 100(number of pregnancies/number of females mated)
- Parturition Index = 100(number of parturitions/number pregnancies)
- Survival Index, 4-day = 100(number pups viable at lactation day 4/number of viable pups born)
- Survival Index, 21-day = 100(number pups viable at lactation day 21/number of viable pups at lactation day 4)

i) Only one copulation counted per estrus cycle.

**Developmental Toxicology**

| | |
|---|---|
| **Type** | Developmental Toxicology |
| **Test Substance** | 1,3-Dioxolane<br>CAS Number: 646-06-O<br>Hoechst Celanese Lot UN 1166<br>Purity verified by analysis |
| **Method** | |
| • Guideline | U.S. Environmental Protection Agency Toxic Substances Control Act Test Guidelines - 798.4900 |
| • GLP | Yes |
| • Year | 1991 |
| • Species | Rat |
| • Strain | Charles River Crl:CD®BR VAF/Plus |
| • Route of administration | Oral gavage in corn oil |
| • Doses | 0, 125, 250, 500 and 1000 mg/kg |
| • Sex | Female, pregnant |
| • Exposure Period | Days 6 to 15 of pregnancy |
| • Frequency of treatment | Daily |
| • Control Group | Corn Oil |
| • Duration of test | 15 days |

- Statistical Methods

Maternal and fetal incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution (1).
Maternal body weights, body weight changes, gravid uterine weights and feed consumption values, and litter averages for fetal body weights, percent male fetuses, percent resorbed conceptuses per litter, fetal ossification sites and percent fetal alterations were analyzed using Bartlett's Test of Hotnogeneity of Variances (2) and the Analysis of Variance (3), when appropriate (i.e., Bartlett's Test was not significant (P>0.05)). If the Analysis of Variance was significant (P<0.05), Dunnett's Test (4) was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate [i.e., Bartlett's Test was significant (P<0.05)], the Kruskal-Wallis Test (5) was used, when less than or equal to 75% ties were present; when more than 75% ties were present, Fisher's Exact Test (6) was used. In cases in which the Kruskal-Wallis Test was statistically significant (P<0.05), Dunn's Method of Multiple Comparisons (7) was used to identify the statistical significance of the individual groups. All other Caesarean-sectioning data were evaluated using the procedures previously described for the Kruskal-Wallis Test (5).


1. Snedecor, G.W. and Cochran, W.G.. Variance test for homogeneity of the binomial distribution. Statistical Methods, 6th Edition, Iowa State University Press, Ames, pp. 240-241.( 1967).
2. Sokal, R.R. and Rohlf, F.J. Bartlett's test of homogeneity of variances. Biometry, W.H. Freeman and Co., San Francisco, pp. 370-371(1969).
3. Snedecor, G.W. and Cochran, W.G. Analysis of Variance. Statistical Methods, 6th Edition, Iowa State University Press, Ames, pp. 258-275(1967).
4. Dunnett, C.W. A multiple comparison procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50: 1096-I 129(1955).
5. Sokal, R.R. and Rohlf, F.J. Kruskal-Wallis Test. Biometry, W.H. Freeman and Co., San Francisco, pp. 388-389 (1969).
6. Siegel, S. Nonparametric Statistics for the Behavioral Sciences, McGraw-Hill, New York, pp. 96-104(1956).
7. Dunn, O.J. Multiple comparisons using rank sums. Technometrics 6(3):241-252( 1964).


- Age at Study Initiation
  - Females were approximately 93 days old at first dosing
  - Males were approximately 24 weeks old when bred to the virgin females
- Number of animals per group
  - 25 mated presumed-pregnant females per dose group
- Test Substance Preparation and Analysis
  - Test substance was prepared weekly at four concentrations (prepared twice overall) was analyzed for content of test material and each batch was analyzed seven days later to establish stability.

- Analysis and Stability

| Target Conc. (mg/gm) | Day-1 Conc (mg/gm) | | 7-Day Conc (mg/gm) | |
|---|---|---|---|---|
| | Prep 1 | Prep 2 | Prep 1 | Prep 2 |
| 2.5 | 26 | 28 | 19 | 27 |
| 50 | 52 | 56 | 48 | 56 |
| 100 | 100 | 114 | 94 | 112 |
| 200 | 198 | 223 | 190 | 219 |

- Vehicle
  - Corn oil
- Clinical Observation Performed and Frequency
  - Viability observed at lest twice daily.
  - Additional observations for clinical signs of test substance effects, abortions, premature deliveries and deaths were also made immediately prior to intubation (days 6 through 15 of presumed gestation) and approximately one hour post-dosage. These additional observations were made once daily during the post-dosage period (days 16 through 20 of presumed gestation).
  - The body weights of the rats were recorded at least once weekly prior to mating. Body weights and feed consumption values were recorded on day 0 and on days 6 through 20 of presumed gestations.
- Mating Procedures

Following the acclimation period (approximately two weeks), 160 healthy virgin female rats were placed in cohabitation with 160 breeder male rats (one male rat per female rat). The remaining 20 virgin female rats were placed in cohabitation with breeder male rats that had mated during the first night of the cohabitation period. Female rats with spermatozoa observed in a vaginal lavage or a copulatory plug observed *in situ* were considered to be at day 0 of presumed gestation and assigned to individual housing. Female rats mated by the same male rat were assigned to different dosage groups.

| | | |
|---|---|---|
| | ◊ Maternal Parameters Assessed During Study | Body weight, feed consumption and clinical signs during life. A gross necropsy was conducted on each rat. The intact gravid uterus was excised and weighed. The number and placement of implantation sites, early and late resorptions, live and dead fetuses, and the number of corpora lutea in each ovary were recorded. Uteri from rats that appeared nonpregnant were stained with ammonium sulfide to confirm pregnancy status |
| | ◊ Fetal Parameters Assessed During Study | Litter size, placental weight, gross malformations, fetal body weight, sex ratio, body cross sections, skeletal examination. Live fetuses were sacrificed by immersion in the appropriate fixative. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations by using a variation of Wilson's sectioning technique. The remaining fetuses in each litter were cleared, stained with alizarin red S(7) and examined for skeletal alterations. |
| | ◊ Organs Examined at Necropsy | List not provided, stated in protocol that visceral organs that are abnormal will be noted and retained |
| | ◊ Rationale for Dose Selection | The doses were selected based on a 14-day gavage study in non-pregnant rats of the same strain. |

**Results**

| | | |
|---|---|---|
| ● NOAEL & LOEL for Maternal Toxicity | ◊ NOAEL = 250 mg/kg/day<br>◊ LOEL = 500 mg/kg/day Body weight gain | |
| • NOAEL& LOEL for Developmental Toxicity | ◊ NOAEL = 500 mg/kg/day<br>◊ LOEL = 1000 mg/kg/day Reduced fetal body weights and gross external, soft tissue and skeletal malformations or variations occurred in the 1000 mg/kg/day dosage group fetuses. | |
| ● Actual Doses Received | Not calculated, but very close to the target doses of 0, 125,250, 1500 and 1000 mg/kg/day | |

- Maternal data

No rats died during the conduct of this study. No clinical or necropsy observations were caused by dosages of the test substance as high as 1000 mg/kg/day. The 500 and 1000 mg/kg/day dosages of Dioxolane caused weight loss in dams on days 6 to 7 of gestation. This initial weight loss was significant ($P<0.05$) for the 1000 mg/kg/day dosage group, and average maternal body weight gain was reduced for this group for the entire dosage period (calculated as days 6 to 16 of gestation), as compared with the control group value. Dosages of 250 and 500 mg/kg/day slightly reduced maternal feed consumption and the 1000 mg/kg/day dosage of the test substance produced a more marked reduction in absolute (g/day) and slightly reduced relative (g/kg/day) maternal feed consumption values during the dosage period as compared with the control group values. Significantly decreased ($P<0.05$) absolute feed consumption values occurred for the 500 and 1000 mg/kg/day dosage groups on days 6 to 7 of gestation. Administration of the 1000 mg/kg/day dosage of Dioxolane significantly decreased ($P<0.05$) absolute maternal feed consumption values for the entire dosage period.

- Fetal data
  - Cesarean Data

Litter size was comparable in all groups. No Caesarean-sectioning parameters were affected by administration of 1,3-Dioxolane to the dams at dosages as high as 1000 mg/kg/day. The averages for corpora lutea, implantation sites, litter sizes, live fetuses and resorptions, and the numbers of dams with any resorptions were comparable for the five dosage groups. No litters consisted of only resorbed conceptuses

  - External and visceral effects

The 1000 mg/kg/day dosage of the test substance resulted in significant increases in the litter and fetal incidences of externally evident tail malformations and vertebral malformations interrelated with the tail malformations and septal defects in the heart. The gross external and soft tissue malformations that were considered effects of the 1000 mg/kg/day dosage of the test substance were eight fetuses (from seven litters) in the 1000 mg/kg/day dosage group litters that had malformed tails. These malformations differed in severity and included constrictions of the tail, thread-like tail, short tail and absent tail; thread-like tail occurred for four fetuses (from four different litters) in this dosage group. Soft tissue examination revealed three fetuses (from three litters) at 1000 mg/kg/day dosage group litters that had ventricular septal defects as their only alteration. One high-dose fetus was found with a cleft palate.

- Major skeletal defects

Skeletal examination of fetuses from the 1000 mg/kg/day group revealed increases in vertebral malformations associated with the tail malformations (five fetuses †† from four litters †† had absent caudal vertebrae) and other alterations in vertebral and rib ossification that were probably interrelated with the more severe effects evident in the fetuses with visible tail malformations. These alterations included "scrambling" or reduced ossification of centra in the thoracic vertebrae (asymmetric, bifid, unilateral or absent ossification, fused ccntrum and arch), the lumbar vertebrae (fused centrum and arch, centra that were bifid or not ossified, and arches that were small or not ossified), absent sacral and caudal vertebrae and rib-vertebral malformations. These vertebral malformations and delays in ossification occurred in 4, 0, 0, 11 and 36" fetuses from 3, 0, 0, 7 and 16 †† litters in the 0, 125, 250, 500, and 1000 mg/kg/day dosage groups, respectively. Of these alterations, significant increases (P<0.01) in the litter and fetal incidences occurred for thoracic vertebrae with bifid, unilaterally ossified or not ossified centra, lumbar vertebra with a bifid centrum, and absent caudal vertebrae. In the 1000 mg/kg/day group, the litter average for the average number of ossified metacarpal bones per fetus was significantly decreased (P<0.01) for this dosage group, a delay in ossification that is expected because of the significantly decreased (P<0.01) fetal body weight that also occurred for this group.

- Fetal and Litter Effects

| Effect | Dose Group | | | | | |
|---|---|---|---|---|---|---|
| | 0 | | | 500 | 1000 | |
| | 9 | 10 | 5 | | | |
| Litters evaluated | 17 | 10 | 6 | 23 | 24 | |
| Fetuses evaluated | | | | 382 | 362 | 376 |
| Litters with altered fetuses | 4.43 | 2.44 | 1.52 | 8 | 2 | 1 †† |
| Fetuses with any alteration | 0 | 0 | 0 | 16 | 52 †† | |
| Percent fetuses with any alteration per litter | 0 4.43 | 0 2.44 | 0 1.52 | 1 3 | 4.16 7 | 8 †† |
| Tail malformations. fetuses | 0 | 0 | 0 | 0 | 8 †† | 8 |
| Tail malformations. litters | 0 | 0 | 0 | 7 | | 0 |
| Heart, ventricular septal defect | 0 | 0 | 0 | 0 | 3 †† | |
| Vertebral scrambling. fetuses | 4 | 0 | 0 | 11 | 36 †† | |
| Vertebral scrambling, litters | 3 | 0 | 0 | 7 | 16 †† | |
| Vertebral/rib malform., fetuses | 0 | 0 | 0 | 0 | 2 | |
| Vertebral/rib malform litters | 0 | 0 | 0 | 0 | 2 | |
| Sternebrae alterations, fetuses | 7 | 5 | 2 | 0 | 5 | |
| Sternebrae alterations, litters | 4 | 5 | 2 | 0 | 4 | |

- Ossification

Statistically significant reductions in the average number of ossified melacarpals per fetus occurred for the 500 and 1000 mg/kg/day dosage group litters, as compared with the control group values. This observation was considered biologically important and an effect of the test substance for only the 1000 mg/kg/day dosage group because: 1) no other statistically significant dosage-dependent delays in ossification occurred for the 500 mg/kg/day dosage group; 2) the values for the 500 and 1000 mg/kg/day dosage groups did not demonstrate expected differences reflecting the magnitude of the difference in the dosages provided (the 500 and 1000 mg/kg/day dosage group litters had averages of 3.12 ± 0.18 † and 3.10 ± 0.21 †† ossified metacarpal sites per fetus, values that are not biologically different); and 3) the statistical significance of the value for the 500 mg/kg/day dosage group reflected the relatively small standard deviation that occurred for this group, as compared with the higher values for the standard deviations of the other dosage groups.

Remarks Field for Results

Food Consumption

Food Consumption per dam (gm/day)

| | Dose Level (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| Time | 0 | 125 | 250 | 500 | 1000 |
| Days 6-7 | 22.2 | 21.7 | 19.2 | 18.6† | 18.2† |
| Days 6-16 | 22.4 | 23.6 | 21.3 | 21.4 | 20.7† |
| Days 6-20 | 23.8 | 25.0 | 23.1 | 23.4 | 23.0 |

† = p<0.05

| | Dose Group (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| **Parameter** | 0 | 125 | 250 | 500 | 1000 |
| Pregnancies | 25/25 | 25/25 | 25/25 | 23/25 | 24/25 |
| Corpora lueta (per liter) | 19.6 | 21.2 | 19.8 | 20.5 | 21.5 |
| Implantations (per liter) | 17.0 | 17.2 | 16.7 | 16.8 | 17.1 |
| Liter size | 16.2 | 16.2 | 15.3 | 15.7 | 15.7 |
| Live fetuses | 405 | 388 | 382 | 362 | 376 |
| Dead fetuses | 0 | 0 | 0 | 0 | 0 |
| Early resorptions (total) | 19 | 23 | 36 | 24 | 31 |
| Late resorptions (total) | 0 | 1 | 0 | 0 | 3 |
| Resorptions per liter | 4.4 | 5.8 | 8.6 | 6.5 | 8.3 |
| Males % | 47.8 | 52.0 | 50.0 | 52.1 | 49.5 |
| Body weight (mean grams) | 3.38 | 3.54 | 3.46 | 3.48 | 3.16 †† |

**Conclusions**

Remarks Field

Based on the results of this studv. Dioxolane is not considered a specific developmental toxin. The developmental NOEL was found to be 500 mg/kg/day while the maternal NOEL was found lo be 250 mg/kg/day. Neither deaths nor adverse clinical signs were observed in any of the animals. No adverse effects are anticipated for the conceptus in the absence of maternal toxicity.

The maternally-toxic 1000 mg/kg/day dosage of the test substance significantly reduced (P<0.05 to P<0.0 1) fetal body weights for male and female fetuses and for the combined sexes. This dosage of the test substance also significantly increased (P<0.05 to P<0.01) the litter and fetal incidences of externally evident tail malformations, vertebral malformations interrelated with the tail malformations and septal defects in the heart, and significantly reduced (P<0.01) the litter average for the average number of ossified metacarpal bones per fetus, an expected delay in ossification related to the significantly decreased (P<0.01) fetal body weights.

**Data Quality**

- Reliability

Klimisch Code 1. Reliable without restriction, study meets GLP standards and/or most requirements

**References**

1,3-Dioxolane: Oral Developmental Toxicity Study In Crl:CDOBR VAF/Plus's Presumed Pregnant Rats, Argus Research Laboratories, Inc. 905 Sheehy Drive Horsham, Pennsylvania 19044 Argus report # 508-002, May 9, 1991, submitted to Hoechst Celanese Corporation.

**Other**

A subsequent study was published which supports this result. In this study Dioxolane was administered by gavage every other day from days 8-20 of gestation as an aqueous solution daily doses of 0.14, 0.58 or 1 .15 g/kg/day. Dioxolane administration was not associated with increased embryo or fetus intrauterine death rates or congenital defects at any dose level. The mid (0.58 g/kg) and high dose (1.15 g/kg) were reported to be associated with dose-related delays in fetal development and the high dose showed clear maternal toxicity. The developmental and maternal NOEL were both judged to be 0.58 g/kg/day under these conditions of every other day dosing.

References for supporting studies

Sitarek K, Baranski B, Berlinska B. The effect of maternal exposure to dioxolane on prenatal and postnatal development in rats. Pol J Occup Med Environ Health 5: 159-66 (1992).

† = p≤0.05, †† = p≤0.01