201-16861A

1803 BUILDING
August 18, 2011

The Dow Chemical Company
Midland, MI [illegible] USA

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OPPT CBIC
2011 OCT 28 AM 10: 01

Mark W. Townsend
Chief HPV Chemicals Branch
Office of Pollution Prevention and Toxics (OPPT)
USEPA Headquarters
Ariel Rios Building
1200 Pennsylvania Avenue, N. W.
Mail Code: 7403M
Washington, DC 20460

Dear Mr. Townsend:

The Dow Chemical Company previously submitted an updated IUCLID file on 2-Amino-2-methyl-1-propanol (AMP, CAS number: 124-68-5) last December 2010. Our intention in the accompanying correspondence may have not been stated clearly therefore I need to clarify that The Dow Chemical Company requests that AMP be removed from the ICCA program and brought back to the EPA HPV Challenge program.

The IUCLID is a fairly comprehensive package of the toxicological data. It is our understanding that under the Challenge Program this dossier will be posted on the EPA website, updating the information that is currently presented. Furthermore, EPA will be conducting a hazard characterization and that AMP may be subject to test rule.

If you have any questions, please call me at 989-636-9870.

Kind Regards,
Brian J. Hughes

Brian J. Hughes, Ph.D./ D.A.B.T.
Toxicology & Environmental Research and Consulting
The Dow Chemical Company
Bhughes2@dow.com

cc: Oscar Hernandez

201-16861B

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2011 OCT 28 AM 11:30

IUCLID

Data Set

Existing Chemical	:	ID: 124-68-5
CAS No.	:	124-68-5
EINECS Name	:	2-amino-2-methylpropanol
EC No.	:	204-709-8
TSCA Name	:	1-Propanol, 2-amino-2-methyl-
Molecular Formula	:	C4H11NO
Producer related part		
Company	:	Dow Chemical, TERC
Creation date	:	01.03.2004
Substance related part		
Company	:	Dow Chemical, TERC
Creation date	:	01.03.2004
Status	:	
Memo	:	AMP
Printing date	:	30.11.2006
Revision date	:	
Date of last update	:	30.11.2010
Number of pages	:	111
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	manufacturer
Name	:	The Dow Chemical Company
Contact person	:	Dr. Brian J. Hughes
Date	:	30/11/2010
Street	:	1803 Building
Town	:	48674 Midland, MI
Country	:	United States
Phone	:	989 636 1000
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	http://www.dow.com/Homepage/index.html

27.04.2005

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

Type	:	manufacturer
Name of plant	:	ANGUS Chemical Company
Street	:	
Town	:	Sterlington, Louisiana
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	http://www.dow.com/angus
Source	:	Frauson, L. (2003). Internal E-mail of the Dow Chemical Company to C. Houtman.
Reliability	:	(2) valid with restrictions 2g

27.04.2005

Type	:	manufacturer
Name of plant	:	ANGUS Chemie GmbH
Street	:	
Town	:	Ibbenburen
Country	:	Germany
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	http://www.dow.com/Homepage/index.html
Source	:	Frauson, L. (2003). Internal E-mail of the Dow Chemical Company to C. Houtman.
Reliability	:	(2) valid with restrictions 2g

27.04.2005

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name : 2-Amino-2-methyl-1-propanol
Smiles Code : OCC(N)(C)C
Molecular formula : C4H11NO
Molecular weight : 89.14
Petrol class :

Reliability : (2) valid with restrictions
2g

28.06.2004 (1)

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type : measured for specific batch
Substance type : Organic
Physical status : Liquid
Purity : ca. 100 % w/w
Colour : Colourless
Odour : faint amine odor

Field

Remark : The test material is sold in formulations of >99% ai, 95% ai, 90% ai, and 75% ai.

AMP-REGULAR is 92% minimum ai, maximum 8% impurities.
Source : MSDS of The Dow Chemical Company. 2003.
Reliability : (2) valid with restrictions
2g

27.04.2005

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

2-Amino-2-methyl-1-propanol

Reliability : (2) valid with restrictions
18.03.2004 (1)

Aminomethylpropanol

Source : ANGUS Chemie GmbH Ibbenburen
European Commission- European Chemicals Bureau Ispra(VA)
Reliability : (2) valid with restrictions
01.03.2004

AMP

Source : ANGUS Chemie GmbH Ibbenburen
European Commission- European Chemicals Bureau Ispra(VA)
Environmental Evaluation Report, DR-0001-9476. The Dow Chemical Company.
Reliability : (2) valid with restrictions
01.03.2004

AMP 100R

27.04.2005

AMP-75

27.04.2005

AMP-90

27.04.2005

AMP-90 Textile

27.04.2005

AMP-95

Source : Environmental Evaluation Report, DR-0001-9476. The Dow Chemical Company.
Reliability : (2) valid with restrictions
01.03.2004

27.04.2005

AMP-95 SG

27.04.2005

27.04.2005

AMP-ULTRA PC 1000 Neutralizing Amine

27.04.2005

AMP-ULTRA PC 2000 Neutralizing Amine

27.04.2005

AVT-75 Amino Alcohol

27.04.2005

CORRGUARD-100 Amino Alcohol

27.04.2005

CORRGUARD-75 Amino Alcohol

27.04.2005

CORRGUARD-95 Amino Alcohol

27.04.2005

CORRGUARD-A Amino Alcohol

27.04.2005

P-1826

27.04.2005

1.3 IMPURITIES

Purity	:	typical for marketed substance
CAS-No	:	124-68-5
EC-No	:	204-709-8
EINECS-Name	:	2-amino-2-methylpropanol
Molecular formula	:	C4H11NO
Value	:	75 - 100 % w/w
Source	:	MSDS of The Dow Chemical Company. 2003.
Reliability	:	(2) valid with restrictions 2g

18.11.2004

Purity	:	typical for marketed substance
CAS-No	:	27646-80-6
EC-No	:	
EINECS-Name	:	MMAMP
Molecular formula	:	
Value	:	< 6.9 % w/w

Field

27.04.2005

1.4 ADDITIVES

Purity type	:	Other
CAS-No	:	7732-18-5
EC-No	:	231-791-2
EINECS-Name	:	Water
Molecular formula	:	H2O
Value	:	0 - 25 % w/w
Function of additive	:	Solvent
Source	:	MSDS of The Dow Chemical Company. 2003.
Reliability	:	(2) valid with restrictions 2g

01.03.2004

1.5 TOTAL QUANTITY

1.6.1 LABELLING

Labelling	:	as in Directive 67/548/EEC
Specific limits	:	Yes
Symbols	:	Xi, , ,
Nota	:	C, ,
R-Phrases	:	(36/38) Irritating to eyes and skin (52) Harmful to aquatic organisms (53) May cause long-term adverse effects in the aquatic environment
S-Phrases	:	(2) Keep out of reach of children (61) Avoid release to the environment. Refer to special instructions/Safety data sets
Source	:	European Commission - European Chemicals Bureau Ispra (VA)

01.03.2004

1.6.2 CLASSIFICATION

Classified	:	as in Directive 67/548/EEC
Class of danger	:	Irritating
R-Phrases	:	(36/38) Irritating to eyes and skin (52) Harmful to aquatic organisms (53) May cause long-term adverse effects in the aquatic environment
Specific limits	:	

Remark : Phrases apply for concentrations of AMP > 25%.
Source : European Commission - European Chemicals Bureau Ispra (VA)
27.04.2005

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : Industrial
Category : Paints, lacquers and varnishes industry

27.04.2005

Type of use : Industrial
Category : other: paper coating, personal care, metalworking fluid

Reliability : (2) valid with restrictions
2g
01.03.2004 (1)

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : Other
Limit value :

Remark : none established
Source : ANGUS Chemie GmbH Ibbenburen
European Commission - European Chemicals Bureau Ispra (VA)
01.03.2004

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

Source of exposure : Human: exposure by production
Exposure to the : Substance

Source : Frauson, L. (2003). Internal E-mail of the Dow Chemical Company to C. Houtman.
Reliability : (2) valid with restrictions
2g
01.03.2004

Source of exposure : Human: exposure of the consumer/bystander
Exposure to the : Substance

Source : Frauson, L. (2003). Internal E-mail of the Dow Chemical Company to C. Houtman.
Reliability : (2) valid with restrictions
2g

01.03.2004

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value : 30 °C
Decomposition :
Sublimation :
Method : OECD 102
Year : 2010
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Reliability : (2) valid with restrictions
Source : Pyzowski. 2010. AMP-ULTRA PC 1000 Melting Point. ANGUS Report ALR 2010-027
Flag : Critical study for SIDS endpoint
01.12.2010

Value : 30.5 °C — Field
Decomposition : no, at 31 °C — Field
Sublimation : No
Method : other: unknown
Year : 1989
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions
2 (data from a collection)
Flag : Critical study for SIDS endpoint
10.05.2006 (2)

2.2 BOILING POINT

Value : 163 to 164 °C at 1013 hPa
Decomposition : No
Method : Vapor pressure was measured up to 138 °C then boiling point was extrapolated to vapour pressure of 760 mm Hg using the Antoine equation determined by the measurement of the vapour pressure at several temperatures.
Year : 2004
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Reliability : (2) valid with restrictions
Source : Morrison. 2004. The vapor pressure of six mixtures containing 2-Amino-2-methyl-1-propanol (AMP). ANGUS Report SC AL SC-2004-000232
Flag : Critical study for SIDS endpoint
01.12.2010

Value : 165.5 °C at 1013 hPa — Field
Decomposition : No
Method : other
Year : 1989
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions
2 (data from a collection)
Flag : Critical study for SIDS endpoint
10.05.2006 (2)

2.3 DENSITY

Type : relative density
Value : = .0.953 at 25 °C — Field

Method	:	other:
Year	:	2005
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Jones. 2005. Specific Gravity and Viscosity on AMP-Regular and AMP-95. ANGUS Report ALR 2005-014
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

01.12.2010

Type	:	relative density
Value	:	= .934 at 40 °C
Method	:	other:unknown
Year	:	1989
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Budavari, S. (1989) The Merck Index (Eleventh Edition). Merck & Co., INC. Rahway, NJ, USA. Not GLP, Published
Reliability	:	(2) valid with restrictions 2 (data from a collection)
Flag	:	Critical study for SIDS endpoint

17.05.2005

Type	:	Density
Value	:	= .928 g/cm³ at 40 °C
Method	:	other:unknown
Year	:	
GLP	:	No
Test substance	:	
Source	:	ANGUS Chemie GmbH Ibbenburen European Commission - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

17.05.2005

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	= .45 hPa at 20 °C
Decomposition	:	
Method	:	other (measured): gas saturation method
Year	:	2005
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)
Flag	:	Critical study for SIDS endpoint

17.05.2005 (3)

Value	:	= 1.33 hPa at 20 °C
Decomposition	:	
Method	:	other (measured): EPA Chemical Fate Test Guidelines, CG-1600, "Vapor Pressure", August 1982 and Federal Register, Vol. 45, 77345, No. 772.122-3 (Nov. 21, 1980)
Year	:	1997
GLP	:	Yes

Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions 2 (data from a collection)

28.06.2004 (4)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	ca. -.63 at 20 °C
pH value	:	
Method	:	OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year	:	1996
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(1) valid without restriction 1 (well-documented and acceptable for assessment)
Flag	:	Critical study for SIDS endpoint

17.05.2005 (5)

Partition coefficient	:	octanol-water
Log pow	:	ca. -.74 at 20 °C
pH value	:	
Method	:	
Year	:	1999
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Meylan, W. (1999) SRC-KOW for Microsoft Windows, v1.65, Octanol/Water partition coefficient estimating software. Not GLP / Published
Reliability	:	(2) valid with restrictions 2 (accepted calculation method)

17.05.2005

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	Miscible
Stable	:	Yes
Deg. product	:	
Method	:	
Year	:	1989
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions 2 (data from a collection)
Flag	:	Critical study for SIDS endpoint

10.05.2006 (6)

Solubility in	:	Water
Value	:	= 0 - 99.9 vol% at 20 °C
pH value	:	= 11.3

concentration	:	.1 other:molar at 20 °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	9.72 at 25 °C
Description	:	Miscible
Stable	:	
Deg. product	:	
Method	:	other:unknown
Year	:	1989
GLP	:	no data
Test substance	:	
Source	:	ANGUS Chemie GmbH Ibbenburen European Commission - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable 4 (original reference not available)

01.03.2004

2.6.2 SURFACE TENSION

Value	:	67.7 mN/m at 20°C at a concentration of 10,000 mg/L
Method	:	ASTM D1590-60
Year	:	1974
GLP	:	
Test Substance	:	as prescribed by 1.1 - 1.4
Source	:	Hunsucker. 1974. AMP - wetting properties. Report C.P.D.M. 119.
Reliability	:	(2) valid with restrictions

01.12.2010

2.7 FLASH POINT

Value	:	82.1 °C at 1013 hPa
Type	:	EU Method A.9
Method	:	other: Tag Closed Cup
Year	:	2004
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Pyzowski. 2004. Physical properties for the AMP-ULTRA PC 1000 and AMP-ULTRA PC 2000. ANGUS Report ALR 2004-04
Reliability	:	(2) valid with restrictions

01.12.2010

Value	:	ca. 78 °C
Type	:	closed cup
Method	:	other: Tag Closed Cup
Year	:	1993
GLP	:	
Test substance	:	no data
Source	:	ANGUS Chemie GmbH Ibbenburen European Commission - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

01.03.2004

2.8 AUTO FLAMMABILITY

Value	:	= 437.7 °C at 1013 hPa
Method	:	other: ASTM D 2155
Year	:	1980
GLP	:	No
Test substance	:	

Field

Source	:	ANGUS Chemie GmbH Ibbenburen European Commission- European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

01.03.2004

2.9 FLAMMABILITY

Method	:	
Year	:	1993
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The material is considered combustible.
Source	:	ANGUS Chemie GmbH Ibbenburen European Commission - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

27.04.2005

2.10 EXPLOSIVE PROPERTIES

Result	:	not explosive
Method	:	
Year	:	1993
GLP	:	
Test substance	:	
Remark	:	Based on the structure of the chemical and our experience this substance is not explosive.
Source	:	ANGUS Chemie GmbH Ibbenburen
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

01.03.2004

2.11 OXIDIZING PROPERTIES

Result	:	no oxidizing properties
Method	:	
Year	:	1993
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Material is considered corrosive to copper, brass, and aluminum.
Source	:	ANGUS Chemie GmbH Ibbenburen
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

27.04.2005

2.12 DISSOCIATION CONSTANT

Acid-base constant	:	9.81
Method	:	Other
Year	:	1993
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Potassium nitrate (5.05g, 0.05 mole) was added to a 500mL volumetric flask, which was subsequently purged with N2 to exclude CO2. Approximately 490mL of purified water that had been previously boiled was added to the flask. A N2 purge ensured a good mixing of the solution. The

test material and addition water were added, bringing the total volume of the solution to 500mL. Half of the solution was transferred to a 400mL jacket beaker containing a magnetic stir bar, the pH electrode, the ATC probe, and a N2 purge. A Neslab headed / refrigerated circulator was used to jeep the temperature of the solution from 25.0-25.3C. The solution was titrated with 0.2000N hydrochloric acid and the pH and temperature was recorded with the addition of each mL of acid.

pKa was calculated from the experimental data.

Reliability : (2) valid with restrictions
2 (calculations based on experimental titration data)

01.03.2004 (7)

Acid-base constant : 9.88 @ 293K
Method : Other
Year : 1990
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : The pKa value of the conjugate acid was determined by potentiometric titration of the basic compounds at constant ionic strength. Titration curves were recorded by computerized equipment. The pH meter was read by a digital voltmeter connected to a computer interface. The motorburet was also driven by this computer interface. The sample and buffer solutions were thermostated at the required temperature before use. CO2 was excluded from the titrations using a N2 purge. The pKa values were calculated from titration data. The experiments were run in triplicate at each temperature, and the mean of the 3 at each temperature are reported.
Result : The triplicates were reproducible within +/-0.02pKa.
Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

01.03.2004 (8)

2.13 VISCOSITY

Value : 435 cps or mPa s at 40°C
Test type : Brookfield viscometer
Test procedure :
Method :
Year : 2005
GLP :
Test substance : as prescribed by 1.1 - 1.4
Remark :
Source : Jones. 2005. Specific gravity and viscosity on AMP-Regular and AMP-95. ANGUS Report ALR 2005-014
Reliability : (2) valid with restrictions
12.1.2010 :

Test type : Other
Test procedure :
Method :
Year : 1993
GLP :
Test substance :

Remark : Viscosity at 30C = 102 centipoise
Source : ANGUS Chemie GmbH Ibbenburen
European Commission - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
4 (documentation insufficient for assessment)

01.03.2004

2.14 ADDITIONAL REMARKS

Memo : Henry's Law Constant

Result : 6.95 x 10^-3 Pa*m3 / mole
29.08.2006 (9)

3.1.1 PHOTODEGRADATION

Type	:	Air
Light source	:	Sun light
Light spectrum	:	ca. 200 - 225 nm
Relative intensity	:	based on intensity of sunlight
Method	:	The experimental technique involved formation of OH radicals in a flow system by pulsed vacuum ultraviolet photolysis of H2O at >105nm and monitoring their resulting decay rates by detection of the OH resonance fluorescence band at 306.4nm with a cooled multiplier tube. The reaction was studied at 50+-1 torr total pressure of Argon. The concentration of the amines in the argon flow was determined by UV absorption at 200-225nm.
Result	:	k1(AMP) = (2.8+-0.5)x10^-11 cm3molecule-1s-1
Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)
Flag	:	Critical study for SIDS endpoint

01.06.2006 (10)

3.1.2 STABILITY IN WATER

Type	:	Abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	
Year	:	2006
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	AMP lacks functional groups that would be subject to hydrolysis under typical environmental conditions.
Reliability	:	(2) valid with restrictions 2 (data from a handbook or collection)
Flag	:	Critical study for SIDS endpoint

Field

Field

Field

01.06.2006 (11)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other: air, water, soil sediments
Air	:	% (Fugacity Model Level I)
Water	:	% (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Method	:	other: Mackay fugacity model
Year	:	2006
Method	:	Input Parameters for Level III Model:

Property	Value
Data Temperature (°C)	25

Chemical Type 1 (can partition into all environmental compartments)
Molecular Mass (g/mol) 89.1
Water Solubility (g/m3) 1 x 10^6
Vapor Pressure @ 25°C (Pa) 78
Melting Point (C) 30.5
Henry's Law Constant (Pa*m3/mole) 6.95 x 10^-3
Log Kow -0.63
Simulated Emission Rate (kg/hr) 1,000 (Default)
Simulated Environment Level III Default environment

Reaction Half-lives (hr) Input to Level III Model:

Air (vapor phase) 5
Water (no susp. solids) 3,600*
Soil 7,200*
Sediment 7,200*
Suspended Sediment 1.0 x 10^11
Fish 1.0 x 10^11
Aerosol 1.0 x 10^11

Result : Predicted Distribution to air, water, soil, and sediments

1000 kg/hr to water (most likely emission scenario)
Air <0.1% (2 kg)
Water 99.9% (8.38 x 10^5 kg)
Soil <0.1% (144 kg)
Sediment <0.1% (333 kg)

1000 kg/hr to air
Air 0.5% (2770 kg)
Water 59.1% (3.58 x 10^5 kg)
Soil 40.5% (2.45 x 10^5 kg)
Sediment <0.1% (142 kg)

1000 kg/hr to soil
Air <0.1% (278 kg)
Water 58.1% (7.49 x 10^5 kg)
Soil 41.8% (5.39 x 10^5 kg)
Sediment <0.1% (298 kg)

1000 kg/hr simultaneously to water, air, and soil
Air 0.1% (3050 kg)
Water 71.2% (1.94 x 10^6 kg)
Soil 28.7% (7.84 x 10^5 kg)
Sediment <0.1% (773 kg)

Conclusion : This substance has high water solubility, a low vapor pressure, and low log Kow. The substance has a low potential for adsorption to soil or sediments, and a low potential to volatilize from water or soil to the atmosphere. If released to air, the substance will react with hydroxyl radicals. If released directly to water, the most probable emission route based on physical properties and use patterns, most of the substance will remain in the water compartment and is expected to be biodegraded. If released to soil, the substance is expected to be biodegraded.

Reliability : (2) valid with restrictions
2 (accepted calculation method)

Flag : Critical study for SIDS endpoint

29.08.2006 (9)

Type : fugacity model level I
Media : other: air, water, soil sediments
Air : .1 % (Fugacity Model Level I)
Water : 99.8 % (Fugacity Model Level I)

Soil	:	.1 % (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Method	:	other: Mackay fugacity model
Year	:	2006
Method	:	Input Parameters for Level I Model:

Property	Value
Data Temperature (°C)	25
Chemical Type	1 (can partition into all environmental compartments)
Molecular Mass (g/mol)	89.1
Water Solubility (g/m3)	1 x 10^6
Vapor Pressure @ 25°C (Pa)	78
Melting Point (C)	30.5
Henry's Law Constant (Pa*m3/mole)	6.95 x 10^-3
Log Kow	-0.63
Simulated Emission Rate (kg/hr)	1,000 (Default)
Simulated Environment	Level I Default environment

Result	:	Predicted Equilibrium Distribution Among Air, Water, Soil, and Sediments 100 000 kg total emissions Air 0.1% (140 kg) Water 99.8% (99800 kg) Soil <0.1% (21 kg) Sediment <0.1% (<1 kg)
Conclusion	:	This substance has high water solubility, a low vapor pressure, and low log Kow. In the absence of advective and reactive processes, these physical properties dictate that the substance will be distributed primarily in the water compartment at equilibrium. The substance has a low potential for adsorption to soil or sediments, and a low potential to volatilize from water.
Reliability	:	(2) valid with restrictions 2 (accepted calculation method)

29.08.2006 (9)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	Aerobic
Inoculum	:	activated sludge, domestic, non-adapted
Concentration	:	11 mg/l related to Test substance
Contact time	:	28 days
Degradation	:	89.3% based on BOD after 28 day(s)
Result	:	Readily biodegradable
Deg. product	:	
Method	:	OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Contact time	:	28 days
Degradation	:	89.3% based on BOD after 28 day(s)
Result	:	Readily biodegradable
Deg. product	:	
Method	:	OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year	:	2010
GLP	:	Yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The study followed OECD Guide-line 301 F "Manometric Respirometry

Test".

AMP was dissolved in mineral nutrient medium at a concentration of 11 mg/L. The mineral medium had been prepared from aerated de-ionized water amended with inorganic nutrient salts. The reaction mixtures were inoculated with 30 mg/L of activated sludge biosolids collected from a municipal wastewater treatment plant. Corresponding control mixtures were prepared with inoculated mineral medium (blanks); inoculated mineral medium with the reference substance aniline (positive controls); inoculated mineral medium with test substance and reference substance (toxicity controls); inoculated mineral medium with test substance that is chemically sterilized (abiotic control). The purpose of the control mixtures was to correct for background oxygen consumption; confirm the viability of the microbial inoculum; determine whether the test substance was inhibitory to the inoculum; and determine whether the degradation observed was biologically mediated. 500-mL volumes of the various reaction mixtures were dispensed into replicate bottles, sealed and incubated in the dark for 28 days at 20-21 ºC.

The headspace oxygen concentration in the reaction vessels was measured every six hours over the duration of the test. The percent biodegradation of the test substance was determined using the following formula:

BOD = (mg O2 uptake by test substance – *mean* mg O2 uptake by blanks)/(mg test substance in vessel)

The percentage of test and reference substance biodegradation (DO2) was determined at each sample interval by dividing this BOD value by the theoretical oxygen demand (ThODNH3) for each reaction mixture as follows:
$DO_2 = (BOD \times 100)/(ThOD_{NH3})$

Result : The results for replicate samples, expressed as percent biodegradation, are as follows:

AMP: 89.3% after 28 days. 10-day window was met (onset biodegradation (10%) occurred after 7.9 days. 60% degradation occurred 1.7 days later.

Aniline: 60% degradation within 6.6 days in the positive controls, confirming viability of inoculum. No toxicity observed in toxicity controls. No degradation observed in abiotic controls, confirming that degradation was due to biological processes.

Reliability : (1) valid without restrictions
(GLP guideline study)
Flag : Critical study for SIDS endpoint

(12)

Type : Aerobic
Inoculum : activated sludge, domestic, non-adapted
Concentration : 2 mg/l related to Test substance
related to
Contact time :
Degradation : ca. 40 (±) % after 28 day(s)
Result : other: not readily biodegradable
Deg. product :
Method : OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year : 1989
GLP : No
Test substance : as prescribed by 1.1 - 1.4

Field

Method	The study followed OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test". AMP was dissolved in mineral nutrient medium at a concentration of 2 mg/L. The mineral medium had been prepared from aerated de-ionized water amended with nutrient salts and trace element stock solutions. The reaction mixtures were inoculated with a few drops of inoculum from the discharge of a community wastewater treatment plant. Corresponding control mixtures were prepared with the nutrient medium; nutrient medium with inoculum; and nutrient medium with inoculum and the reference substance sodium acetate to correct for background oxygen consumption and confirm the viability of the microbial inoculum. Equal volumes of the various reaction mixtures were dispensed into replicate bottles, sealed and incubated in the dark for 28 days at 20-21 ºC. The dissolved oxygen concentration in duplicate samples was measured with an oxygen electrode on days 0, 5, 15, and 28. The percent biodegradation of the test substance was determined using the following formula: Dt = (mg of BODx/L) o 100 (mg of substance/L) o TOD Dt = percent biodegradation of the test substance BODx = biological oxygen demand after x days. TOD = Theoretical oxygen consumption
Result	The results for replicate samples, expressed as percent biodegradation, are as follows: AMP: 5 days (0.1%, 0.1%); 15 days (1.9%, 1.9%); 28 days (40.7%, 38.9%) Average biodegradation after 28 days was 40%. Sodium acetate: 5 days (51.5%, 51.5%); 15 days (70.9%, 58.0%); 28 days (77.3%, 83.6%)
Reliability	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)
Flag	Critical study for SIDS endpoint
01.06.2006	(13)

Type	Aerobic
Inoculum	activated sludge, domestic, non-adapted
Concentration	560 mg/l related to COD (Chemical Oxygen Demand) related to
Contact time	
Degradation	ca. 50 (±) % after 28 day(s)
Result	other: moderate degradation
Deg. product	
Method	other: DIN 38 412 part 25 (static test)
Year	1986
GLP	No
Test substance	as prescribed by 1.1 - 1.4

Field

Source	:	ANGUS Chemie GmbH Ibbenburen European Commission - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)
19.05.2006		
Type	:	Aerobic

Inoculum	:	other: soil
Contact time	:	
Degradation	:	ca. 50 (±) % after 35 day(s)
Result	:	
Deg. product	:	
Method	:	other: GSF-Test
Year	:	1989
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	ANGUS Chemie GmbH Ibbenburen
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

Field

19.05.2006

Type	:	Anaerobic
Inoculum	:	other: soil
Contact time	:	
Degradation	:	ca. 15 (±) % after 35 day(s)
Result	:	
Deg. product	:	
Method	:	other: GSF-Test
Year	:	1989
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	ANGUS Chemie GmbH Ibbenburen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

Field

19.05.2006

Type	:	Anaerobic
Inoculum	:	activated sludge, domestic, non-adapted
Concentration	:	1 g/l related to Test substance related to
Contact time	:	
Degradation	:	ca. 4 (±) % after 5 day(s)
Result	:	other: low under test conditions
Deg. product	:	
Method	:	other: degradation in activated sludge
Year	:	1989
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	ANGUS Chemie GmbH Ibbenburen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

Field

19.05.2006

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5		
Method	:	other:unknown
Year	:	1979
Concentration	:	1 g/l related to Test substance
BOD5	:	< 10 mg/l
GLP	:	No
COD		
Method	:	other: dichromate method
Year	:	1979

COD	:	= 2050 mg/g substance
GLP	:	No
RATIO BOD5 / COD		
BOD5/COD	:	ca. .005
Source	:	ANGUS Chemie GmbH Ibbenburen
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

19.05.2006

3.7 BIOACCUMULATION

Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	3 day(s) at °C
Concentration	:	50 µg/l
BCF	:	< 1
Elimination	:	no data
Method	:	other: GSF-test; BCF-3-days
Year	:	1989
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Five Gold orfes were raised in a test vessel with 8L of water, in which the 14-C-labeled chemical was dissolved at a concentration of 50ppb. The fish were not fed during the 3-day test. The vessel was closed and the water surface was stirred with a blade stirrer gently to provide air exchange and movement of water. At the end of the experiment, the concentration of AMP was determined by measuring the radioactivity of the fish and in the water and, from that, the accumulation factor was determined.
Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

01.06.2006 (13)

Field

Species	:	other: algae (Chlorella fusca)
Exposure period	:	1 day(s) at °C
Concentration	:	50 µg/l
BCF	:	ca. 320
Elimination	:	no data
Method	:	other: GSF-Test, BCF-1-day
Year	:	1989
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	ANGUS Chemie GmbH Ibbenburen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

Field

19.05.2006

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	Static
Species	:	Lepomis macrochirus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 100 ▲ measured/nominal
LC50	:	= 190 ▲ calculated
Limit test	:	
Analytical monitoring	:	No
Method	:	
Year	:	1980
GLP	:	Yes
Test substance	:	as prescribed by 1.1 - 1.4

Field

Field

Method : Bluegill sunfish were obtained from a commercial supplier, and held in culture tanks with a 16-hour light cycle, and acclimated for 14 days prior to exposure to the test material. Fish received a standard commercial fish food until 48 hours prior to testing, at which time feeding was discontinued. The fish had a mean weight of 0.21 grams and a mean standard length of 22 mm.

The static bioassay was conducted in 5-gallon glass vessels containing 15 liters of reconstituted water (48mg $NaHCO_3$, 30mg $CaSO_4$, 30mg $MgSO_4$, and 2mg KCl). Vessels were kept in a water bath at 21-23C, and the fish were acclimated to the dilution water and test temperature, and fasted for 48 hours prior to testing. Dissolved oxygen was 8.8 mg/L, pH was 7.0, total hardness was 45 mg/L as $CaCO_3$, and total alkalinity was 25 mg/L $CaCO_3$.

Dose levels for the definitive 96-hour study were set in a 72-hour range-finding test at 1, 10, 100, 560, 1000 mg/L. Dose levels for the definitive assay were 32-560 mg/L in logarithmic series. Concentrations are reported based on nominal dose.

Result : The LC50 data was reported as follows:

AMP 24-hour LC50 = 220 (170-280) mg/L
AMP 48-hour LC50 = 220 (170-280) mg/L
AMP 96-hour LC50 = 190 (150-250) mg/L

Antimycin A 24-hour LC50 = 0.000056 mg/L
Antimycin A 48-hour LC50 = 0.000037 mg/L
Antimycin A 96-hour LC50 = 0.000028 mg/L

There were no sublethal effects reported.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

Flag : Critical study for SIDS endpoint

18.09.2006 (14)

Type	:	Semistatic
Species	:	Pleuronectes platessa (Fish, marine)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= 184 ▲ calculated
Limit test	:	No
Analytical monitoring	:	No
Method	:	
Year	:	1983
GLP	:	No

Field

Test substance	:	other TS:AMP-95
Result	:	LC50 values were calculated based on nominal concentrations and observed mortality, at a 95% CI: 24 hour LC50 = 231 mg/L 48 hour LC50 = 184 mg/L 72 hour LC50 = 184 mg/L 96 hour LC50 = 184 mg/L Nominal temperature of the test solution was 15C (14.4-15C during the test). Dissolved O2 range during the test was 7.0-8.2 mg/L. The pH range during the test was 8.1-10.3. Hardness was not reported.
Test condition	:	In a 96-hour, semi-static seawater test system, the toxicity of AMP-95 to Plaice was studied. Fish (20 per dose level, mean weight of 4.1g and mean length of 63.2mm) were placed in 18L glass vessels containing 10L of the test solution. The pH of the system was 8.08-8.14, and salinity was 34.76-34.82 o/oo. Concentrations tested were 100, 320, 560, 1000 mg/L.
Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

01.06.2006 (15)

Type	:	Static
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC0	:	= 320 Field
LC50	:	= 331 Field
LC100	:	= 340 Field
Method	:	other: DIN 38 412 L 15
Year	:	1986
GLP	:	
Test substance	:	no data
Source	:	ANGUS Chemie GmbH Ibbenburen European Commission - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

19.05.2006

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	Static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= 100 measured/nominal Field
EC50	:	= 193 calculated Field
Analytical monitoring	:	Yes
Method	:	other
Year	:	1983
GLP	:	Yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Test procedures per "Methods of Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (USEPA, 1975) were followed. The daphnia used in the test were cultured at the testing facilities, and the adult daphnia were fed a suspension of trout chow and alfalfa daily until 24 hours prior to testing. The static Daphnia bioassay was conducted in 250mL beakers containing 200mL of test solution. The vessels were kept at 19-21C, with a 16-hour

light / 8 hour dark photocycle.

An initial range-finding study was conducted using 10 Daphnia per concentration level. The range was found by beginning at 0.1 mg/L and increasing the amount of test material by a factor of 10 until a toxic level was found. Once this level had been determined, five concentrations, in duplicate, of the test compound with 10 Daphnia per beaker were selected for their respective bioassay. These concentrations were a logarithmic series ranging from 100 to 1000 mg/L.

Remark : There was no additional data provided on the results of the range-finding study referenced in the methods section.

Result : A computerized program calculated the LC50 for Daphnia based on the method of Stephan et.al. with a 95% CI using nominal concentrations. The 24-hour LC50 was calculated to be 240 mg/L, and 48-hour LC50 was calculated to be 193 mg/L.

Examination of water quality parameters revealed dissolved oxygen levels ranging from 8.3 mg/L in the control at study start, and levels of 8.5 (control), 8.5 (100mg/L AMP), 8.6 (320 mg/L AMP), and 8.6 mg/L O2 (1000 mg/L AMP) at 48 hours. The pH of the control at study start was 7.8. At 48 hours, the pH data collected was: 8.1 in the control, 8.3 (100mg/L AMP), 8.9 (320 mg/L AMP), and 9.4 (1000 mg/L AMP). Hardness was 225 ppm as CaCO3, and held at a temperature of 19-20C.

Test condition : Daphnias (Daphnia magna) first instar < 24 hours old. Test conditions were a temperature range of 19-20C, and a photoperiod of 16 hours light. Test solution (200mL) was placed in 250mL beakers, and ten daphnias were added per test solution. Concentrations tested were in duplicate at 0, 100, 180, 320, 560, and 1000 mg/L AMP-95.

Test substance : AMP-95 (94.31% Active, 5.69% water, NVM 0.0004%.

Lot 216, the same as referenced in the report, "Acute Toxicity Effects of AMP-95 on Bluegill Sunfish, Ninety-Six Hour LC50" (C. Parekh, 1980).

Received as a clear liquid and stored at room temperature.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

Flag : Critical study for SIDS endpoint

01.06.2006 (16)

Type : Semistatic
Species : Crangon crangon (Crustacea)
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : ca. 179 calculated
Limit Test : No
Analytical monitoring : no data
Method :
Year : 1983
GLP : No
Test substance : as prescribed by 1.1 - 1.4

Field

Method : Concentrations of AMP-95 tested were 0, 56, 100, 320, and 560 mg/L under semi-static conditions for 96 hours. The 18L glass vessel was folled with 10L of the test solution, and 20 brown shrimp were added to each vessel after an on-site acclimation period of 5 days on a specially-prepared diet. Food was withheld during the test. The test solution was comprised of local filtered seawater (pH=8.10-8.16, salinity 34.86-34.90 o/oo), and was maintained within a temperature range of 14.7-15.8C. The solutions were aerated with compressed air and dissolved O2 ranged from 7.2-8.4 mg/L. The pH range during the test was 8.0-10.1. Hardness was not reported.

Remark	:	Detailed information regarding pH and dissolved oxygen were not provided. Only target ranges were given.
Result	:	Results were as follows, with a 95% CI, based on nominal concentrations of test material: 24-hour LC50 = 241 mg/L 48-hour LC50 = 179 mg/L 72-hour LC50 = 179 mg/L 96-hour LC50 = 179 mg/L The control mortality was 0% at 96 hours.
Test substance	:	The purity and identification of the test material is not noted. The test material was confirmed by the authors to be AMP-95 (2-Amino-2-methyl-1-propanol).
Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

01.06.2006 (17)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus sp. (Algae)
Endpoint	:	growth rate
Exposure period	:	72 hour(s)
Unit	:	mg/l
EC50	:	ca. 520
Limit test	:	
Analytical monitoring	:	No
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	1989
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4

Field

Method	:	The investigations according to the guideline give false results in the case of volatile substances due to the decreasing concentration. Closing of the test vessel led to growth inhibition or death in the absence of the test material. By previous passage of CO2-enriched air and variation of the air space in the test vessel, the conditions could be optimized for the growth of the algae in the closed vessel during the 96 hour test so that the growth rate was comparable to that in an open vessel. The chemical was added at increasing concentrations from a test solution prepared from a continuously illuminated algae stock culture, prepared with nutrient solution, and was placed in front of a light wall. The test vessels were shaken slightly from time to time in order to prevent sedimentation of the algae. The determination of the algae concentrations was carried out, based on a calibration curve and nominal concentrations, by measuring the extinction at time 0, 24, 48, 72, 96 hours. The inhibition was calculated with the trapezoidal formula in the Guideline. The EC50 after 72 hours was determined graphically by plotting the percent inhibition linearly against the concentration expressed logarithmically.
Result	:	No data other than EC50.
Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)
Flag	:	Critical study for SIDS endpoint

01.06.2006 (13)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	Activated sludge
Species	:	Unknown
Exposure period	:	3 hours
Unit	:	mg/L
EC50	:	343 calculated

Analytical monitoring	:	No
Method	:	
Year	:	2010
GLP	:	Yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The effect of AMP on activated sludge microorganisms maintained in an aerobic environment was assessed by the Activated Sludge Respiration Inhibition Test Method (OECD Guideline 209). The test contained control, reference and treatment groups. The control replicates were used to determine the background respiration rate of the sludge and were not dosed with the test or reference substance. The reference group was dosed with 3,5-dichlorophenol, a known inhibitor of respiration, at concentrations of 3, 15 and 50 mg/L. The treatment group was dosed with AMP at concentrations of 10, 30, 100, 300 and 1000 mg/L. After an exposure period of three hours, the respiration rates of the test solutions were measured using a YSI Model 50B-115 Dissolved Oxygen Meter. The respiration rates in the two controls were 86.4 and 86.4 mg O2/L/hr. The difference between the two control respiration rates was 0.0 %, and was within the 15% difference limit established for the test. The validity of the test was further supported by the results from the 3,5-dichlorophenol reference group, which resulted in an EC50 value of 11.8 mg/L, with 95 percent confidence limits of 3 mg/L and 15 mg/L. The EC50 for the reference substance was within the 5 to 30 mg/L range considered acceptable for the test. An inhibitory dose response effect was observed for the treatment group. The EC50 value for AMP was determined to be 342.9 mg/L.
Reliability	:	(1) Reliable without restriction (GLP guideline study)

(18)

Type	:	Aquatic
Species	:	Pseudomonas putida (Bacteria)
Exposure period	:	120 hour(s)
Unit	:	mg/l
EC50	:	ca. 101 - 202 calculated
Analytical monitoring	:	No
Method	:	
Year	:	1986
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	No detailed method information was provided in the report.
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

01.06.2006 (13)

Type	:	Aquatic
Species	:	other bacteria: activated sludge filtrate
Exposure period	:	120 hour(s)
Unit	:	mg/l
EC50	:	ca. 132
Analytical monitoring	:	No
Method	:	other: Sapromat
Year	:	1989
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	ANGUS Chemie GmbH Ibbenburen European Commission - European Chemicals Bureau Ispra (VA)

Field

Reliability : (4) not assignable
4 (documentation insufficient for assessment)

19.05.2006

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

Species : other avian: Redwinged Blackbird
Endpoint : Mortality
Exposure period :
Unit : mg/kg bw
LC50 : ca. 102 measured/nominal
Method :
Year : 1983
GLP : no data
Test substance :

Method : Wild-trapped birds were preconditioned to captivity for 2-6 weeks and were dosed via gavage with solutions of the test chemical in propylene glycol. LD50 values were calculated by the method of Thompson (1948) and Thompson and Weil (1952).
Remark : There was no discussion of any in-life observations or pathological findings.
Result : The calculated LD50 based on experimental results was 102 mg/kg.
Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)
Flag : Critical study for SIDS endpoint
01.06.2006 (19)

Species : Musca domestica (arthropod (Diptera))
Endpoint : other: incorporation into phospholipids of larvae
Exposure period :
Unit : other: umol/g
Method :
Year : 1965
GLP : no data
Test substance :

Method : Larvae were grown in diets with and without choline, and growth rate and mortality were observed.
Result : When no choline is included in the diet, growth retardation is noted. The test material in the diet causes death of the larvae before the sixth day after hatching. If choline chloride is added to the diet with the test material, growth is normal.

Decreasing the level of choline in the diet causes an increase by 7-24% (of total lipids) in the proportion of phosphatidyl-aminobutanols found in larval lipids.
Reliability : (4) not assignable
4 (documentation insufficient for assessment)
26.07.2006 (20)

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo : In vivo
Type : Metabolism
Species : Rat
Number of animals
Males : 42
Females :
Doses
Males : The test material at a specific activity of 5uCi/umole was delivered via an IP dose of 5.1x10^6 counts/minute in 0.2mL water per rat
Females :
Vehicle : water
Route of administration : i.p.
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product : no
Method : other
Year : 1970
GLP : no
Test substance : other TS: 14C-AMP

Method : The 42 rats were divided into 2 equal groups. One group of 50g Sprague-Dawley rats were given a standard rodent diet, and the other group was given a choline-deficient diet (via a supplement of 13.2mL of ADC drops).

The test material ((3H)2-amino-2-methylpropanol) at a specific activity of 5uCi/umole was delivered to the rats via one intraperitoneal injection of 5.1x10^6 counts/min in 0.2mL water. The rats were sacrificed at 30 minutes, and at 1, 2, 3, 6, 24, 96 hours following dosing.

The rats were sacrificed via decapitation. Serum was stored at -20 and the organs and tissues were removed and immediately frozen in tubes and immersed in a dry-ice / acetone bath. Urine and feces were collected on paper towels and the matter was extracted from the paper using a 95% ethanol solution until no more than 5% of the initial radiation on the paper remained. The given tissues (0.5-1g each of the brain, heart, kidney, liver, intestine, spleen, and skeletal muscle) were homogenized with 3mL of 95% ethanol and the homogenate was centrifuged. To extract lipids from the brain tissue, the homogenate was was mized with a 2:1 chloroform : methanol solution, and the filtrate was stored until use. To fractionate the livers and kidneys, the mitochondria were prepared via peer-reviewed method (Hogeboom et al.) and extracted using 95% ethanol. Microsomes were isolated by centrifugation, and the microsomes and mitochondria were washed with sucrose, then repeatedly extracted using ethanol. The particulate-free supernatant fluid was extracted reoeatedly with ethanol mixtures to insure the maximum recovery possible from the cytosol. Determining the radioactivity of the serum was possible using trichloroacetic acid to make the serum protein-free, and transferred into an ethanol solution. Aliquots of urine and tissue extracts were transferred into ethanol and were similarly treated. Isolation and hydrolysis of phospholipids of mitochondria and microsomes of the liver was accomplished by removing the mitrochondrial and microsomal solvents in vacuo, and residues taken up in a chloroform : methanol (2:1) mixture. Aliquots from each sample were developed on a silicic acid plate and

stained with I2. Stained areas were scraped from the plates, and extracted with ethanol, then chloroform:methanol. Solvents were removed, and the phospholipid residues were hydrolyzed with HCl and stored at 105 degrees for 6hr. Petroleum ether extraction removed the fatty acids and unhydrolyzed lipids. The aqueous layer was dried, and residues were extracted in ethanol. Individual components were separated via paper chromatography, and the location of the compound was identified by staining them with I2 vapor. The papers were placed in scintillation liquid for radioactive determinations.

Remark : Technical experts that reviewed the paper noted that there are 2 main deficiencies in the methods employed by the authors. An IP dose, as administered in this study, gave the reviewers no data regarding the oral bioavailability of AMP, and it is assumed that accidental ingestion is one of the most likely routes of exposure to the compound. It is also noted that a lack of mass balance determination is a significant methodological deficiency, as it is impossible to determine the extent of storage of the test material, or the extent of excretion of the material. While the metabolic fate of the AMP in the tissues and urine is known (AMP is stored unchanged and excreted unchanged), the relative per cent of material stored versus excreted cannot be determined due to lack of a mass-balance method.

The reviewers note that this is a reputable study with acceptable analytical and experimental methods except where noted, and is generally a useful study to use as a reference.

Result : Serum
Tritium appeared in the serum 30 minutes post-dosing. After an initial uptake of the tritium, a large portion disappeared quickly. The radioactivity in the serum of rats fed the choline-deficient diet was always lower than rats on a normal diet, except at 6 hours, when the levels were about the same.

Urine
The tritium levels in urine (cumulatively) for the normal-diet group was greater than the choline-deficient group up to 6 hours, at which time the levels were essentially the same. From 6-24 hours, the accrued radioactivity levels in the normal-diet group exceeded that of the choline-deficient group. Data analyses showed that in both groups, the test material was excreted in the urine unchanged.

Tissues & Organs
Tissues from the choline-deficient diets had a greater retention of the amino alcohol than the normal-diet group. The radioactivity incorporated into the mitochondria and microsomal phospholipid fractions of liver from choline-deficient rats increased with time, while the radioactivity in the corresponding fractions in normal rats decreased with time. The distribution of tritium among the liver & kidney fractions was different and suggested an incorporation of the test material into various types of phospholipids. The livers from the choline-deficient group showed a higher level of tritium in all subcellular fractions than the normal-diet group at all times. Most of the tritium was found in the cytosol of both liver and kidney. While the amount of tritium decreased with time in the mitochondrial and microsomal fractions of livers from animals fed normal diet, the amount of radioactivity increased with time in the corresponding subcellular fractions of the livers from animals receiving the choline-deficient diet.

Test substance : The authors confirm the test substance as 2-amino-2-methylpropanol, but do not give purity or lot.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

Flag : Critical study for SIDS endpoint
01.06.2006 (21)

In vitro/In vivo		In vitro
Type	:	Dermal absorption
Species	:	Rat and Human skin
Strain	:	Spraque-Dawley
Sex	:	
Number of Animals	:	
Vehicle	:	40% aqueous solution and test lotion
Doses	:	
Method	:	Equivalent to OECD Guideline 428 (Skin Absorption: In Vitro Method)
Route of Administration	:	
Exposure time	:	24 hours
Product Type	:	
Guidance		
Decision on results on acute tox. tests	:	
Adverse effects on prolonged exposure	:	
Half-lives	:	
Toxic behavior	:	
Deg. Product	:	No
Method	:	Details on study design

The objectives of this study were to evaluate the rate and amount of [14C]-AMP absorption across fresh human and Sprague-Dawley rat skin after in vitro exposure, and to evaluate the disposition of [14C]-aminomethylpropanol ([14C]-AMP) in the various layers (stratum corneum, epidermis, and dermis) of fresh human and Sprague-Dawley rat skin after in vitro exposure.

Tissue Preparation

Fresh skin was obtained from two human donors and one preparation of Sprague-Dawley rats. The Sprague-Dawley rats were shaved one day prior to preparation of skin disks to allow for skin recovery following shaving. Human skin obtained from the abdominal region of two human donors was cut with a dermatome to a split thickness of 200 to 500 µm. Full thickness rat skin was obtained from the abdominal, back, or thigh regions of Sprague-Dawley rats. At least 18 skin disks (16 to 20 mm in diameter) per species were used for the incubations. The disks were prepared from the skin using a cork-boring tool and stored in receptor fluid kept on ice prior to incubation.

Mounting of Skin into Perfusion Apparatus

Bronaugh flow-through diffusion cells and accessory percutaneous absorption/metabolism apparatus were used for the experiment, as described by Bronaugh and Stewart (2). A 37 ± 1°C water-jacketed metal block was used to maintain the skin surface temperature at approximately 32°C. The prepared skin disks were mounted between the upper and lower chambers of each diffusion cell (epidermis facing the upper chamber), leaving an exposure area of 0.636 cm2 (0.9 cm diameter). The receptor fluid was pumped at a rate of 1.5 to 2.0 mL/hour through the lower chamber.

Barrier Function Test

Barrier function was confirmed in each skin disk used in the study. Tritiated water (200 µL; ~1.5 × 106 dpm) was applied to the epidermal surface of the skin disks. The receptor fluid from the lower chamber was collected for 20 minutes. Liquid scintillation cocktail (10 mL) was added to the barrier function test samples and the samples were analyzed in a liquid

scintillation counter. Skin disks with sufficient barrier function (< 0.3% penetration of tritiated water for human skin disks, < 1% penetration of tritiated water for rat skin disks) were considered acceptable for use in this study.

Tissue Incubation

Skin disks with acceptable barrier function were dosed with a target volume of 20 µL/cm2 of the [14C]-AMP formulations. [14C]-AMP was applied to the epidermal surface of each skin disk. The receptor fluid pumped through the lower chamber was collected into scintillation vials at 1, 2, 4, 8, 12, 18, and 24 hours. At the end of the 24-hour incubation period, the skin disks were wiped with cotton swabs and washed twice with detergent and twice with water. The skin disks were removed and separated into component layers (stratum corneum, epidermis, and dermis). Each skin layer was digested in 1 mL of 1 N sodium hydroxide and retained for liquid scintillation counting. The sides of each diffusion chamber were wiped with cotton swabs to obtain any [14C]-AMP remaining in the diffusion chamber after each skin disk was removed. Cotton swabs used to remove [14C]-AMP from the skin surface or the sides of the diffusion chamber, and swabs used in the washes, were analyzed by liquid scintillation counting.

PTFE Disk Controls

The evaporative loss of test article from the diffusion cell apparatus was determined for the three formulations. Each formulation was applied to PTFE disks mounted in the flow-through diffusion cells, with receptor fluid flowing through the apparatus and temperature maintained at approximately 32°C. After 24 hours the dose was recovered from the apparatus, and the percent recovery of the applied dose was determined by liquid scintillation counting of the various fractions recovered from the apparatus.

Analysis

Liquid scintillation fluid was added to the entire volume or an aliquot of each of the fractions (cotton swab extractions, skin layer digests, and receptor fluid collections). The fractions were placed in the dark at ambient temperature for at least 24 hours to allow chemiluminescence to decay. The fractions were analyzed using a liquid scintillation counter.

Calculations

The transport flux rate for and the amount of [14C]-AMP absorbed across the skin barrier were calculated. The percent distribution in each of the skin layers was determined. Descriptive statistics of the absorbance data were calculated using Microsoft® Excel 2000 and are expressed as mean ± standard deviation.

Criteria for data acceptance

Data were accepted if skin disks used in the study demonstrated acceptable barrier function (< 0.3% penetration of tritiated water for human skin disks, < 1% penetration of tritiated water for rat skin disks).

Year : 2007
GLP Yes
Test Substance 2-amino-2-methylpropanol
Remark
Result Percutaneous absorption rate
Time point 24 hours Dose 20 µL/cm2 Absorption (%) ca. 43
Remarks Rat skin, AMP Ultra PC 2000

Time point 24 hours Dose 20 µL/cm2 Absorption (%) ca. 50.9
Remarks Rat skin, 40% Aqueous AMP solution

Time point 24 hours Dose 20 µL/cm2 Absorption (%) ca. 30
Remarks Rat skin, Neat Control Lotion B, 4% AMP

Time point 24 hours Dose 20 µL/cm2 Absorption (%) ca. 16.7
Remarks Human skin, AMP Ultra PC 2000

Time point 24 hours Dose 20 µL/cm2 Absorption (%) ca. 14.1
Remarks Human skin, 40% Aqueous AMP solution

Time point 24 hours Dose 20 µL/cm2 Absorption (%) ca. 6.6
Remarks Human skin, Neat Control Lotion B, 4% AMP

Rat

The barrier function tests showed that for full thickness Sprague-Dawley rat skin, less than 1% of the tritiated water applied on the epidermal surface was absorbed through the skin disks in 20 minutes. These results indicate that all the skin disks used in the experiments possessed acceptable barrier function.

Absorption of [14C]-AMP in the Ultra PC 2000™ formulation gradually increased throughout the incubation, and appeared to approach a plateau, with a maximum mean flux rate of 291 µg/cm2/hr at 24 hours. Absorption of [14C]-AMP in the 40% aqueous formulation increased progressively, but had not reached a plateau by 24 hours. The mean flux rate at 24 hours was 411 µg/cm2/hr. Absorption of [14C]-AMP in the Neat Control Lotion B formulation gradually increased throughout the incubation, and appeared to approach a plateau, with a maximum mean flux rate of 197 µg/cm2/hr at 24 hours. The absorption of [14C]-AMP was comparable in the three formulations, although unlike the Ultra PC 2000™ and Neat Control Lotion B formulations, the 40% aqueous formulation does not appear to have reached a steady-state by 24 hours.

In general, the total recovered dose for all three formulations was relatively low. After 24 hours, the mean total absorbed dose of [14C]-AMP with the Ultra PC 2000™ formulation was 43.0%, and the mean total recovered dose was 59.6%. The dose was primarily recovered from the skin wipes (16.7%), the epidermis (14.3%), and the receptor fluid (21.2%). With the 40% aqueous formulation, the mean total absorbed dose of [14C]-AMP was 50.9%, and the mean total recovered dose was 60.6%. The dose was primarily recovered from the skin wipes (9.68%), the epidermis (18.9%), and the receptor fluid (19.7%). With the Neat Control Lotion B formulation, the mean total absorbed dose of [14C]-AMP was 30.0%, and the mean total recovered dose was 56.8%. The dose was primarily recovered from the skin wipes (26.8%) and the receptor fluid (17.3%), with smaller recoveries in the epidermis (5.33%) and the dermis (3.44%). No evidence of marked accumulation of [14C]-AMP in skin tissue was evident.

Human

The barrier function tests showed that for human skin from Donors 1 and 2 used in this study, less than 0.3% of the tritiated water applied on the epidermal surface was absorbed through the skin disks in 20 minutes. These results indicate that all of the skin disks used in the study possessed acceptable barrier function.

Absorption of [14C]-AMP in the Ultra PC 2000™ formulation increased progressively throughout the incubation, and appeared to approach a

plateau in donor 1 while it appeared continue upward in donor 2, with a maximum mean flux rate of 128 µg/cm2/hr at 24 hours. Absorption of [14C]-AMP in the 40% aqueous formulation also increased progressively, and appeared to approach a plateau, with a maximum mean flux rate of 111 µg/cm2/hr at 24 hours. The pattern of absorption of [14C]-AMP in the Neat Control Lotion B formulation was variable between the replicate skin disks. Two of the replicates (skin disks 13 and 14) showed much higher flux rates at 2 hours than the other 4 replicates. Reduced barrier function does not appear to adequately explain these observations, as no correlation between barrier function values and high flux rates is evident. Using all 6 replicate skin disks (skin disks 13–18), the mean maximal flux rate of 107 µg/cm2/hr occurred at 2 hours, followed by a gradual reduction in flux rates through 24 hours. The absorption of [14C]-AMP is similar in the Ultra PC 2000™ and 40% aqueous formulations, gradually increasing or approaching a steady-state by 24 hours, while the Neat Control Lotion B formulation shows a rapid absorption phase followed by a gradual decline in flux rates through 24 hours.

As with rat skin incubations, the total recovered dose for all three formulations was relatively low, particularly with the Ultra PC 2000™ formulation. After 24 hours, the mean total absorbed dose of [14C]-AMP with the Ultra PC 2000™ formulation was 16.7%, and the mean total recovered dose was 25.1%. The dose was primarily recovered from the skin wipes (8.32%) and the receptor fluid (8.30%), with lesser amounts recovered in the dermal and epidermal skin layers. With the 40% aqueous formulation, the mean total absorbed dose of [14C]-AMP was 14.1%, and the mean total recovered dose was 59.2%. The dose was primarily recovered from the skin wipes (45.1%) and the receptor fluid (6.48%). With the Neat Control Lotion B formulation, the mean total absorbed dose of [14C]-AMP was 6.65%, and the mean total recovered dose was 52.1%. The dose was primarily recovered from the skin wipes (45.5%), the dermis layer (2.26%), and the receptor fluid (2.71%). No evidence of excessive accumulation of [14C]-AMP in skin tissue was evident.

The reduced recoveries of dose with the three formulations may be explained by the results of control incubations in which the recovery of applied dose from PTFE disks was determined after a 24-hour incubation in diffusion cells. This control was intended to evaluate the evaporative loss of test article from the diffusion cell apparatus when no absorption could take place. The percent recovery of the applied dose was 26.0, 68.2, and 64.7 for the Ultra PC 2000™, 40% aqueous, and neat control lotion B, indicating that significant loss of the test article due to evaporation had occurred, and accounting for the low recovery of applied dose in the main study.

Conclusions

The absorption and cutaneous disposition of [14C]-AMP in an Ultra PC 2000™ formulation, a 40% aqueous formulation, and a Neat Control Lotion B formulation were determined in rat and human skin. Rat skin showed gradually increasing flux rates for the three formulations, approaching a steady state through the 24-hour incubation. Maximal flux rates ranged from 197 to 411 µg/cm2/hr for the three formulations. The total absorbed dose as a percent of the applied dose ranged from 30.0 to 50.9 %. Human skin showed gradually increasing flux rates or rates approaching a steady state through the 24-hour incubation for the Ultra PC™ 2000 and 40% aqueous formulations. The Neat Control Lotion B formulation showed a rapid increase in flux rates through 2 hours, followed by a gradual decline through 24 hours. Maximal flux rates ranged from 107 to 128 µg/cm2/hr for the three formulations. The total absorbed dose as a percent of the applied dose was less than in rats, and ranged from 6.65 to 16.7 %. Recovery of the applied dose was low, however, evaporative loss of the test article from the test system could account for the low recovery of dose in both species.

Rat skin had consistently higher flux rates than human skin, and the total absorbed dose was markedly higher in rat skin. This may explain the higher recovery of the applied dose with the Ultra PC 2000™ formulation in rat skin relative to human skin (43.0% vs. 25.1%). That is, less evaporation of the dose could occur from rat skin because the dose was absorbed more rapidly and completely than human skin, so less test article was available for evaporation from rat skin. In both species, absorbed [14C]-AMP was primarily recovered from the receptor fluid. No evidence of marked accumulation of [14C]-AMP in skin tissue was evident.

Executive summary

The objectives of this study were to evaluate the rate and amount of [14C]-aminomethylpropanol ([14C]-AMP) absorption across fresh human and Sprague-Dawley rat skin after in vitro exposure and to evaluate the disposition of [14C]-AMP in the various layers (stratum corneum, epidermis, and dermis) of fresh human and Sprague-Dawley rat skin after in vitro exposure.

Human and rat skin samples were incubated for 24 hours with the epidermal surface exposed to [14C]-AMP prepared in three formulations. The amount of [14C]-AMP absorbed across the skin and the disposition of [14C]-AMP in the various skin layers following this 24-hour incubation period were determined by liquid scintillation counting.

The absorption and cutaneous disposition of [14C]-AMP in an Ultra PC 2000™ formulation, a 40% aqueous formulation, and a Neat Control Lotion B formulation were determined in rat and human skin. Rat skin showed gradually increasing flux rates for the three formulations, approaching a steady state through the 24-hour incubation. Maximal flux rates ranged from 197 to 411 µg/cm2/hr for the three formulations. The total absorbed dose as a percent of the applied dose ranged from 30.0 to 50.9 %. Human skin showed gradually increasing flux rates or rates approaching a steady state through the 24-hour incubation for the Ultra PC 2000 and 40% aqueous formulations. The Neat Control Lotion B formulation showed a rapid increase in flux rates through 2 hours, followed by a gradual decline through 24 hours. Maximal flux rates ranged from 107 to 128 µg/cm2/hr for the three formulations. The total absorbed dose as a percent of the applied dose was less than in rats, and ranged from 6.65 to 16.7 %. Recovery of the applied dose was low, however, evaporative loss of the test article from the test system accounted for the low recovery of dose in both species. In both species, absorbed [14C]-AMP was primarily recovered from the receptor fluid. No evidence of marked accumulation of [14C]-AMP in skin tissue was evident.

Test Substance

The test article was identified in this study as follows:[14C]-AMP (molecular weight = 91.1 g/mol; specific activity = 25 mCi/mmol)[14C]-AMP was provided by The Dow Chemical Company prepared in three formulations, identified in this study as follows:
1. AMP ULTRA PC 2000™ (Batch No. TA074801Z1) – Contains 94.85 mg AMP/g and 0.27 mg 14C-AMP/g, for a total calculated value of 95.12 mg AMP/g (Paste)
2. 40% aqueous solution (w/v, pH 9.5) prepared from AMP ULTRA PC2000™ – Contains 0.26 mg 14C-AMP/g (Liquid)
3. Control Lotion B with pure AMP (Lot BP200500759.06) – Contains 40 mg AMP/g and 0.26 mg 14C-AMP/g, for a total calculated value of 40.26 mg AMP/g (equivalent to the level of AMP in cosmetic lotions)

Ultra PC 2000™, 40% aqueous, and Neat Control Lotion B formulations were evaluated for radiochemical concentration, which was determined to be, respectively, 79.3, 86.2, or 87.6% in a first shipment of formulations, and 80.3, 88.9, or 92.4% in a second shipment of formulations.

Reliability

1 (reliable without restriction)

Flag Critical study for SIDS endpoint
9.9.2010 22

5.1.1 ACUTE ORAL TOXICITY

Type	:	LDLo
Value	:	ca. 1000 - 2000 ml/kg bw
Species	:	rabbit
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	:	1940
GLP	:	
Test substance	:	no data

Method : Oral administration was carried out on rabbits, and the test material was delivered by stomach tube. The materials were administered in 25% w/v aqueous solutions. The solutions were neutralized in repeat experiments with HCl due to high alkalinity. Animals were fasted 24 hours prior to dosing. Following administration, animals were observed for 2-3 hours prior to caging, and were kept for observation more than 5 weeks after initial dosing.

Result : The lowest lethal dose was observed to be 1000-2000 mg/kg with both the alkaline and neutral solutions. Twenty to 40 minutes following dosing, animals exhibited progressive weakness to collapse, unsteadiness and incoordination ending in complete ataxia, and changes in respiration first by slowing and later by increasingly rapid rate. No deviations in blood findings were noted, and no methemoglobin formation. There were no other findings in the post-exposure observation period.

Reliability : (4) not assignable
4 (documentation insufficient for assessment)

30.11.2006 (23)

Type	:	LD50
Value	:	ca. 2150 ml/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: Miller and Tainter, Proc.Soc.Exper.Biol. and Med. 57:261. 1944
Year	:	1955
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Field

Source : ANGUS Chemie GmbH Ibbenburen
European Commission - European Chemicals Bureau Ispra (VA)

Reliability : (4) not assignable
4 (documentation insufficient for assessment)

30.11.2006 (24)

Type	:	LD50
Value	:	= 2175 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	

Field

Doses	:	1250, 1875, 2500, 3750 mg/kg
Method	:	
Year	:	1976
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Mice were dosed once at dose levels of 1250, 1875, 2500, or 3750 mg/kg AMP. No further information was provided.
Result	:	All dose levels produced irritation, slowed activity and response to a tail pinch. All mice were grossly normal at necropsy.
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

30.11.2006 (25)

Type	:	LD50
Value	:	= 2400 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	female
Number of animals	:	
Vehicle	:	
Doses	:	1875, 1950, 2100, 2400, 3000 mg/kg
Method	:	
Year	:	1976
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Mice were dosed perorally with doses of 1875, 1950, 2100, 2400, or 3000 mg/kg AMP-95 (5% added water). No further methods information was provided.
Result	:	The calculated LD50 was 2400 +/- 89 mg/kg.
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

Field

30.11.2006 (26)

Type	:	LD50
Value	:	ca. 2900 mg/kg bw
Species	:	rat
Strain	:	no data
Sex	:	male
Number of animals	:	50
Vehicle	:	physiol. Saline
Doses	:	2200, 2400, 2800, 3600, 4000 mg/kg
Method	:	
Year	:	1976
GLP	:	no data
Test substance	:	other TS: P1826 (6C29-9B)
Method	:	The acute oral toxicity of P-1826 was determined using young adult male fasted rats. Five groups of 10 animals each were administered orally a single dose of P-1826 diluted in saline using equal volumes. Animals were observed closely for four hours and daily thereafter for 14 days.
Result	:	Doses exceeding 2800 mg/kg orally results in rapid absorbtion into the circulatory system resulting in gross damage to the liver, kidney, spleen, and respiratory system, followed by respiratory collapse. The compound produces irritation to the stomach and duodenum at doses of 2800 mg/kg and greater. In life observations included: traces of blood on the nostrils, labored breathing, ataxia, and death due to respiratory collapse. Necropsy findings (animals that died during the observation period): gas in

Field

stomach, inflamed and gaseous duodenum, liver and spleen dark, blood clot in heart.

Necropsy findings (survivors until a scheduled necropsy): No findings in dose levels less than 2800 mg/kg. At 2800 mg/kg, 3 were grossly normal and 3 had a small amount of gas in the stomach with a slightly inflamed duodenum. At 3600 mg/kg, 1 was grossly normal and 1 was grossly normal except a stomach adhered to the liver.

The following data are reported:

LD0 = 2200 mg/kg
LD50 = 2900 +/-140 mg/kg
LD100 = 4000 mg/kg

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

Flag : Critical study for SIDS endpoint

01.06.2006 (27)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : > 2000 mg/kg bw
Species : rabbit
Strain : no data
Sex : male/female
Number of animals : 12
Vehicle : other: no vehicle
Doses : 1000, 1500, 2000 mg/kg
Method : other: Pharmacology Lab Protocol
Year : 1980
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Field

Method : Each group of rabbits was treated with 1000, 1500, or 2000 mg of test material per kg body weight (mg/kg). The desired dose was spread over the prepared abdominal skin area (abraded or smooth as designated). The skin was covered with a gauze and a sheet of impervious rubberized cloth to prevent any loss of the test material. The trunk was further enclosed with a flexible wire screen held in place by tape. The animals were returned to individual cages.

After 24 hours dermally exposed, the bindings and patches were removed, the exposed areas gently cleaned, and observed for skin irritancy. The animals were observed for another 14 days for any gross symptoms of toxicity. At the end of the 14 day observation period, the animals were weiged, sacrificed, and the organs examined for gross pathology.

Result : At the end of the 24 hour exposure period, the intact and abraded treated skin sites were severely irritated and black in color. The sites became necrotic within two to three days and remained necrotic for the 14 days. The treated sites had severe eschar formation by the 14th day. The rabbits in the three treatment groups lost body weight over the 14 day observation period. The animals in all treated groups showed no signs of toxicity or abnormal pharmacological behavior. At necropsy, all organs in all rabbits were grossly normal. The treated skin sites in all rabbits were necrotic.

Test condition : 12 Rabbits weighing 3.0 +/- 0.5 kg were divided into 3 groups of 4 each, and their abdomens were shaved free of hair. The skin of 2 rabbits were further prepared by abrasions. The abrasions were made 2-3 cm apart over the area of exposure with a blunt hypodermic needle without bleeding.

Conclusion	:	The acute dermal LD50 for P-1826 for the rabbit was >2000 mg/kg. The test material was dermally nontoxic, but was a severe skin irritant.
Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)
Flag	:	Critical study for SIDS endpoint

01.06.2006 (28)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	other
Value	:	
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male
Number of animals	:	
Vehicle	:	water
Doses	:	0.76-1.52 mmol : 100g body weight
Route of admin.	:	i.p.
Exposure time	:	
Method	:	
Year	:	1979
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Field

Method	:	The test material was obtained from Aldrich Chemical Co., Milwaukee, WI. The material was used as received, no additional purification. The compound was dissolved in distilled water, and neutralized to a pH 7-7.2, and injected intraperitoneally. All compounds were freshly prepared, and used immediately after neutralization. The compound was tested at 0.76mmol / 100g body weight. The dosage is equivalent to that used for d-sterine in previous experiments, which always resulted in proximal tubular necrosis. Evidence of any cellular necrosis was evaluated by light microscopic examination of hemotoxylin- and eosin-stained sections of kidneys from animals sacrificed at 24 hours after administration of the test material.
Result	:	2-Amino-2-methyl-1-propanol did not cause noticeable tubular necrosis under these test conditions.
Test condition	:	Male Sprague-Dawley rats (Charles River), 100-120g, were used. The animals had free access to feed and water.
Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

01.06.2006 (29)

Type	:	LC50
Value	:	ca. 325 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	Miller and Tainter, Proc. Soc. Exper. Biol. and Med. 57:261. 1944
Year	:	1955
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Field

Source	:	ANGUS Chemie GmbH Ibbenburen European Commission - European Chemicals Bureau Ispra (VA)

Reliability	:	(4) not assignable 4e (documentation insufficient for assessment)

06.03.2006

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	
Exposure	:	
Exposure time	:	4 hour(s)
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	highly irritating
Classification	:	irritating
Method	:	
Year	:	1940
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Animals were prepared by closely clipping the skin over the anterior abdominal wall. After securing the animal in the supine position, a measured amount of the test material was pipetted onto the surface of the skin. The test material was allowed to evaporate in still air, and the animal was released to the cage after the skin was dry. The treatments were repeated daily until 5 had been made.
Result	:	No illness resulted from skin applications of the test material, and observations were limited to irritation. Two animals showed chemical dermititis, infiltration of the skin and subcutaneous tissues, superficial redness, and a considerable loss of hair. Burrowing lesions in the subcutaneous tissues were produced by the introduction of the test material into small wounds on an abraded dermal test site.
Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, and acceptable for assessment)
Flag	:	Critical study for SIDS endpoint

01.06.2006 (23)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	100 %
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	none
Result	:	highly irritating
Classification	:	risk of serious damage to eyes
Method	:	
Year	:	1975
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Animals were dosed with the test material, in one study the eyes were flushed 15-30 seconds after application, and in the other, they were not. No further methods information was given.
Remark	:	Based on known toxicity and use patterns, it is assumed that the free amine was tested in this case and that the high pH was likely the cause of the serious eye damage noted.
Result	:	Unflushed eyes scored a 110 on the grading scale used, the highest possible. The test material had destroyed the vision. Flushed eyes scored

69.3/89.3 when flushed at 15 and 30 seconds, respectively. A score of 16 or higher is considered a severe irritant.

Conclusion	:	The test material is considered to be a severe eye irritant, and there is little beneficial effect even when eyes are almost immediately flushed with water.
Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)
Flag	:	Critical study for SIDS endpoint

01.06.2006 (30)

Species	:	rabbit
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	none
Result	:	highly irritating
Classification	:	risk of serious damage to eyes
Method	:	
Year	:	1975
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The data source does not specify other than citing Draize Techniques for rabbits.
Remark	:	Based on known use patterns and toxicity of the compound, it is assumed that the serious eye damage noted in this evaluation was due to testing of the free amine at a pH~11. The high pH is likely the cause of the eye damage.
Result	:	Animals treated with AMP scored a 110 for eye irritation. It is considered a severe eye irritant, with a high potential for eye damage.
Conclusion	:	The potential for eye damage is high.
Reliability	:	(4) not assignable 4 (documentation insufficient for assessment)

01.06.2006 (31)

5.3 SENSITIZATION

Type	:	Patch-Test
Species	:	guinea pig
Concentration	:	1st: Induction 5 % 2nd: Challenge 2.5 % 3rd: Challenge 5 %
Number of animals	:	30
Vehicle	:	water
Result	:	not sensitizing
Classification	:	not sensitizing
Method	:	other: Buehler
Year	:	1982
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	One group was treated with 0.5mL of 10% P-1826 solution, a negative control group was treated with saline, and a positive control group was treated with dinitrochlorobenzene (DNCB solubilized in alcohol and made to volume with saline). After 24 hours, the patches were removed, and sites were cleaned and scored at 24 and 48 hours for erythema and edema according to Draize (Draize, JH, "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics". Assoc. of Food and Drug Officials of the United States, p. 48, 1957). At 48 hours, the application was repeated with

each group, and continued 2-3 times per week until 10 applications were made. Animals were allowed a 2 week recovery period, and then challenged at a virgin site. The test and negative control animals were challenged with 0.5mL of 2.5% and 5% solutions of P-1826. Positive and negative control animals were also challenged with 0.03% DNCB solution. After 24 hours, the test material was cleaned away, depilated, and three hours later scored for erythema and edema. Sites were scored again at 48 hours.

Test material is considered a sensitizer if the challenge elicits skin reactions in a large number of test animals when compared to the negative control.

Result : During the induction, the 10% P-1826 solution was found to be mildly irritating to all animals in the test group, so the remaining 8 doses during the induction were made with a 5% solution. The positive control, DCNB, elicited a mild to strong reaction during the 10 applications. There was one death in the positive control group, but was deemed not treatment-related via necropsy.

At challenge with 2.5% and 5% solutions of P-1826, none of the animals in the test or negative control groups showed any skin reactions at 24 hours, but the positive control animals showed mild skin reactions at 48 hours.

Nine of ten positive controls when challenged with DNCB at 24 hours showed skin reactions, and 7/10 showed reactions at 48 hours. Only 4/10 negative controls showed reactions when challenged with DNCB at 24 hours, and none at 48 hours.

Test condition : Thirty male guinea pigs (250-300g each) were divided into 3 groups of 10 each. The animals' backs and flanks were shaved free of hair. The guinea pigs were topically treated with the solutions applied under an occlusive patch.

Conclusion : 2-Amino-2-methyl-1-propanol was a non-sensitizer in the topical sensitization test in guinea pigs, under these test conditions.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

Flag : Critical study for SIDS endpoint

18.09.2006 (32)

Type : Intracutaneus test
Species : guinea pig
Concentration : 1st: Induction .1 %
2nd: Challenge .05 %
3rd: Challenge .01 %
Number of animals : 30
Vehicle : water
Result : not sensitizing
Classification : not sensitizing
Method :
Year : 1982
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : One group was treated with 0.05mL of 1% P-1826 solution, a negative control group was treated with saline, and a positive control group was treated with dinitrochlorobenzene (DNCB solubilized in alcohol and made to volume with saline). After 24 hours, sites were cleaned and scored for erythema and edema according to Draize (Draize, JH, "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics". Assoc. of Food and Drug Officials of the United States, p. 48, 1957). At 48 hours, the application was repeated with each group, and continued 2-3 times per week until 10 applications were made. Animals were allowed a 2 week

recovery period, and then challenged at a virgin site. The test and negative control animals were challenged with 0.1mL of 0.05% and 0.01% solutions of P-1826. Positive and negative control animals were also challenged with 0.3% and 0.03% DNCB solution. After 24 hours, they were depilated, and three hours later scored for erythema and edema. Sites were scored again at 48 hours.

Test material is considered a sensitizer if the challenge elicits skin reactions in a large number of test animals when compared to the negative control.

Result : During the induction phase, the first injection at 1% and second injection at 0.5% P-1826 induced necrotic lesions, so the remaining 8 injections were made with 0.1% solutions. The 0.3% DNCB sites were necrotic for the entire 10 injections.

At challenge with 0.05% and 0.01% P-1826, one animal in the test group showed mild reactions with 0.05%, but none of the negative controls challenged with P-1826 showed any reactions at 24 or 48 hours. In the repeat challenge, none of the animals in the test group showed any reactions with P-1826, but of the negative control group, 4 animals at 0.05% and 1 animal at 0.01% showed skin reactions at 24 hours.

A challenge with the positive control induced skin reactions in the positive control group. The 0.03% solution did not elicit any skin reaction at 48 hours.

Test condition : Thirty male guinea pigs (250-300g each) were divided into 3 groups of 10 each. The animals' backs and flanks were shaved free of hair. The guinea pigs were intradermally-injected with the solutions.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

Flag : Critical study for SIDS endpoint

01.06.2006 (33)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	other: CD
Route of admin.	:	gavage
Exposure period	:	13 weeks
Frequency of treatm.	:	5 days / week
Post exposure period	:	
Doses	:	0, 500, 750, 1100, 1700 mg/kg at pH of 7 & 11+
Control group	:	yes, concurrent vehicle
LOAEL	:	< 500 mg/kg
Method	:	
Year	:	1977
GLP	:	no
Test substance	:	other TS: P-1826 (assumed to be AMP-Regular)

Method : Dose Preparation
The dose solution for pH=11+ was prepared by adding the neat test material to distilled water in order to achieve a concentration adequate to dose the animals 1-5mL of dose solution according to the animals' body weights and dose group. The dose solution of pH=7 was prepared by adding the neat test material to distilled water, neutralizing the solution to pH=7. Because the resulting stock solution is purple in color, the solution was decolored using activated charcoal. The stock solution was then diluted as appropriate to obtain solutions adequate to dose the animals with 1-5mL solution according to their body weights and the targeted dose.

Dose Administration
The solutions were administered 5 days per week for 13 weeks, once per day by oral gavage. The dose was adjusted to the body weight of the rat by administration of different volumes of solution (1-5mL).

In-life Observations & Measures
Animals were observed 5 days/week for pharmacological effects of the test material. The observations were general, including signs of altered food and water consumption, irritability, sedation, hair and cutaneous alterations, external inflammation, hyperventilation, convulsions, neuromuscular paralysis, and altered activity. Body weights were recorded weekly, and dead animals were recorded and autopsied as soon as discovered.

Hematology & Clinical Chemistry Measures
The following blood parameters were measured: hemaglobin, packed cell colume (hematocrit), erythrocyte count, leucocyte count, differential count, transaminases, glutamic-oxalacetic and glutamic-pyruvic, total protein, blood urea nitrogen (BUN), Sodium, Potassium, Chloride, and Creatinine. For urine, Ph, protein, occult blood, glucose, bilirubin, sediment, and specific gravity were measured.

Pathology
Only gross pathology was reported.

Result : Mortality
A high mortality rate was recorded for animals with the pH=11 doses. No treatment-related mortality was noted for the pH=7 groups.

Behavior and Appearance
General observations on the pH=11 groups included hyperventilation and respiratory difficulty, neither of which could be confirmed at necropsy. Non-treatment related pneumonia was noted in some rats. Rats generally showed hyperirritability and hyperactivity at the time of AMP administration. In the pH=11 rats receiving more than 750mg/kg, abdominal swelling, nasal and mouth bleeding were noted the first few days of the study. The abdominal swelling was always followed by death. Some loss of hair around the mouth and face was noted for pH=11 rats, and assumed to be treatment-related. General observations for rats receiving the dose at pH=7 were hyperventilation (lesser extent than pH=11 rats), and limited hyperactivity and hyperirritability. Some of the rats also showed loss of hair around the mouth and face. In a few rats, the effect was accompanied by skin ulceration due likely to excessive salivation, or a local irritancy by the compound.

Body Weight, Food Consumption Trends
There was no significant difference in the rate of body weight change between all dose groups relative to the controls. Food and water consumption were qualitatively noted. Food consumption appeared normal, while AMP-administered rats appeared to drink more frequently than controls.

Hematology & Clinical Chemistry
Neither the pH=7 or pH=11 females showed significant differences in hematocrit, hemoglobin, erythorcyte counts, or leukocytes. The males from the pH=11 group in the 1100 mg/kg dose group showed a significant decrease in hematocrit, hemoglobin, and red blood cell count due to a loss of blood.

pH 11 group males

Group	HCT	HgB	RBC(x10^6)	WBC
Control (10)	44+-1	16.5+-0.4	7.41+-0.29	8.2+-1.2

500mg/kg (8)	43+-1	16.7+-0.9	6.20+-0.47	13.6+-2.6
750mg/kg (7)	43+-1	16.2+-0.5	6.31+-0.34	9.4+-2.5
1100mg/kg(2)	40+-1	14.9+-0.3	5.94+-0.10	9.0+-1.6
1700mg/kg (none surviving)				

A slight but significant decrease is seen in the RBC of rats receiving 500 and 750 mg/kg AMP. There was no significant effect on hematocrit at these exposure levels. The pH=7 group (only at 1700mg/kg) showed significant decreases in hematocrit and hemoglobin.

pH 7 group

Group	HCT	HgB
Control (10)	46+-1	17.1+-0.1
500mg/kg (9)	43+-2	16.4+-0.5
750mg/kg (10)	42+-1	16.4+-0.6
1100mg/kg(10)	42+-1	16.4+-0.8
1700mg/kg (9)	40+-1	15.1+-0.2

No significant differences were measured in Na, K, Cl, Ca, total serum protein, BUN, or creatinine for either pH group at any dose level. SGPT values were within normal ranges for all dose groups except for pH=7 females at 1700mg/kg, and pH=7 males at 1000 and 1700 mg/kg, where the values were significantly elevated.

Urinalysis
Urinalysis was performed only for the pH=11 groups. The data indicated some proteinuria.

Gross Pathology
There were no treatment-related grossly-recognizable changes observed at necropsy.

Test condition : CD rats were received from Charles River weighing 90-100g, and were acclimated a minimum of one week to the laboratory prior to the experimental start. The rats were divided into groups of 10 per sex per dose level, for each of 2 pH's. They were housed in groups of 2-3 per plastic cage, and fed via overhead racks. Food and water was provided ad libitum and animals were housed in rooms with controlled temperature, humidity, and photocycles.

Conclusion : LOAEL and NOAEL cannot be established because the authors do not specifically state, or provide detailed data regarding the lowest dose level at which in-life observations (respiratory difficulty, hyperirritability, hyperactivity, etc.) were seen.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

Flag : Critical study for SIDS endpoint

01.06.2006 (34)

Type : Sub-chronic
Species : dog
Sex : male/female
Strain : Beagle
Route of admin. : oral feed
Exposure period : 90 days
Frequency of treatm. : continuous
Post exposure period :
Doses : 0, 25, 600, 2500 ppm
Control group : yes, concurrent vehicle
NOAEL : = 25 ppm
LOAEL : = 600 ppm
Method :
Year : 1981

Field
Field

GLP : no
Test substance : other TS: AMP-95/HCl 48.6% ai. Lot 109-1

Method : The AMP was administered orally via the diet as AMP-95/HCl to 32 Beagle dogs (4/sex/dose group) at dose levels of 0, 25, 600, and 2500 ppm for a three month period. The control animals received an untreated diet. Body weights were measured pretest, weekly thereafter, and after fasting at termination; food consumption was measured daily and presented at weekly intervals. Feed samples of each dose level were assayed for homogeneity, stability, and verification of dose concentration. Results were presented in a subsequent report from the performing laboratory, and not presented in this report. Animals were observed for mortality and gross signs of toxicologic or pharmacologic effects twice daily, and dogs were given a detailed physical examination pre-test and weekly thereafter. Ophthalmoscopic examinations were performed pretest and at termination. Hematology, clinical chemistry, and urinalysis parameters were analyzed twice pre-test, and Month 1 and 3. Blood was obtained via jugular venipuncture. Urine was collected in stainless steel metabolism pans attached to each animal's permanent cage. Dogs were fasted overnight prior to blood collections, and were not dosed until after samples were collected. Hematology parameters evaluated included: hemoglobin, hematocrit, erythrocytes, platelets, clotting time, prothrombin time, and total and differential leukocytes. Clinical Chemistry parameters included: serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, lactic acid dehydrogenase, blood urea nitrogen, fasting glucose, cholesterol, total protein, albumin, globulin, A/G ratio, total bilirubin, direct bilirubin, sodiom, potassium, chloride, and calcium. Urine was evaluated for the following: Gross appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, occult blood, urobilinogen, and microscopic analysis.

On days 96-99, animals were necropsied and tisses were preserved for weights (brain, ovaries, pituitary, thyroid, heart, adrenals, spleen, testes, kidneys, liver) and microscopic evaluation (the above plus aorta, bone, sternum, epididimus, esophagus, eye, colon, duodenum, ileum, lungs, mesenteric lymph node, mammary gland, sciatic nerve, pancreas, parathyroids, prostate, salivary gland, skeletal muscle, skin, spinal cord, stomach, thymus, thyroid, trachea, urinary bladder, and gross lesions).

Statistical analyses were performed on data collected for body weight, food consumption, hematology, clinical chemistry, organ weights, and organ:body weight ratios. Mean values of all dose groups were compared to control at each time interval (where appropriate). Statistically significant differences from the controls are indicated.

Species and strain of test animals, route and method of test substance administration, and dose levels were established by the sponsor. The study was performed at Bio/dynamics, INC.

Remark : Results section references Appendix tables and data, but none is included in the Dow Chemical copy of the report. Test material intake, therefore, cannot be estimated.

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

Physical Observations
Included emesis, loose stool, alopecia, and slight penile or vaginal discharge. These findings occurred sporadically in the control and/or treated animals and are not considered by the authors to be treatment-related.

Ophthalmology
Pretest and terminal examinations revealed no significant ocular changes

through the study. There is no evidence of compound-induced ocular damage.

Body Weight: No statistically-significant differences were found in any dose group when compared to animals of the same sex in the control group. There was a 10% mean body weight reduction noted in the high dose group relative to the control for males and females in latter phases of the study, suggesting to the authors a slight compound-related effect.

Food Consumption
There was no treatment-related effect of the test material on food consumption at any dose level.

Hematology
Pretest hematology values were within the normal physiological range for all animals. There were no statistically-significant differences between the control and treated animals at Month 1 testing. At Month 3, there was a trend toward reduced prothrombin time observed in the treated males and females with statistically significant differences noted in the top two dose levels for males only ($p<0.01$). In addition, a slight trend torward reduced hemoglobin concentrations was found in the treated females. Only the mean value for the high dose, which was slightly below the normal physiological range, was statistically significant ($p<0.05$). No other biologically significant differences were observed in any other hematology parameter evaluated.

Clinical Chemistry
Pretest mean clinical chemistry values were within the normal physiological range for males and females. At both the Month 1 & 3 evaluations, statistically significant elevations in serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and alkaline phosphatase activities occurred in the high dose males and females. The magnitude of the increases ranged from 2-4 fold for serum glutamic oxaloacetic transaminas, 12-28 fold for serum glutamic pyruvic transaminase, and 5-6 fold for alkaline phosphatase. These significant increases were limited to the high dose animals only, and were similar at months 1 & 3 for both sexes. Other findings included reduced mean cholesterol values in both sexes at the above intervals, while direct bilirubin concentrations were slightly higher in the treated animals. No other toxicologically significant differences occurred in the other clinical chemistry parameters evaluated. The above effects observed in the high dose animals suggest a high degree of hepatocellular damage in the high dose animals with no significant changes in the low or mid dose groups. The combination of increased serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase levels, both of which are present in high concentrations in hepatocytes, are indicative of hepatocellular necrosis. The increased alkaline phosphatase and direct bilirubin levels are suggestive of cholestasis and/or bile duct epithelial necrosis. It appears, therefore, that the liver is the promary target organ, based on the clinical chemistry data, when the test substance is administered via the diet.

Urinalysis
There is no evidence of a compound-related effect on any of the urinalysis parameters studied.

Organ Weight/ Organ:Body Weight Ratios
A trend towards increased liver and/or liver/body weight rations was ovserved in both the mid and high dose male and female dogs. These differences were slight but tend to support the conclusion that the liver is the principle site of toxic action. Also, mean kidney weights and kidney/body weight ratios were increased in the high dose males, and in

mid and high dose females. Other organ weight and organ/body weight differences were marginal and appear unrelated to the administration of the test material.

Pathology
Two females and one male from the high dose group were found to have tan discoloration and mottling in liver upon gross postmortem evaluations. No other such findings were present in any of the other treated or control animals. Other gross findings occurred sporadically or were present in the treated and control animals to a similar degree. Microscopic findings included vacuolization, periportal cirrhosis, and bile duct hyperplasia in the liver sections in all high-dose males and females. Lipid deposition within the cellular vacuoles was confirmed by staining. The periportal cirrhosis was characterized by hepatocellular necrosis and fibrosis. These findings were more severe in the high-dose females as compared to the males. In addition, one low dose female had liver findings similar to the above, while 2 mid-dose males exhibited minimal fatty changes in the liver. Other microscopic findings occurred sporadically in the treated and/or control animals and were not related to the administration of the test material. The above microscopic findings correlate well with the clinical chemistry data, as both indicate hepatotoxicity induced by the test material in the high dose animals. The findings noted in the liver of the one low dose female, in the absense of altered clinical chemistry data, as well as the absence of similar microscopic findings in the mid dose group, are considered to be of uncertain significance (this may represent a response to treatment in an unusually sensitive animal, or may be a spontaneous finding).

Test condition : Study was run with Beagle dogs, 4/sex/dose. They were 4.5-5 months of age at arrival to the Bio/dynamics laboratory, and 5.5-6 months of age at the initiation of the study. Weight range at the start of treatment was 7.7-9.6kg for males (mean 8.7kg), and was 6.3-9.7 for females (mean 8.1kg). The dogs were immunized previously against distemper, hepatitis, leptospirosis, and rabies by the supplier. Fecal examinations were conducted on all dogs by the performing lab during the one month acclimation period. Baseline clinical laboratory tests were also performed. They were housed in elevated metal grid cages, and fed 400g of Purina Canine Meal Diet (#5007) presented for 4.5 hours. Fresh food was presented daily. Water was provided ad libitum by an automated water system. The dogs were maintained on a 12-hour photocycle, and temperature was monitored twice daily.

Dogs were ranked by body weight and distributed into 4 groups of 4/sex so that body weights in each group were comparable. Groups were assigned to control and dose levels randomly. Each dog was assigned a unique identification number on an ear tag and on the animal's cage. In addition, each dog had an ear tatoo bearing the USDA number.

Test substance : AMP-95/HCl (48.6% AI). Lot #109-1 (11/14/80) provided by International Minerals & Chemical Corporation (the study sponsor).

Conclusion : The results of this study agree with those from a concurrently-run study (Bio/dynamics #80-2503), utilizing the same test material and similar dose levels in rats, which also indicated the liver is the principle target organ from dietary administration of the test material. The degree of changes in the present study clearly indicates that dogs are more sensitive to the hepatotoxic effects of the test material.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

Flag : Critical study for SIDS endpoint
01.06.2006 (35)

Type : Chronic
Species : dog
Sex : male/female

Strain	:	Beagle
Route of admin.	:	oral feed
Exposure period	:	1 year
Frequency of treatm.	:	continuous
Post exposure period	:	
Doses	:	0, 1.1, 11, 110 ppm
Control group	:	yes, concurrent vehicle
NOAEL	:	> 110 ppm
Method	:	
Year	:	1990
GLP	:	no data
Test substance	:	other TS: AMP-HCl (47.1% AMP)

Field

Method :

Animals were dosed via the diet, which was tested by the supplier (Purina) for contaminants. The test article was incorporated into the diet on a weight/weight basis using a premix. Fresh food was prepared with the test article weekly. The animals were offered 400g of the diets daily. Any uneaten food was weighed and recorded. Drinking water was provided throughout the study ad libitum. Six males and six females were assigned to each of 4 dose groups (0, 1.1, 11, 110 ppm).

Daily observations were made of the general appearance, behavior, the presence of any signs of toxicity or pharmacologic effects and mortality. Body weights were recorded prior to dosing, and weekly thereafter. The fasting terminal body weight was also recorded. While weighing, the animals were examined for lesions or other signs of toxicity. Each animal had an ophthalmoscopic examination once pre-study, weeks 1, month 3,6,9,12 by a veterinarian.

Blood samples were obtained pre-study, and at 3, 6, 9, 12 months via venipuncture of fasted animals. The following parameters were recorded: hematocrit, hemoglobin, erythrocyte count, leukocyte count, platelet count, prothrombin time, MCH, MCHC, and MCV, Calcium, Phosphorous, Chloride, Sodium, Potassium, glucose, serum alkaline phosphatase, serum aspartate aminotransferase, serum analine aminotransferase, gamma glutamyl, transpeptidase, blood urea nitrogen, total protein, albumin, globulin, creatinine, bilirubin, and serum protein. Urine was examined for pH, specific gravity, volume and appearance, protein, glucose, acetone bodies, creatinine, and microscopic sediment.

Post-mortem examinations included a complete gross examination of the external surfaces, all orifices, the cranial cavity, the external surface of the brain, the thoracic, pelvic, and abdominal cavities and their viscera, cervical tissues and organs, and the carcass. Two animals per sex per dose were sacrificed at 6 months, with the remainder at 12 months. Organ weights were recorded for the liver, kidneys, testes, thyroids, adrenals, and brain. Tissues that were removed and preserved are the same as current OECD guidelines.

Experimental dates: 5/19/1988 to 7/25/1989

Remark : Test material intake is estimated based on week 36 body weights and food consumption.

Males

	estimated (mg/kg/day)
Control	0
1.1ppm	.031
11ppm	0.31
110ppm	2.98

Females

		estimated (mg/kg/day)
	Control	0
	1.1ppm	.029
	11ppm	0.31
	110ppm	2.55

Result : There were no general in-life observations made for any treated or control animal. Likewise, there were no ophthalmoscopic observations noted for any animal. Neither an ANOVA or a Kruskal-Wallis non-parametric ANOVA revealed any statistical significance in food consumption patterns for any dose level in either sex. There was no statistically significant differences in body weights for any of the animals throughout the study. Clinical chemistry findings for males revealed a slight decrease in the low and mid-dose groups' albumin-globulin ratio. Since there was no dose-response (the high dose group was not different than the control), it was judged to be a spurious finding and not treatment-related. High-dose males at 9 and 12 months showed differences in serum albumin via serum electrophoresis, but was not corroborated by actual clinical chemistry measurements, and was not considered by the authors to be biologically-significant. Throughout the study, findings for females included differences in serum sodium, serum ALT, and serum AST, but were transient, did not appear dose-related, and were judged by the authors to be not related to the administration of AMP. There was no effect of AMP administration on hematology, and likewise the urinalysis revealed no treatment-related effects. There were no effects on organ weights or organ/body weight ratios that were attributed to test material administration by the authors. At necropsy, evaluation of the tissues revealed several isolated observations, none of which were attributed to the administration of the test material. There were no neoplastic observations in any of the dogs sacrificed at either 6 months or one year of administration in their diets.

Test condition : Twenty nine male and 29 female beagle dogs were received from Laboratory Research Enterprises, Kalamazoo, MI, and 25 of the healthiest of each sex were used for study. The animals were selected based on criteria of the US FDA (1982). The dogs were 4 months of age at arrival to the testing laboratory. Animals were identified by an assigned identification number on a collar, housed singly for an acclimation period, and were examined by a laboratory veterinarian for general health status. Animals were stratified by body weight, and assigned randomly to their treatment groups.

Test substance : 2-amino-2-methyl-1-propanol supplied by ANGUS Chemical Co, Northbrook, IL. Supplied as an aqueous solution of AMP-HCl. Concentration of AMP in the solution was 47.1%.

Conclusion : Based on the findings under these study conditions, there is no effect at any dose level on general appearance, behavior, body weight, food consumption, ophthalmoscopic exams, clinical chemistry, hematology, organ weights, or tissue histopathology. Based on the absence of statistically and biologically significant findings in dose-response patterns, the No-Observed Effect Level for AMP in the diets of Beagle dogs in greater than 110 ppm.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

Flag : Critical study for SIDS endpoint

05.06.2006 (36)

Type : Sub-acute
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 8 weeks
Frequency of treatm. : continuous

Post exposure period	:	
Doses	:	0, 1000, 2000, 4000, 8000, 16000 ppm
Control group	:	yes, concurrent vehicle
LOAEL	:	ca. 1000 ppm
Method	:	
Year	:	1976
GLP	:	
Test substance	:	other TS: P-1826 (assumed to be AMP-regular)

Field

Method : The test material was fed in the diet at dosage levels of 1000, 2000, 4000, 8000, 16000 ppm. Ten male and 10 female rats were used at each dose level and also in a control group. The compound was mixed with distilled water so that the same total volume of liquid was mixed with the food for each group. The compound-water premix was mixed with a small amount of diet, and the food premix was mixed with the total diet. Control rats also received distilled water in their diet at the same volume as the treated diets.

Rats were observed daily for changes in general behavior and appearance. Individual body weights and sex group food consumption were recorded weekly.

At the termination of the study, all surviving rats at the 8000ppm and 16000ppm doses were necropsied. In addition, 2 males and 2 females at each of the other dose levels plus control were likewise necropsied. Livers plus any gross lesions from all animals necropsied were paraffin embedded, sectioned, and stained and examined microscopically.

Remark : Estimated test material intake on week 4, based on mean body weights and food consumption:

MALES

Dose Group	Material Intake (mg/kg/day)
Control	0
1000ppm	244
2000ppm	474
4000ppm	912
8000ppm	1901
16000ppm	4698

FEMALES

Dose Group	Material Intake (mg/kg/day)
Control	0
1000ppm	436
2000ppm	806
4000ppm	1628
8000ppm	3424
16000ppm	7579

Result : In-life observations made beginning week 3 included emaciation, rough hair coat, and scattered 1x1mm lesions over the dorsal surfaces of a few rats in the 16000ppm group. After week 3, the incidences increased so that all rats in the high dose showed similar clinical signs. Hair loss on the dorsal surface was also noted in rats in this group beginning at week 5 and continuing thereafter. One female rat at the high dose showed general paleness and tremors prior to death at week 2. Two female rats in the 16000ppm group died during the study.

Rats in the 16000ppm group gained slightly less weight as compared to control rats. Gains in the other dose groups were similar to the control. Food consumption patterns were similar for all animals.

At necropsy, all 16000ppm surviving rats, and all rats at the 8000ppm dose level were necropsied. Two males and two females from each of the other

groups were also examined. Alopecia and focal skin erosions and ulcerations found in rats at the 16000ppm dose level were viewed as possibly compound related. Hepatocyte vacuolation descrived in rats from all the treated groups, with increasing severity at the higher dose levels, was considered compound related. Microscopic skin lesions found at the 16000ppm dose level, like the corresponding gross lesions, were viewed as possibly compound related.

Test condition : Sixty male (133-183g at study start) and female (115-146g at study start) CD rats were received from Charles River Labs, and were housed individually in hanging canges, maintained in a temperature and humidity controlled room. Purina rodent chow and water were provided to the animals ad libitum.

Conclusion : LOAEL = 1000ppm
LOAEL under these test conditions was 1000ppm. Observed effects at 1000 ppm included alopecia and focal skin erosions.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

01.06.2006 (37)

Type : Sub-acute
Species : mouse
Sex : male/female
Strain : CD-1
Route of admin. : oral feed
Exposure period : 8 weeks
Frequency of treatm. : continuous
Post exposure period :
Doses : 0, 200, 400, 800, 1600, 3200 ppm
Control group : yes, concurrent vehicle
NOAEL : > 3200 ppm
Method :
Year : 1976
GLP :
Test substance : other TS: P-1826 (assumed to be AMP-regular)

Field

Method : The test material was fed in the diet at dosage levels of 200, 400, 600, 800, 1600, 3200 ppm. Ten male and 10 female mice were used at each dose level and also in a control group. The compound was mixed with distilled water so that the same total volume of liquid was mixed with the food for each group. The compound-water premix was mixed with a small amount of diet, and the food premix was mixed with the total diet. Control mice also received distilled water in their diet at the same volume as the treated diets.

Mice were observed daily for changes in general behavior and appearance. Individual body weights and sex group food consumption were recorded weekly.

At the termination of the study, all surviving mice at the 3200ppm dose were necropsied. In addition, 2 males and 2 females at each of the other dose levels plus control were likewise necropsied. Livers plus any gross lesions from all animals necropsied were paraffin embedded, sectioned, and stained and examined microscopically.

Remark : Estimated test material intake for the animals were calculated based on 4 week body weight and food consumption:

MALES

	Estimated mg/kg/day
Control	0
200ppm	1.244

400ppm 2.088
800ppm 3.360
1600ppm 8.950
3200ppm 14.380

FEMALES
Estimated mg/kg/day
Control 0
200ppm 1.600
400ppm 3.146
800ppm 6.070
1600ppm 12.870
3200ppm 27.742

Result : No changes considered to be related to compound were seen in the general behavior and appearance. There was no treatment-related mortality. Increases in body weight over the course of the study were comparable for control and all treated animals. Food consumption values were also similar for the control and treated animals.

There were no compound-related gross lesions observed in any of the necropsied mice. There were likewise no microscopic lesions that were considered treatment-related in the livers of any mice necropsied.

Test condition : Sixty male (20-31g at study start) and female (18-27g at study start) CD-1 mice were received from Charles River Labs, and were housed individually in hanging canges, maintained in a temperature and humidity controlled room. Purina rodent chow and water were provided to the animals ad libitum.

Conclusion : The NOAEL for CD-1 mice under these conditions is >3200ppm.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

01.06.2006 (38)

Type : Sub-acute
Species : dog
Sex : male/female
Strain : Beagle
Route of admin. : oral feed
Exposure period : 4 weeks
Frequency of treatm. : continuous
Post exposure period :
Doses : 0, 600, 1800, 5400, 16200 ppm
Control group : yes, concurrent vehicle
NOAEL : ca. 600 ppm
Method :
Year : 1976
GLP :
Test substance : other TS: P-1826 (lot 6C29-9B), purity not given, supplied by the study sponsor

Method : P-1826 was administered in the diet at doses 600, 1800, 5400, and 16,200 ppm. One male and one female was assigned to each dose level. The compound was mixed with distilled water and a small amount of chow to create a premix. The premix was used to mix the total diets at each dose level. Dogs were observed daily for changes in general behavior and appearance. Individual body weights and food consumption were recorded weekly. Blood and urine was collected pre-study and at week 4. Hematology studies included hemoglobin, hematocrit, total erythrocyte count, total and differential leukocyte counts and erythrocyte sedimentation rate. Clinical chemistry parameters included: fasting glucose, urea nitrogen, serum glutamic oxalacetic and pyruvic transaminase activities,

serum alkaline phosphatase activity, total protein and albumin. Albumin to globulin ratios were calculated. Urinalysis included description of color and appearance, measurement of volume, pH, specific gravity, occult blood, and microscopic examination of the sediment.

Remark : Estimated test material intake was calculated based on week 2 body weights and food consumption data:

Males

Control	estimated (mg/kg/day)
600ppm	21.9
1800ppm	59.6
5400ppm	25.37 (food consumption extremely low)
16200ppm	(1 gram of food consumption reported)

FEMALES

Control	estimated (mg/kg/day)
600ppm	19.0
1800ppm	70.86
5400ppm	198.0
16200ppm	168.8 (food consumption low)

Result : Soft stool and/or diarrhea frequently were noted for the female dog at 5400ppm, and for both dogs at 1800 and 16200 ppm. A dry nose and mouth were noted the second week of study for both dogs at the 16200 ppm. There was no mortality during the study. Marked losses in body weight were seen for the male dog at the 5400ppm dose level and for both dogs at the 1800 and 16200 ppm dose levels. All other dogs maintained or showed gains in body weight. A marked decrease in food consumption was noted for the male dog at the 5400ppm dose level, and almost total anorexia was noted for both dogs at the 16200ppm dose level. Slightly elevated hemoglobin, hematocrit, and erythrocyte count were noted for the female high-dose female at 4 weeks. Also at 4 weeks, a slight neutropenia for the male dog at the 5400 and 16200 ppm dose levels was noted. No other changes were seen in hematological studies. At the 600ppm dose level, the female showed a slight increase in SGPT activity. Moderate to marked increases were seen in alkaline phosphatase and SGPT activity for all dogs at the 1800, 5400, and 16200ppm dose levels. Slight to moderate increases in SGOT activity also were seen for all dogs at the 5400 and 16200ppm dose levels. No other changes were seen in clinical chemistry parameters. There were no unusual values seen in urinalyses. There were no compound related gross pathologic lesions observed at necropsy in any dog. There appeared to be a compound related decrease in absolute liver weights at 5400 and 16200 ppm and several dogs also had decreases in relative liver weights as compared to values from untreated dogs of similar age and weight. Microscopically, all dogs except the male at 600ppm had evidence of toxic liver injury, characterized by hepatocyte vacuolation, necrosis of scattered individual hepatocytes and accumulations of a greenish-brown pigment in Kuppfer cells. Sinusoids in the centrolobular areas of some livers appeared dilated, suggesting that atrophy of some liver parenchymal tissue had occurred. Decreased liver weight noted in some dogs would be consistent with atrophy of hepatic parenchymal tissue; with only one dog of each sex, the authors had difficulty assessing such. Microscopic lesions in tissues other than the liver were those which commonly occur spontaneously in dogs and were not considered significant.

Test condition : Four male and four female beagle dogs (6.9-10 kg) were housed individually in metal metabolism cages, maintained in a temperature and humidity controlled room, and offered Purina dog chow and water ad libitum.

Test substance : P-1826 (lot 6C29-9B) purity not given, supplied by the study sponsor

Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)
01.06.2006		(39)
Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Long-Evans
Route of admin.	:	gavage
Exposure period	:	5 days
Frequency of treatm.	:	once per day
Post exposure period	:	
Doses	:	0, 500, 1000, 2500 mg/kg
Control group	:	yes
NOAEL	:	ca. 500 - 1000 mg/kg
LOAEL	:	ca. 1000 - 2500 mg/kg
Method	:	
Year	:	1977
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Four groups of rats were gavaged daily for 5 days with AMP in distilled water at dose levels of 0, 500, 1000, 2500 mg/kg. These were administered from stock solutions at a rate of 1mL/100g body weight. The pH's of the dosing solutions all exceeded 11. The rats were observed daily and body weights were taken through the 5 days of dosing and up to the 15th day of the study. The survivors were sacrificed and necropsied on the 15th day. Tissues were taken for further study. Body weights and organ weights were measured for livers and kidneys. Representative samples of livers and kidneys were selected for microscopic examination.
Result	:	Mortality All five males at 2500mg/kg were dead by test day 3. Two females died by test day 11 at 1000mg/kg, and all five females at 2500mg/kg were dead by test day 3. Bodyweight There were slight decreases in bodyweight noted for surviving rats in all dose groups, relative to the controls. There were no statistically significant findings. Necropsy All livers of the surviving rats looked normal grossly. Upon further examination of tissues, there was no data presented in the report that suggest a harmful effect of the compound under these experimental conditions. The gross and microscopic findings represent the usual profile of spontaneous diseases that occur in rats. There were no significant findings in kidney and liver organ weights or organ:body weight ratios for any dose level relative to the controls.
Source	:	Albany Medical College, unpublished data. FOI# F86-38214.
Test condition	:	Forty Long-Evans rats (90-100g), 5/sex/dose, were acclimated to the lab for more than one week. Rat chow and water were provided ad libitum.
Conclusion	:	In general, no dose-related pathological effects were seen in the livers of rats. The mortality of the higher dose animals appeared to be associated with GI tract effects. The clinical chemistry studies show significant changes in some of the parameters, and cannot be disregarded. There exists, however, a strong possibility that the clinical chemical differences as well as GI tract pathology is associated with the strong alkalinity (pH>11) of the dose preparation, rather than an effect of the AMP alone. Under conditions of this study, the following LOAEL and NOAEL are noted: Males

LOAEL = 2500 mg/kg
NOAEL = 1000 mg/kg

Females
LOAEL = 1000 mg/kg
NOAEL = 500 mg/kg

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

19.05.2006 (40)

Type : Sub-acute
Species : monkey
Sex : female
Strain : other: Rhesus
Route of admin. : drinking water
Exposure period : 5 days
Frequency of treatm. : once daily
Post exposure period :
Doses : 500, 1000 mg/kg
Control group : no
Method :
Year : 1977
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : The monkeys were dosed with AMP in distilled water at dose levels of 500 and 1000 mg/kg. Dosing continued for 5 days or until the higher dose monkey died. Body weights were taken daily. The low dose monkey was observed for 14 days prior to necropsy on the 15th day, and the high dose monkey was necropsied at the time of death. Hematology and clinical chemistry parameters were evaluated, and animals were examined at necropsy for gross and microscopic findings.

Result : The high dose female died 2 hours after the 3rd dose. Cause of death was recorded as gastrointestinal hemorrhaging. Both monkeys showed an approximately 10% decrease in body weight from the first to last measurement.

Hematology results showed no significant changes in RBC, HgB, or Hct and only a slight increase in WBC counts. In the surviving monkey, significant differences in Ca++, BUN, Creatinine, SGPT, CPK, and OCT were seen.

The liver in both the low and high dose animals, upon necropsy evaluation, were found to be grossly and microscopically within normal limits.

Source : Albany Medical College, unpublished data. FOI #F86-38214.
Test condition : Two Rhesus monkeys were acclimated to the conducting laboratory for over one week. Baseline values for clinical chemistry, hematology, and urinalysis were performed prior to dosing.
Conclusion : In general, no dose-related pathological effects were seen in the livers of monkeys. The mortality of the high dose animal appeared to be associated with GI tract effects. The clinical chemistry studies show significant changes in some of the parameters, and cannot be disregarded. There exists, however, a strong possibility that the clinical chemical differences as well as GI tract pathology is associated with the strong alkalinity (pH>11) of the dose preparation, rather than an effect of the AMP alone.
Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

19.05.2006 (40)

Type : Sub-acute

Species	:	mouse
Sex	:	male
Strain	:	Swiss Webster
Route of admin.	:	inhalation
Exposure period	:	180 minutes
Frequency of treatm.	:	once
Post exposure period	:	20 minute recovery in room air
Doses	:	185-1160 mg/m3
Control group	:	yes
Method	:	
Year	:	1996
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : The method of aerosol generation for AMP is not specified in detail. The animals were placed in plethysmographs attached to the generation apparatus such that their heads were extended into the interior of the chamber. A latex collar served as a seal around the neck of the mouse and held its head in place. Pressure transducers permitted the measurement of pressure changes created by mice during inhalation and exhalation. Relative tidal volume changes and respiratory frequency were measured. Each experiment consisted of a 20 minute control period in air alone, exposed to air (sham exposed), or exposed to the test material for 180 minutes. A 20-minute recovery period was given immediately following exposure. Animals were observed for 1 week for any mortality.

Using the breathing patterns measured during the experiment by the plethysmograph, sensory irritation and pulmonary irritation can be identified.

Remark : The article failed to cite detailed atmosphere generation methods. The atmosphere concentration is reported only as a range of 185-1160 mg/m3; the absence of a time-weighted average concentration makes interpretation of the results difficult, as the actual dose to the animal is not known. The analysis of the atmosphere by Miran IR does not preclude the possibility aerosol exposure at a higher concentration than reported.

Result : In general, exposure to the test material resulted in immediate sensory irritation, and persisted throughout the 3 hour exposures. The test material also produced pulmonary irritation, but the effect was not immediate (2-3 hour delay in onset). Animals exposed to AMP failed to return to control levels during the 20 minute recovery period following exposure, thus recovery was considered poor. The test material is considered moderate in potency relative to other test materials tested concurrently under these test conditions.

Test substance : Supplied to the conducting lab by the United Auto Workers and General Motors Corporation National Joint Committee om Health and Safety.

Conclusion : AMP may be considered a sensory and pulmonary irritant at the concentration range tested in mice under these study conditions.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

01.06.2006 (41)

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	3 months
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	0, 25, 150, 250, 2500 ppm in feed
Control group	:	yes, concurrent vehicle

Field

LOAEL	:	= 2500 ppm
Method	:	
Year	:	1981
GLP	:	no
Test substance	:	other TS: AMP-95/HCl 66.1ai (Lot 109-1)

Method :

Appropriate amounts of test material were mixed with standard lab rodent diet weekly, to provide 0, 25, 150, 250, or 2500 ppm to the animals. The animals received the test material continuously in the diet for 90-92 days prior to necropsy. Homogeneity of the test material in the feed was assayed by sampling all dose levels (from the top, middle, and bottom of the containers). Samples from all dose levels were assayed for stability in the feed at day 0, 3, 4, 7, 10, and 14. Concentration of the test material in the feed was analyzed for all dose levels on test weeks 1, 2, 3, 4, 6, 8, 12, and 13.

Animals were observed twice daily for mortality and gross signs of toxicologic or pharmacologic effects. A detailed physical examination for signs of local or systemic toxicity, pharmacologic effects, and palpable masses was performed weekly. Ophthalmoscopic examinations were performed pretest and at the end of month 1 and 3. Body weights were recorded twice pretest, weekly during treatment, and terminally after fasting. Food consumption was recorded weekly beginning the week prior to study start, and test material was calculated from food consumption data based on nominal concentration of the test material in the feed.

Blood was analyzed for the following parameters (pretest, month 1 & 3): hemoglobin, hematocrit, erythrocytes, platelets, total & differential leukocytes, erythrocyte morphology, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, blood urea nitrogen, fasting glucose, total protein, albumin, globulin, A/G ratio, cholesterol, sodium, potassium, calcium, total bilirubin, and lactic acid dehydrogenase.

Urine was analyzed at month 1 and 3, and gross appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, occult blood, urobilinogen, and microscopic analysis were evaluated.

Animals dying spontaneously received a gross postmortem exam. All others surviving to necropsy were euthanized under anesthesia, underwent a gross postmortem exam, and select tissues were weighed and/or preserved for examination. Organ weight was collected for adrenals, brain, gonads, heart, kidneys, liver, pituitary, and spleen and they were also preserved for examination. In addition, the following were preserved: aorta, blood smear, bone and bone marrow, epididymus, eye, cecum, ileum, duodenum, jejunum, lungs, mesenteric & mediastinal lymph nodes, mammary glands, sciatic nerve, pancreas, parathyroid, prostate, salivary gland, seminal vesicle, skeletal muscle, skin, spinal cord, stomach, thymus, thyroid, trachea, bladder, uterus, gross lesions, and tissue masses.

Statistical analysis included body weight, food consumption, hematology, and clinical chemistry parameters, organ weights and organ:body weight ratios. Mean values of all dose groups were compared to control at each time interval where appropriate. Statistically significant differences from control are indicated in appendices.

Remark : Results & methods sections referenced appendix data, which is not provided in Dow's copy of the report. As such, test material intake (mg/kg/day) could not be estimated.

Result :

Mortality
All animals survived the test material administration, with the exception of 2 females- one died following Month 1 blood collection, and the other was sacrificed on test day 18 due to poor food consumption, constipation,

lethargy, and swollen abdomen. The condition was not considered due to the test material.

Physical Observations
All observations noted were interpreted by the author to be related to a viral infection (sialdacryoadenitis) in all dose groups at approximately the same frequency, primarily in males except where noted, and included: alopecia, swollen salivary glands (both sexes), opacities, excessive lactimation, and chromodacryorrhea.

Ophthalmoscopic Examinations
Pretest exams revealed no notable abnormalities. At months 1 & 3, a significant increase in a variety of lesions, which appeared to be infectious in origin, were noted. The observations were likely secondary to the sialodacryoadenitis infection which can lead to opacity and other ocular lesions. The incidence of the abnormalities was similar in all dose groups and both sexes, and therefore does not appear to be treatment-related.

Body Weight
Body weight differences between the treated and control animals of both sexes were slight during the course of the study. No consistent pattern related to the administration of the test substance was evident. It is concluded by the authors that the dose levels selected did not affect body weights significantly.

Food Consumption
Among treated males, slight reductions in food consumption were evident during weeks 1-3 as compared to the control. Thereafter, mean food consumption values were comparable in all groups. Mean food consumption values tended to be slightly higher in the treated females as compared to the controls during the course of the study. The increases were slight and are not considered to be of toxicological significance.

Test Substance Intake
Test substance intake values based on nominal dietary concentrations were approximately proportional to the dietary concentrations. Test substance intakes were highest for all groups during the early phase of the study and gradually declined reaching a plateau by approximately week 10. Since the females consumed more feed on a body weight basis, their test substance intake calues were highter as compared to the males within a given group.

Hematology
Pretest hematology values were within normal limits for both sexes. Occasional statistically significant differences were noted in the group mean hematology data for the treated animals as compared to the control groups, for both males and females. However, as all means were generally within the normal physiological range and no consistent treatment-related pattern evident, it was concluded by the authors that the administration of the test material did not significantly alter the hematology parameters evaluated.

Clinical Chemistry
Pretest values were normal for both sexes. At month 1, total bilirubin values were significantly decreased in the 2 highest dose groups while total protein and globulin levels were significantly increased in only the highest dose group. Among the females, month 1 alkaline phosphatase was slightly elevated in all but the control and low dose groups. Statistical significance was noted for only the 250ppm group. At Month 3, a trend toward increased SGOT levels was found among the treated males, while BUN levels tended to be lower in dosed animals as compared to the controls. In addition, LDH was significantly increased in the high dose only.

Among the females, SGOT, SGPT, and LDH were significantly elevated in the high dose only. In addition, total protein and globulin values were significantly decreased in the high dose. Other significant differences were noted in both sexes at the 1 and 3 month evaluations. However, these differences were generally slight and the values within the normal physiological range for animals of this species and strain. The combination of increases in SGOT, SGPT, and LDH in the males and decreased BUN in the females at Month 3 suggest that the liver may be a target organ. The magnitude of these differences was slight and generally confined to the high dose group. Therefore any alterations in liver function would be expected to be minimal in degree.

Urinalysis
There were no effects on urinalysis parameters noted in any animal over the course of the study.

Organ Weight and Organ:Body weight ratios
Among the males, the high dose kidney/body weight ratio was increased, while the absolute liver weights were decreased in the 150 and 250 ppm groups. Among the females, the absolute and relative liver and spleen weights were significantly increased in the 25 and 2500 ppm groups, respectively. The changes were slight and appear due to biological variation as no dose-response pattern emerged. The possible exception is the tendency toward increased liver weights in the treated females. As previously discussed, the clinical biochemistry values suggest the liver as a target organ for the test material. Frequently, liver weight changes are secondary to biochemical altherations in liver morphology and/or function.

Pathology
Gross post-mortem examinations revealed no significant compound-related abnormalities. Lesions noted occurred sporadically and/or were found in both the control and treated animals at approximately the same incidence. Microscopic evaluations revealed patchy hepatocellular vacoulization in the livers of 3/20 males and 4/20 females in the high dose group This finding ranged from nomimal to moderate in degree. These vacuoles, presumably containing lipid, suggest that the liver may be a target organ for the test compound, as the clinical chemistry evaluations showed increased serum concentrations of hepatic enzymes.

Test condition : Male and female Sprague-Dawley rats were received from Charles River Breeding Laboratories and were 4 weeks of age at arrival to the Bio/dynamics Lab. They were allowed to acclimate to the laboratory for a minimum of 14 days, and were housed individually in elevated stainless steel cages. They were fed Purina Certified Lab Chow (#5002) ad libitum, and fresh food was presented weekly. Water was provided ad libitum via an automated water system. Photocycle was 12 hours light per 24 hour cycle, and temperature was monitored twice daily. They were approximately 7.5 weeks of age at the start of treatment. Animals were given a pre-assignment physical examination and unsuitable animals were excluded from assignment to test groups. The animals were assigned randomly to groups (20/sex/dose), and were identified with an numeric metal ear tag.

Reliability : (3) invalid
3 (methodological deficiencies, high incidence of viral infection in test animals)

01.06.2006 (42)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Bacterial reverse mutation assay
System of testing : S. typhimurium (tester strains TA98, TA100, TA1535, TA1537) and E.coli (tester strain WP2 uvrA)
Test concentration : 100, 333, 1000, 3333, 5000 ug per plate

Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	OECD Guide-line 471
Year	:	1996
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method :

Positive controls, appropriate to the given tester strains, were used as follows:
All strains, activated - 2-aminoanthracene
WP2uvrA, nonactivated - methyl methanesulfonate
TA98, nonactivated - 2-nitrofluorene
TA100, TA1535, nonactivated - sodium azide
TA1537, nonactivated - 9-aminoacridine

The concentration varied according to positive control compound. The sterility of the test article was verified.

Preliminary Toxicity Assay
Ten dose levels of the test article were plated, one plate per dose, with overnight cultures of TA100 and WP2 uvrA on selective minimal agar in the presence and absence of rat liver S9 activation.

Mutagenicity Assay
An initial and confirmatory assay was used to evaluate the mutagenic potential of the test material. A minimum of 5 dose levels of the test article, with vehicle and positive controls, were plated with tester strains in the presence and absence of rat liver S9 activation. All dose levels of test article, vehicle controls, and positive controls were plated in triplicate. The test systems were exposed to the test article via the plate incorporation methodology described by Ames, et.al.((1975) and updated by Maron and Ames (1983). Plates were coded according to the testing laboratory's standard procedures. Plates were incubated for 48-72 hours at 35-39C. Plates not counted immediately following the incubation period were stored at 2-6C until the colonies could be counted.

Toxicity and degree of precipation were scored relative to the vehicle control plate using a well-defined 9-point scale. Colonies were counted either by an automated colony counter or by hand unless the assay was preliminary or the plate exhibited toxicity. Plates exhibiting test article precipitate that interferes with an automated colony counter were counted by hand.

Mean and standard deviation of the number of revertants per plate were calculated and reported. For a positive finding, the test material must cause a dose-related increase in mean revertants per plate of at least one tester strain with a minimum of 2 increasing concentrations of test article. Positive finding for TA1535 and TA1537 is an increase in mean revertants at the peak of dose-response greater than, or equal to, 3x mean control value. Positive finding for TA98, TA100, WP2 uvrA is an increase in mean revertants at the peak of dose-response greater than, or equal to, 2x mean control value.

Remark :

GLP's were followed except for the following exceptions:
1. The identity, strength, purity, and composition or other characteristics to define the test or control article were not determined by the testing facility.
2. Analyses to determine the uniformity, concentration, or stability of the test or control mixtures were not performed by the testing facility.
3. The stability of the test or control article under the test conditions was not determined by the testing facility.

Result :

Water was selected as the solvent of choice. The test material was soluble in water at approximately 100ug.mL, the maximum concentration tested.

Preliminary Toxicity Assay
The maximum dose tested was 5000ug/plate, achieved by using a concentration of 100mg/mL and a 50uL plating aliquot. Based on the findings, the maximum dose plated in the mutagenicity assay was 5000ug/plate. Neither precipitate nor appreciable toxicity was observed.

Mutagenicity Assay
Neither precipitate nor appreciable toxicity was observed in the initial or confirmatory assays. In neither the initial nor confirmatory assay was there a positive response with any of the tester strains at any dose level, in the presence or absence of S9 activation. The mean of each positive control exhibited at least a 3-fold increase in the number of revertants over the mean value of the respective vehicle control, indicating a valid test.

Test condition : Salmonella tester strains were recieved directly from Dr. Bruce Ames, University of California, Berkeley, and the E.coli was received from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland.

Tester strains TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens. Tester TA1535 is reverted by mutagens that cause basepair substitutions. Tester TA100 is reverted by mutagens that cause both frameshift and basepair substitution mutations. Specificity of the reversion mechanism in E.coli is sensitive to basepair substitution mutations, rather than frameshift mutations.

To assure that cultures were harvested in late log phase, the length of incubation was controlled and monitored. Each culture wasmonitored spectrophotometrically for turbidity and was harvested at a percent transmittance yielding a titer of approximately 10^9 cells per milliliter.

Acoclor 1254-induced rat liver S9 was used for the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single IP injection of Acroclor 1254, 500mg/kg, five days prior to sacrifice. Each batch was assayed for its ability to metabolize.

Conclusion : The results of this study indicate that, under the conditions of this study, the test material did not cause a positive response with any of the tester strains in the presence and absence of Acrclor-induced rat liver S9.

Reliability : (1) valid without restriction
1 (guideline, GLP study)

Flag : Critical study for SIDS endpoint

01.06.2006 (43)

Type : Mammalian cell gene mutation assay
System of testing : L5178Y/TK+- Mouse Lymphoma
Test concentration : 0.5, 1.5, 5.0, 15, 50, 150, 500, 1500, 5000 ug/mL
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 476
Year : 1996
GLP : yes
Test substance : other TS: AMP-95

Method : Aroclor 1254-induced rat liver S9 was used as the metabolic activation system.

A preliminary toxicity assay was used to establish the optimal dose levels for the mutagenesis assay. L5178Y cells were exposed to the vehicle alone and to concentrations of the test article ranging from 0.5-5000 uL in both the absence and presence of S9-activation. Cell population density was determined at 24 and 48 hours after exposure to the test article.

Toxicity was measured as suspension of growth relative to the growth of the solvent controls. The mutagenesis assay, initial and independent repeat, was used to evaluate the mutagenic potential of the test article. L5178Y mouse lymphoma cells were exposed to the vehicle alone and 10 concentrations of the test article in both the absence and presence of S9. Positive controls, with and without S9, were tested concurrently.

Tubes containing L5178Y/TK+- cells with F0P medium or S9 activation mixture, and 100uL of test solution at concentrations of 0.5, 1.5, 5.0, 15, 50, 150, 500, 1500, 5000 ug/mL were prepared. Positive controls treated with MMS and 7,12-DMBA were also prepared. Treatment tubes were gassed with CO2 and incubated in the dark for 4 hours. Cells were washed twice, resuspended, gassed with CO2 in air again, and placed on a roller. Cells were counted, and numbers were adjusted if necessary. For expression of TK+- cells, cells were placed in a cloning meduim of granulated agar, mixed with Trifluorothymidine (TFT) or viable count, prewarmed and filled with cloning media. The tubes were centrifuged, supernatant decanted, and cells resuspended in cloning media. Tests were conducted in duplicate. TFT or Viable Count were added to the flask and placed on a shaker. The suspension was placed in petri dishes, placed in cold storage for 30 minutes, then incubated for 10-14 days.

Diameters and numbers of colonies were recorded, and analyzed relative to positive and solvent controls. Previous published data indicates that the large colony mutants received localized damage, likely in the form of a point mutation or small deletion within the TK locus, and small colony mutants received damage to collateral loci concordant with the loss of TK activity.

Remark : Study followed GLP's except for the following:
1. The identity, strength, purity, and composition or other characteristics to define the test or control article were not determined by the testing facility.
2. Analyses to determine the uniformity, concentration, or stability of the test or control mixtures were not performed by the testing facility.
3. The stability of the test or control article under the test conditions was not determined by the testing facility.

Result : Sterile distilled water was the solvent of choice. The test material was miscible in water at 500 mg/mL, the maximum tested concentration.

Preliminary Toxicity Assay
The high dose tested was 5000ug/mL. Osmolality of the solvent control was 300mmol/kg and of the highest dose was 396mmol/kg. Suspension growth relative to the solvent control was 13% at 5000ug/mL without S9 activation, and 31% with S9 activation. Based on these results, the dose levels chosen for the mutagenesis assay ranged from 500-5000ug/mL for both the non-activated and S9-activated cultures.

Mutagenesis Assay
In the non-activated system, cultures treated with 1000-500ug/mL were cloned and produced a range in suspension growth of 11-55%. In the S9-activated system, cultures treated with 500-5000ug/mL were cloned and produced a range in suspension growth of 9-98%.

No treated cultures exhibited mutant frequencies more than 55 mutants per 10^6 clonable cells over the solvent control. A dose-response trend was not observed in either the activated or non-activated systems. Over the concentration ranges, total growth for the non-activated cultures ranged from 12-60%, and 10-137% for the S9-activated cultures. Results of the independent repeat assay are similar (12-62% and 32-124% for non-activated and S9-activated system suspension growth, respectively; 14-67% and 29-191% for non-activated and S9-activated system total growths, respectively). There was likewise no increase in mutant frequencies over the solvent control. Colony sizing for the positive control

yielded the expected increase in small colonies, thereby verifying the methods used to detect small colony mutants.

Conclusion : The results of the L5178Y/TK+- Mouse Lymphoma Mutagenesis Assay indicate that, under the conditions of this study, 2-amino-2-methyl-1-propanol did not cause a positive response in the non-activated and S9-activated systems, and was concluded to be negative.

Reliability : (1) valid without restriction
1 (guideline, GLP study)

Flag : Critical study for SIDS endpoint

27.07.2006 (44)

Type : Ames test

System of testing : S.cerevisiae (D-4) and S.typhimurium (TA98, TA100, TA1535, TA1537, and TA1538)

Test concentration : 0.01, 0.1, 1.0, 5.0 microliters per plate

Cycotoxic concentr. :

Metabolic activation : with and without

Result : negative

Method :

Year : 1976

GLP : no

Test substance : other TS:Referred to as P-1826 (AMP) Lot #6029-9B. No purity information was included in the final report.

Method : P-1826 was evaluated for its potential mutagenic effect using the microbial procedure of Ames, et.al. Saccharomyces cerevisiae D-4 and five strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537, and TA1538) were used for the evaluation in which the reaction mixture was tested with and without rat liver cell 9000Xg supernate metabolic activation.

Male rats were euthanized and the organs were immediately dissected and placed in cold sucrose buffered solution with TRIS at a pH of 7.4. The organs were washed twice with the buffered solution and homogenized. The homogenate was centrifuged, the supernatant was extracted, and retained in cold storage, and were used for the activation study.

Approximately 10^9 cells from a log phase culture of each indicator strain were added to test tubes containing 2mL of molten agar supplemented biotin and histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selected agar plates. In activation tests, the tissue supernatant and required cofactors were added to the overlay tubes. Four dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar plate and allowed to solidify. Plates were incubated for 48 to 72 hours at 37C, and scored for colonies. Positive (nonactivated assay methylnitrosoguanidine, 2-nitrofluorene, quinacrine mustard; activated assay 2-anthramine, 2-acetylaminofluorene, 8-aminoquinoline, dimethylnitrosamine) and solvent controls (water, DMSO, or saline depending on the positive control used) were run with each assay.

The numbers of colonies on each plate were counted and recorded. The positive and the solvent controls are provided as reference points.

Result : The test compound, P-1826, was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The appropriate positive controls for each system and strain elicited a strongly-positive result in all cases, validating the assay.

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced

physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of the compound was from 0.01uL to 5uL per plate.

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative as well.

Test substance : Referred to as P-1826 (AMP) Lot #6029-9B. No purity information was included in the final report.

Conclusion : The test compound did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

01.06.2006 (45)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : ICR
Route of admin. : i.p.
Exposure period : 1 ip injection
Doses : vehicle control, 16, 30, 60 mg/kg, and positive control CP (50mg/kg)
Result : negative
Method : OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year : 1998
GLP : yes
Test substance : other TS: AMP-95 (lot 35662)

Method : In each the Pilot & Toxicity Studies (designed to set appropriate dose levels for the micronucleus assay), and the Micronucleus Assay, mice were weighed immediately prior to dose administration and the dose volume was based on individual body weights. They were observed after dosing, and daily thereafter for 3 days for clinical signs of chemical effects. Body weights were recorded prior to dose administration and 1 and 3 days after dosing. Mice were given a single IP injection of the test article in water, the vehicle alone, or the positive control substance.

Pilot Study: 2 male mice were dosed with 1, 10, 100, or 1000 mg test material /kg body weight, and 5 males and 5 females were dosed with 2000 mg/kg.

Toxicity Study:
5 male and 5 female mice were dosed with 200, 400, 600, 800 mg/kg.

Based on the results of the pilot and toxicity studies, the high dose for the micronucleus test was set at 60 mg/kg, which was estimated to be approximately 80% of the LD50/3.

Micronucleus Assay:
Male and female mice were dosed with the positive control (5/sex/dose), vehicle control (5/sex/dose), 16 mg/kg (5/sex/dose), 30 mg/kg (5/sex/dose), or 60 mg/kg (5/sex/dose with an additional 5/sex/dose in the event of mortality) via a single IP injection of the test material or the positive control (cyclophosphamide). At the scheduled sacrifice times (24 and 48 hours), up to 5 mice / sex / dose were sacrificed, the femurs exposed, and bone

marrow aspirated into a syringe of fetal bovine serum. The bone marrow cells were pelleted by centrifugation and the supernatant drawn off. The cells were resuspended and a small amount of suspension spread onto a glass slide. Two to four slides were prepared per animal. The slides were fixed in methanol, stained, and permanently mounted. Slides were coded and scored blindly. Using oil immersion, 2000 polychromatic erythrocytes were scored for the presence of micronuclei. The number of micronucleated normochromatic erythrocytes in the field of 2000 polychromatic erythrocytes was enumerated. The proportion of polychromatic erythrocytes to total erythrocytes was also recorded per 1000 erythrocytes. Statistical significance was determined using the Kastenbaum-Bowman tables which are based on the binomial distribution.

The test article was considered to induce a positive response if a dose-responsive increase in mocronucleated polychromatic erythrocytes was observed and one or more doses were statistically-elevated relative to the vehicle control ($p>0.05$) at any sampling time. If a single dose group was significantly elevated without a dose-response trend, the assay was considered a suspect or unconfirmed positive and a repeat assay recommended. The test article would be considered negative if no statistically significant increase in micronucleated polychromatic erythrocytes above the concurrent vehicle control was observed at any sampling time.

Remark : Exceptions to GLP's included:
1. The identity, strength, purity, and composition or other characteristics to define the test or control article were not determined by the testing facility.
2. Analyses to determine the uniformity, concentration, or stability of the test or control mixtures were not performed by the testing facility.
3. The stability of the test or control article under the test conditions was not determined by the testing facility.

Result : Pilot:
Mortality occurred within 4 hours following dosing: 5/5 males and 5/5 females at 2000 mg/kg, and 2/2 males at 1000 mg/kg. There were no clinical observations noted.

Toxicity:
Mortality occurred within 3 days of dosing: 5/5 males and 5/5 females at 400, 600, and 800 mg/kg, and 3/5 males and 5/5 females at 200 mg/kg. Clinical signs included: lethargy in males and females at all dose levels, piloerection in males and females, and crusty eyes in males at 200 mg/kg. The LD50/3 was calculated by probit analysis to be approximately 73.7 mg/kg for male and female mice.

Micronucleus Assay:
There was no treatment-related mortality at any dose level. Clinical signs noted on the day of dosing included: lethargy in males and females at 60 mg/kg (highest dose). All other mice treated with test material and control articles appeared normal during the study. The number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes in test article-treated groups was not statistically increased relative to their respective vehicle control in either male of female mice, regardless of dose level or bone marrow collection time.

Test condition : ICR mice from Harlan Sprague-Dawley, INC, were 6-8 weeks old at study initiation. For the pilot, toxicity, and MNT assay, males ranged in weight from 27.4g to 31.9g, and females ranged from 23.0g to 28.0g. After arrival to the lab, the animals were monitored for parasites and infections, and were quarantined a minimum of 5 days. They were observed daily for general health, food and water consumption patterns, and other conditions. The animals were deemed healthy prior to placement on study.

Mice were housed in an AALAC-accredited facility with appropriate temperature, humidity, and photocycle for the species. They were housed

up to 5 per cage, and given food and water ad libitum. The feed and water contained no contaminants that influenced the study.

The mice were randomized and placed into assigned dose groups, and were identified by a uniquely numbered ear tag.

Conclusion : Under the conditions of the assay described in this report, 2-amino-2-methyl-1-propanol did not induce a sugnificant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow, and was concluded to be negative in the micronucleus test using male and female ICR mice.

Reliability : (1) valid without restriction
1 (guideline, GLP study)

Flag : Critical study for SIDS endpoint

01.06.2006 (46)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type	:	other: OECD 421
Species	:	rat
Sex	:	male/female
Strain	:	other: CD
Route of admin.	:	oral feed
Exposure period	:	2 weeks before breeding through postpartum day 4
Frequency of treatm.	:	continuous
Premating exposure period		
Male	:	2 weeks
Female	:	2 weeks
Duration of test	:	Approximately 7 weeks
No. of generation studies	:	
Doses	:	0, 100, 300, 1000 mg/kg/day
Control group	:	yes, concurrent vehicle
other: NOEL (repro effects)	:	= 100 mg/kg bw
other: NOEL (males, toxicity)	:	= 300 mg/kg bw
other: NOEL (females, toxicity)	:	< 100 mg/kg bw
Result	:	No effects on mating or conception. Liver effects in females at 100 mg/kg/day and higher, increased post-implantation loss at 300 mg/kg/day and higher.
Method	:	OECD Guide-line 421
Year	:	2005
GLP	:	yes
Test substance	:	other TS: AMP-HCl

Field

Field

Field

Method : CD (Crl:CD(SD)IGSBR) rats were obtained from a commercial supplier and were approximately eight weeks of age at the time of study initiation. Each animal was evaluated by a laboratory veterinarian or a trained animal/toxicology technician, under the direct supervision of a lab veterinarian to determine their general health status and acceptability for study purposes upon arrival at the laboratory. The animals were housed 2-3 per cage in stainless steel cages, in rooms designed to maintain adequate conditions (temperature, humidity, and photocycle), and acclimated to the laboratory for approximately two weeks prior to the start of the study. They were offered a commercial diet and water ad libitum. During the study, animals were housed one per cage (prebreeding) or two per cage (one male and one female during breeding) in stainless steel cage in rooms designed to maintain adequate conditions (temperature, humidity, and photocycle). Dams were housed one per cage (with their

litter) in plastic cages provided with corn cob nesting material from approximately day 19 of gestation and throughout the lactation phase of the study.

Animals were stratified by body weight and then randomly assigned to treatment groups using a computer program designed to increase the probability of uniform group mean weights and standard deviations at the start of the study. Animals placed on study were uniquely identified via subcutaneously implanted transponders (BioMedic Data Systems, Seaford, Delaware) which were correlated to unique alphanumeric identification numbers.

Groups of 12 male and 12 female CD rats were fed diets supplying 0 (control), 100, 300, or 1000 mg/kg/day of AMP. Males were exposed for at least two weeks prior to breeding and continuing throughout breeding for 37 days. The females were exposed for two weeks prior to breeding, continuing through breeding (up to two weeks), gestation (three weeks), and lactation (four days). Effects on gonadal function, mating behavior, conception, development of the conceptus, parturition, litter size, pup survival, sex, pup body weight and the presence of gross external morphological alterations were assessed. In addition, a gross necropsy and histopathology of the adults was conducted with an emphasis on organs of the reproductive system. Males were dosed via the diet for at least 14 days prior to mating, continuing throughout mating, for 37 days. Females were dosed by dietary exposure for 14 days prior to breeding, and continuing through breeding (up to two weeks), gestation (three weeks), and lactation (four days).

Representative samples from the test diets were evaluated concurrently with the concentration verification analyses (see below) to ensure homogeneous distribution of the test material at the lowest and highest concentrations in the feed at least once during the study. Preliminary stability analyses of the test material in rodent diets at concentrations of 0.0005% and 0.005% and 5.0% were initiated prior to the start of the range-finding study. Analysis of all test diets from the first mix of the main study were initiated prior to the start of dosing using gas chromatography-mass spectrometry (GC-MS) incorporating an internal standard to determine target concentrations.

Breeding Procedure
Breeding of the adults commenced after approximately two weeks of treatment. Each female was placed with a single male from the same dose level (1:1 mating) until pregnancy occurred or two weeks had elapsed. During the breeding period, daily vaginal lavage samples were evaluated for the presence of sperm as an indication of mating. The day on which sperm were detected or a vaginal copulatory plug was observed in situ was considered day 0 of gestation. The sperm or plug-positive (presumed pregnant) females then were separated from the male and returned to their home cages. If mating did not occur after two weeks, the animals were separated without further opportunity for mating.

Daily Observations
A cage-side examination was conducted twice daily, designed to detect significant clinical abnormalities that were clearly visible upon a limited examination, and to monitor the general health of the animals for all males pre-exposure and weekly throughout the study. Clinical examinations were conducted on all females pre-exposure and weekly throughout the pre-breeding and breeding periods. Mated (sperm-positive or plug-positive) females received clinical examinations on GD 0, 7, 14 and 20. Females that delivered litters were subsequently evaluated on LD 0, 1 and 4, and on additional days if warranted by observations made during daily cage-side examinations. Females that failed to mate or deliver a litter were examined

weekly. Clinical observations included a careful, hand-held examination of the animal with an evaluation of abnormalities in the eyes, urine, feces, gastrointestinal tract, extremities, movement, posture, reproductive system, respiration, skin/hair-coat, and mucous membranes, as well as an assessment of general behavior, injuries or palpable mass/swellings.

Body Weights/Body Weight Gains
Body weights for males were recorded on test days -1, 1, 4, 7, and weekly thereafter. Females were weighed on test days 1, 4, 7, and 14 during the pre-breeding period. During gestation, females were weighed on GD 0, 7, 14, and 20. Females that delivered litters were weighed on LD 1 and 4. Females that failed to mate or deliver a litter were not weighed during the gestation or lactation phases. Body weight gains were determined for the following intervals: GD 0-7, 7-14, 14-20, 0-20, and LD 1-4.

Feed Consumption
Feed consumption for all animals was measured on test days 1, 4, 7 and 14 during the pre-breeding period by weighing feed containers at the start and end of a measurement cycle. During breeding, feed consumption was not measured in males or females due to co-housing. Following breeding, feed consumption was measured weekly for males. For mated females, feed consumption was measured on GD 0, 7, 14, and 20. For females delivering litters, feed consumption was measured on LD 1 and 4. Feed consumption was not recorded for females that failed to mate or deliver a litter.

Litter Data
Females were observed for signs of parturition beginning on or about GD 20. In so far as possible, parturition was observed for signs of difficulty or unusual duration. The day of delivery was recorded as the first day the presence of the litter was noted and was designated as LD 0. Litters were examined as soon as possible after delivery. The following information was recorded on each litter: the date of parturition, litter size on the day of parturition (day 0), the number of live and dead pups on LD 0, 1, and 4, and the sex and the weight of each pup on LD 1 and 4. Any visible physical abnormalities or demeanor changes in the neonates were recorded as they are observed during the lactation period. Any pups found dead or sacrificed in moribund condition were sexed and examined grossly, to the extent possible, for external and visceral defects and discarded.

Adult Necropsy
A complete necropsy of all the adults was performed. All males were necropsied on test day 38, while females that delivered litters were necropsied on LD 4. Females that did not deliver a litter were necropsied at least 24 days after the last day of the mating period. In all cases, dosing continued until the day prior to sacrifice at which time the animals were fasted overnight. Fasted adult rats submitted alive for necropsy were anesthetized by the inhalation of carbon dioxide, weighed, and their tracheas exposed and clamped. The animals were then euthanized by decapitation.

A complete necropsy was conducted on all animals by a veterinary pathologist assisted by a team of trained individuals. The necropsy included an examination of the external tissues, and all orifices. The head was removed, the cranial cavity opened and the brain, pituitary and adjacent cervical tissues were examined. The eyes were examined in situ by application of a moistened microscope slide to each cornea. The nasal cavity was flushed via the nasopharyngeal duct and the lungs were distended to an approximately normal inspiratory volume with neutral, phosphate-buffered 10% formalin using a hand-held syringe and blunt needle. The skin was reflected from the carcass, the thoracic and abdominal cavities were opened and the viscera examined. All visceral

tissues were dissected from the carcass, re-examined and selected tissues were incised. The uteri of all females were stained with a 10% solution of sodium sulfide stain for approximately two minutes and were examined for the presence and number of implantation sites (Kopf et al., 1964). After evaluation, the uteri were gently rinsed with saline and preserved in neutral phosphate 10% formalin. Weights of the epididymides, kidneys, liver, and testes were recorded, and organ:body weight ratios calculated.

Offspring Necropsy
All pups surviving to LD 4 were euthanized by oral administration of sodium pentobarital solution, examined for gross external alterations, and then discarded. Any pups found dead were examined to the extent possible.

Histopathology
Tissues with relevant gross lesions was conducted on all adult rats from the control and high-dose groups. The histopathological examination of the testes included a qualitative assessment of stages of spermatogenesis. Examination of tissues from the remaining groups was limited to the liver (males and females), cervix, ovaries, oviducts, uterus, vagina, and relevant gross lesions. The histopathological examination of the testes included a qualitative assessment of stages of spermatogenesis. The presence and integrity of the 14 stages of spermatogenesis was qualitatively evaluated following the criteria and guidance of Russell et al. (1990). Microscopic evaluation included a qualitative assessment of the relationships between spermatogonia, spermatocytes, spermatids, and spermatozoa seen in cross sections of the seminiferous tubules. The progression of these cellular associations defined the cycle of spermatogenesis. In addition, sections of both testes were examined for the presence of degenerative changes (e.g., vacuolation of the germinal epithelium, multinucleated giant cells, a decrease in the thickness of the germinal epithelium, a preponderance of Sertoli cells, sperm stasis, inflammatory changes, mineralization, and fibrosis).

Selected histopathologic findings were graded to reflect the severity of specific lesions to evaluate: 1) the contribution of a specific lesion to the health status of an animal, 2) exacerbation of common naturally occurring lesions as a result of the test material, and 3) dose-response relationships for treatment related effects. Very slight and slight grades were used for conditions that were altered from the normal textbook appearance of an organ/tissue, but were of minimal severity and usually with less than 25% involvement of the parenchyma. This type of change would not be expected to significantly affect the function of the specific organ/tissue nor have a significant effect on the overall health of the animal. A moderate grade was used for conditions that were of sufficient severity and/or extent (up to 50% of the parenchyma) that the function of the organ/tissue may have been adversely affected, but not to the point of organ failure. The health status of the animal may or may not have been affected, depending on the organ/tissue involved, but generally lesions graded as moderate would not be life threatening. A severe grade was used for conditions that were extensive enough to cause significant organ/tissue dysfunction or failure. This degree of change in a critical organ/tissue may be life threatening.

The above grading criteria were not sufficiently flexible to accurately characterize the liver microscopic vacuolization consistent with fatty change found in this study. Therefore, the following criteria were applied only to the observation of "liver vacuolization, consistent with fatty change, hepatocyte, multifocal": grade 1 - infrequently observed, grade 2 - occasionally observed, and grade 3 - readily observed.

STATISTICS AND CALCULATIONS
Parental body weights and gestation and lactation body weight gains, litter

mean body weights, feed consumption, and organ weights were first evaluated by Bartlett's test for equality of variances (= 0.01; Winer, 1971). Based upon the outcome of Bartlett's test, either a parametric or nonparametric analysis of variance (ANOVA) was performed. If the ANOVA was significant at alpha = 0.05, a Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction (Miller, 1966) was performed (experiment-wise alpha = 0.05). Statistical outliers were identified by a sequential test at alpha = 0.02 (Grubbs, 1969), and were excluded only for documented scientifically sound reasons. Feed consumption values were excluded from analysis if the feed had been spilled or scratched. Gestation length, average time to mating (precoital interval) and litter size were analyzed using a nonparametric ANOVA (Hollander and Wolfe, 1973). If the ANOVA was significant at alpha=0.05, the Wilcoxon Rank-Sum test (Hollander and Wolfe, 1973) with Bonferroni's correction was performed at experiment-wise alpha = 0.05, two-sided. The mating, conception, fertility and gestation indices (defined below) were analyzed by the Fisher exact probability test (Siegel, 1956) with Bonferroni's correction at experiment-wise alpha=0.05, two-sided. Evaluation of the neonatal sex ratio on postnatal day 1 was performed by the binomial distribution test (Steel and Torrie, 1960) at alpha = 0.05, two-sided. Gender was determined for pups born dead on postnatal day 0 and these data were included in sex ratio calculations. Post-implantation loss, pup survival indices and other incidence data among neonates were analyzed using the litter as the experimental unit by a censored Wilcoxon test (alpha = 0.05; Hollander and Wolfe, 1973) as modified by Haseman and Hoel (1974) with Bonferroni's correction. Non-pregnant females, females with resorptions only, or females found to be pregnant after staining of their uteri were excluded from the appropriate analyses. Both the Dunnett's test and Bonferroni's correction correct for multiple comparisons to the control to keep the experiment-wise error rate at 0.05. Both were reported at the experiment-wise alpha level. Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final toxicologic interpretation of the data considered other factors, such as dose-response relationships, biological plausibility and consistency, and historical control values.

Calculation of Reproductive Indices
Reproductive indices were calculated for female and male mating indices, male conception index, female and male fertility indices, gestation index, gestation survival index, post-implantation loss, and days 1 and 4 pup survival indices.

Result : All animals survived until scheduled termination. No treatment-related effects on behavior or demeanor were observed at any dose level during the treatment-period.

There were no treatment related differences in the amount of feed consumed by any of the treated males or females when compared to their respective controls throughout the study. Gestation feed consumption of dams given 1000 mg/kg/day could not be compared due to a lack of viable litters at this dose level.

No significant differences in body weights were observed for males at any dose level tested. Pre-mating body weights of treated females were not different from controls. Dams given 300 mg/kg/day exhibited a non-statistically significant decrease (5.5%) in gestation day 20 body weight, and a statistically significant decrease in body weight gain between GD 14 and 20. This also led to a slight decrease (not statistically identified) in body weight gain for the GD 0-20 interval. The mean GD 14-20 body weight gain in the 300 mg/kg/day group was 40% less than the control body weight gain during this period, which correlated closely with an approximate 50% reduction in mean litter sizes observed in this group.

Gestation body weights and body weight gains of dams given 100 mg/kg/day were not different from controls. Gestation body weights and body weight gains of females given 1000 mg/kg/day could not be compared due to a lack of viable litters at this dose level. There were no treatment-related differences in body weight or body weight gains during the lactation phase of the study.

There were no treatment-related effects at any dose level on mating, conception, fertility, time to mating, gestation length or sex ratio. However, a marked increase in percent post-implantation loss (embryo resorption) occurred in the 300 and 1000 mg/kg/day group females, with both the incidence, severity and likely timing of this effect being dose-related. In the 1000 mg/kg/day group, all twelve pregnant females showed evidence of complete litter resorption. In the 300 mg/kg/day group, four of twelve females exhibited complete litter loss, with the remaining eight females showing partial litter losses to varying degrees. Judging from the gross and microscopic appearance of the resorbed implantation sites, as well as the pattern of individual animal body weight gains during gestation, it is estimated that the embryonic deaths occurred sometime toward the end of the second week of gestation (implantation is completed on GD 6; full term is 21-22 days), with some of the high dose animals perhaps occurring slightly earlier. In the 100 mg/kg/day group, all reproductive parameters including percent postimplantation loss were comparable to control values. One of 12 females in the 100 mg/kg/day group was initially considered non-pregnant based on the absence of implantation sites following sodium sulfide staining at necropsy. However, histological examination of the uterus revealed a very small amount of hemosiderin pigment in only one implantation site, suggesting that an embryo had implanted and then was resorbed almost immediately. This single case of very early resorption does not fit with the timing of resorption seen in the 300 and 1000 mg/kg/day group, which occurred somewhat later. As pregnancies are not normally diagnosed histologically, there are no historical control data to directly address the significance of this observation. However, the likelihood that this was a spurious finding is supported by the fact that isolated occurrences of females that mate, but do no show evidence of embryo implantation following sodium sulfide staining are commonly encountered in reproductive toxicity studies. Considering this, and the fact that all reproductive parameters in this group were unaffected by treatment, this single incident of litter resorption in the low dose group was considered spurious and unrelated to treatment

Observations recorded in the offspring occurred at low frequency and bore no relationship to treatment. There were no visible external morphologic alterations noted in any of the offspring delivered. There were no treatment-related effects on litter size in dams given 100 mg/kg/day. In the 300 mg/kg/day group, there were statistically identified decreases in mean number of pups born live, and mean number of pups on day 1 and 4 postpartum. These decreases in litter size were a function of the increase in post-implantation loss, as noted previously. No litters were produced by the 1000 mg/kg/day group females.

There were no treatment-related effects on mean pup body weights in the 100 mg/kg/day group. Mean pup body weights in the 300 mg/kg/day group were increased on days 1 and 4 postpartum, with the male mean pup weights identified as statistically different. These increases were most likely due to the decreased litter size at this dose level, as pup body weight varies inversely with litter size (Agnish and Keller, 1997). Mean pup body weights of dams given 1000 mg/kg/day could not be evaluated due to a lack of viable litters.

In the 1000 mg/kg/day males, mean body weight was increased approximately 5% when compared to the control group. Although not

statistically identified, it was associated with statistically identified increases in absolute and relative liver and kidney weight. Similar body or organ weight effects were not observed in any of the female groups. However, it should be noted that terminal body weight and organ weight data from the 1000 mg/kg/day group were excluded from analysis because they did not produce any viable litters and, therefore, could not legitimately be compared to the controls, which were in a post-partum state. Also excluded from analysis were four females in the 300 mg/kg/day group and one in the 100 mg/kg/day group which did not produce viable litters. The increased absolute and relative liver and kidney weights of the 1000 mg/kg/day males were statistically identified and were outside of the historical control range for several studies, suggesting a treatment related effect.

There were no treatment-related gross pathologic observations. All animals survived to the scheduled termination of the in-life phase of the study.

Males given 1000 mg/kg/day and females given 100 mg/kg/day had treatment-related liver effects. Hepatocellular changes in 12/12 males of the 1000 mg/kg/day group were characterized by very slight, diffuse, cytoplasmic microvacuolization of periportal hepatocytes. In general, the very slight effect was present in most of the periportal hepatocytes; however, in the three liver sections examined from different lobes (left lateral, middle, and right lateral), one was generally more noticeably affected. In general, the left and right lateral lobes were more affected than the middle lobe. The gradation of the effect was based upon the most severely affected lobe. The livers of a few male rats in the control, low and middle dose group were also affected. It is likely the microscopic hepatocellular change in the 1000 mg/kg/day males was related to the significantly increased absolute and relative liver weight observed in this group.

There were 3/12 female control rats, and nearly all treated female rats with a similar microscopic hepatic effect. In addition to the aforementioned microscopic liver change in the various groups, another observation characterized by individual hepatocytes with vacuolated cytoplasm, suggestive of fatty change, was noted. The fatty change generally involved either the left or right lateral lobe most extensively. In no case was the middle lobe the most severely involved. In rats with a grade 2 or 3 involvement of hepatocytes with fatty change, the effect extended from the periportal region to the midzone of the lobule. Even in the most severely affected livers of either sex, the hepatocytes surrounding the central vein were microscopically normal. The fatty change in livers of 1000 mg/kg/day males was observed at a higher frequency and severity when compared to lower dose groups and their respective control group. In the female rats of all treated groups, the incidence and severity of hepatic fatty change was increased compared to their respective controls.

In the female rats of all treated groups, the incidence and severity of hepatocellular fatty change was increased compared to their respective controls. The number of female rats in the 100 and 300 mg/kg/day groups was more frequently affected with a grade 2 and 3 hepatocellular fatty change in the 1000 mg/kg/day group. This may be due to the fact no litters were being nursed in the highest dose group. A clear dose response for the hepatic effect was not present in female 100 and 300 mg/kg/day groups. The preferential involvement of the left and right lateral lobes verses the middle lobe, in hepatocellular fatty change, may be due to normal variation in portal blood flow and/or normal variation in metabolic enzymes involved in lipid and triglyceride metabolism. The microvacuolization and the fatty change in hepatocytes of even the most severely affected rats were not associated with evidence of increased

hepatocellular necrosis or death. The observed microscopic hepatic effects were interpreted as reversible.

In the 1000 mg/kg/day male rats the microscopic liver effects observed were associated with increased absolute and relative liver weights. However, in the female rat livers of all dose levels were affected, despite the absence of an increased liver weight in this sex.

The microscopic hepatic fatty change was noted in essentially all 1000 mg/kg/day males and all treated groups of females; because a low incidence was observed in controls of both sex, and low and middle dose males, it may represent normal variation associated with fasting. The microscopic vacuolization in the periportal hepatocytes seen in various groups of rats of both sexes may also be partially attributable to a variable degree of fasting prior to necropsy. The normal activity of coprophagy in the rat could also contribute to periportal hepatocellular vacuolization. Therefore, greater amounts of glycogen would still remain in the periportal hepatocytes. Whether the effects within the livers of male and female rats can be totally attributed to indirect nutritional effects is uncertain. It is likely that the response in the liver was due to nutritional considerations and the test chemical. The presence of the test chemical in their feed likely accentuated this effect in high dose males, and all dose group of females, with subsequent fatty change also being observed.

An alternative explanation for the observed liver fatty change is related to the known potential of a number of aliphatic alcoholic amines to cause a deficiency in the nutrient choline in rats and mice. AMP has been shown to disrupt phospholipid synthesis (Cosmetic Ingredient Review, 1990). As reviewed by Zeisel et al. (1995), choline requirements of pregnant rats in particular are substantially increased as the developing fetus is dependent upon maternal supplies of choline. The latter would explain the observed sensitivity of pregnant females to the liver fatty change relative to males. AMP-induced choline deficiency may also have accounted for the increased incidence of fetal resorbtions in the present study consistent with findings of resorptions in mice fed choline-deficient diets.

There were no histopathological lesions per se in the uterus. However, in light of the aforementioned effect on post-implantation loss, four sections of uterus were microscopically examined from each female rat to ascertain the presence of one or more implantation sites. In the control, low, and middle dose animals which delivered a litter, the uterus contained small numbers of individual pigment laden macophages within the deep layer of the endometrial stroma and the myometrium, with a large aggregation of these pigmented cells in the myometrium associated with the mesometrial attachment. This is the normal appearance of a post-partum uterus. In the remaining middle and high-dose females which had totally resorbed litters, the uteri did not have the typical microscopic findings of an implantation site usually located in the region of the myometrium associated with the mesometrial attachment. However, a clearly recognizable change was present in the endometrium likely associated with a previous implantation. Immediately beneath the uterine mucosa, within the endometrial stroma, there were increased numbers of pigment laden macrophages. In five high-dose females, the pregnancies were detected by microscopic examination of the uterus. Overall, the microscopic uterine findings in rats that did not litter were consistent with embryonic death and subsequent resorption.

Normal estrous cyclic activity in all the control and all treated female rats was observed microscopically in the tissues examined. Microscopic findings in the tissues examined from the adults did not reveal a likely mechanism for the observed effects on post-implantation loss. However, the fatty changes in the liver of all dose levels of female rats and the 1000

mg/kg/day males does suggest a possible effect on normal lipid and triglyceride metabolism. Whether similar effects may have been present in tissues of the rapidly developing fetus or involved in the normal formation of the placenta to maintain pregnancy was not determined in this study. Certainly the normal metabolism of lipids and triglycerides is a critical function for cellular membrane synthesis, and could have affected fetal viability. Endocrine glands were not microscopically examined in this study to determine whether a morphologic change was present.

Male reproductive organs and their accessory sex glands were unaffected by treatment. The microscopic observations in tissues other than liver and uterus, were not interpreted to be due to treatment with the test chemical.

Conclusion : Dietary exposure of male rats to 1000 mg/kg/day of AMP caused increases in absolute and relative liver weights, accompanied by a very slight degree of microvacuolization of periportal hepatocytes, with or without vacuolization of hepatocytes consistent with fatty change. Females in all treatment groups exhibited similar histopathological changes in the liver, but in the absence of an organ weight change. Absolute and relative kidney weights were increased in the 1000 mg/kg/day males, but these were not considered toxicologically significant due to the absence of histopathological changes. AMP had no effect on mating performance or conception, but caused marked, dose-related increases in post-implantation loss (embryo resorption). At the high dose level, all 12 pregnant females showed evidence of complete litter resorption (100% post-implantation loss), while at 300 mg/kg/day, post-implantation loss was 70% (vs. 10% in controls). Effects associated with, or secondary to the post-implantation loss increase at 300 mg/kg/day included decreased litter size, increased pup body weight, and decreased gestation body weight and body weight gain. There were no treatment related effects on reproductive performance in the 100 mg/kg/day group.

The no-observed effect level (NOEL) for general toxicity in males was 300 mg/kg/day, while the general toxicity NOEL for females could not be determined, based upon the presence of very slight microscopic liver effects. The NOEL for reproductive effects was considered to be 100 mg/kg/day.

Reliability : (1) valid without restriction
1 (GLP, guideline study)

Flag : Critical study for SIDS endpoint

18.09.2006 (47)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	other: CRL:CD(SD)
Route of admin.	:	dermal
Exposure period	:	Gestation Day 6-20
Frequency of treatm.	:	6 hours per day
Duration of test	:	15 days
Doses	:	0, 30, 100, 300 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 100
other: NOEL (developmental tox)	:	= 300
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	2006
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Groups of 26 time-mated female Crl:CD(SD) rats were administered AMP once daily by the dermal route at dose levels of 0, 30, 100, or 300

Field

mg/kg/day for approximately 6 hours from GD 6-20. Dose solutions were prepared in deionized water at concentrations of 30, 100, and 300 mg/ml and pH adjusted to approximately 9.5 with HCl. The solutions were administered at a dose volume of 1 ml/kg body weight in order to achieve the targeted dose levels. Dose volumes were adjusted daily based on individual body weights. Dose solutions were prepared weekly during the course of the study. Homogeneity and stability were determined prior to study initiation, and concentration of the dose suspensions was determined from the first mix.

Time-mated female rats were obtained from a commercial supplier. Sexually mature adult rats were 10-11 weeks of age and weighed approximately 200-250 grams at study start.

Each animal was evaluated by the laboratory veterinarian to determine the general health status and acceptability for study purposes upon arrival at the laboratory. Rats were housed one per cage in stainless steel cages, in rooms designed to maintain adequate conditions (temperature, humidity, and photocycle) and acclimated for at least three days. Prior to dosing animals were acclimated to the wrapping material for approximately 2, 4, and 6 hours on GD 3, 4, and 5, respectively.

Animals were housed one per cage in stainless steel cages in rooms designed to maintain adequate environmental conditions for the species. Animals were provided feed and municipal water ad libitum. Rats were stratified by GD 0 body weight and then randomly assigned to treatment groups using a computer program designed to increase the probability of uniform mean group weights and standard deviations at the start of the study. Animals that were placed on study were uniquely identified via subcutaneously implanted transponders (BioMedic Data Systems, Seaford, Delaware) that were correlated to unique alphanumeric identification numbers.

Sexually mature, adult virgin females were naturally mated with males of the same strain at the supplier's facility. Females were checked for in situ copulation plugs the following morning and those found with such a plug were removed from the males' cages. The day on which a vaginal plug was detected was considered GD 0. GD 0 body weights were provided by the supplier, and maintained in the study record. Rats arrived in the testing laboratory on GD 1 or 2.

A cage-side examination was conducted twice daily, at approximately the same time each day to detect significant clinical abnormalities that were clearly visible upon a limited examination, and used to monitor the general health of the animals. The animals were not hand-held for these observations unless deemed necessary. In addition, all animals were observed for morbidity, mortality, and the availability of feed and water at least twice daily.

Following removal of the wrapping material at the end of each daily six-hour exposure period, the test sites were graded for erythema, edema, scaling, and fissuring.

Body weights were recorded on GD 0 by the supplier, and daily during the dosing period, and at necropsy (GD 21).

Feed consumption were measured and statistically analyzed for the following intervals: GD 3-6, 6-9, 9-12, 12-15, 15-18, and 18-21.

On GD 21, all surviving females (not fasted) were euthanized by carbon dioxide inhalation and a limited gross pathologic examination (necropsy) was performed. The sequence of the maternal necropsies was

counterbalanced across groups (e.g., control, high, middle, low) to control for potential confounding influences of timing on fetal growth and skeletal ossification. The maternal necropsy included an examination of the external tissues and all orifices. The stomach, liver, and kidneys were dissected from the carcass and were incised. Any obvious gross pathologic alterations were recorded, and the weight of the liver, kidneys, and gravid uterus were recorded. The ratios of liver and kidney weights to terminal body weight were calculated. Representative sections of liver, kidneys, and gross lesions were preserved in neutral, phosphate-buffered 10% formalin. Microscopic examination of tissues was not conducted.

A detailed examination of the reproductive tract was performed and the number and position of implantations, viable fetuses, dead fetuses, and resorptions were recorded. Resorptions were classified as either "early" or "late" based on the presence (late resorption) or absence (early resorption) of grossly recognizable embryonic/fetal form, while a "dead fetus" would indicate a very recent death as evidenced by a lack external degenerative changes. For females with one or more viable fetuses, the number of ovarian corpora lutea were counted. The uteri of females lacking visible implantations were stained with a 10% aqueous solution of sodium sulfide (Kopf et al., 1964) and examined for evidence of early resorptions in order to verify pregnancy status.

The sex of all fetuses was determined and the body weight of all viable fetuses recorded. All fetuses were given an external examination that included observations on body proportions, the head and face (including closure of the palate), abdomen, spine, extremities, genitalia, rectum, and tail. At least one half of all the fetuses in each litter were chosen randomly via computer for visceral examination conducted by dissection under a low power stereomicroscope for evidence of visceral alterations. The visceral examination included observation of the thymus, trachea, esophagus, lungs, great vessels, heart (external and internal), liver, gastrointestinal tract, pancreas, spleen, kidney (sectioned), adrenal glands, ureters, bladder, and reproductive organs. The heads of these fetuses were removed, placed in Bouin's fixative and serially sectioned to allow for inspection of the eyes, brain, nasal passages and tongue. The remaining fetuses not selected for visceral examination were then skinned, eviscerated, preserved in alcohol and double stained with Alcian Blue and Alizarin Red S for cartilage and bone. After staining, skeletons were macerated and cleared. A thorough evaluation of the fetal skeleton was conducted on the remaining fetuses not selected for visceral examination.

All fetal alterations were classified as a variation or malformation. A variation is defined as a divergence beyond the normal range of structural constitution that may not adversely affect survival or health. A malformation is defined as a permanent structural change that may adversely affect survival, development or function and/or which occurs at a relatively low incidence in the specific species/strain. The fetal examinations were conducted such that investigators were blind to treatment group assignment.

Toxicokinetic Subgroup
The first four presumed pregnant females from each dose group were selected for blood collection to evaluate systemic exposure following dermal administration of AMP. On the last day of dosing (GD20), following approximately three hours of dermal exposure, the designated animals were anesthetized using isoflurane and blood was collected from the orbital sinus. Approximately 0.5 ml of blood from each animal was collected into heparinized tubes. The samples were submitted to the analytical chemistry department for analysis and were stored frozen at approximately -80 C. AMP present in the blood was derivatized with pentafluorobenzoyl chloride under basic conditions and extracted in toluene. Quantitation was

performed utilizing an isotopically labeled internal standard (D6-AMP) and matrix standards prepared in control blood. The limit of quantitation was 15 ng/g blood AMP.

STATISTICS AND CALCULATIONS
Maternal body weights, maternal body weight gains, organ weights (absolute and relative), fetal body weights, and feed consumption were evaluated by Bartlett's test (alpha = 0.01; Winer, 1971) for equality of variances. Based on the outcome of Bartlett's test, a parametric (Steel and Torrie, 1960) or nonparametric (Hollander and Wolfe, 1973) analysis of variance (ANOVA) was performed. If the ANOVA was significant at alpha = 0.05, analysis by Dunnett's test (alpha = 0.05; Winer, 1971) or the Wilcoxon Rank-Sum test (alpha = 0.05; Hollander and Wolfe, 1973) with Bonferroni's correction (Miller, 1966) were performed, respectively. Feed consumption values were excluded from analysis if the feed was spilled or scratched.

Frequency of pre- and post-implantation loss (calculations shown below), and fetal alterations were analyzed using a censored Wilcoxon test (Haseman and Hoel, 1974) with Bonferroni's correction. The number of corpora lutea, implantations, and litter size were evaluated using a nonparametric ANOVA (alpha = 0.05) followed by the Wilcoxon Rank-Sum test (alpha = 0.05) with Bonferroni's correction. Pregnancy rates were analyzed using the Fisher exact probability test (alpha = 0.05; Siegel, 1956) with Bonferroni's correction. Fetal sex ratios were analyzed using a binomial distribution test. Females lacking visible implantations at the scheduled necropsy were excluded from the appropriate analyses. Statistical outliers were identified, using a sequential method (alpha = 0.02; Grubbs, 1969), and if excluded, were excluded for sound scientific reasons. Both Dunnett's test and Bonferroni's correction correct for multiple comparisons to the control group to keep the experiment-wise alpha at 0.05. Both were reported at the experiment-wise alpha level.
Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data will consider statistical analyses along with other factors, such as dose-response relationships and whether the results are consistent with other biological and pathological findings and historical control values.

Remark : Test material was utilized at a pH of approximately 9.5, reflecting the highest pH of most personal care and industrial formulations containing AMP. The test material represents an equilibrium between the HCl salt of AMP and the nonionized base ("AMP-base"). As AMP-base is expected to be the primary form absorbed by skin, the test material in this study reflects the form of the material resulting in maximal exposure of humans from dermal contact with an AMP-containing product.

Result : In-Life Observations
Examinations performed on all animals during the course of the study revealed no treatment-related clinical findings. Dermal grading of the test site revealed a pattern of localized effects most pronounced in the 300 mg/kg/day dose group. The 300 mg/kg/day females showed significant dermal effects during the grading period, as compared to the other treatment groups. One 300 mg/kg/day female was found with very slight edema from GD 9-13. This finding was not observed in any other dose group. Almost all of the 300 mg/kg/day females (92%) were observed with slight scaling, with nine animals (36%) progressing to moderate to severe scaling during the last half of the dosing period. One, two, and seven females at 0, 30, and 100 mg/kg/day, respectively, were also found with slight scaling, but this was not considered adverse, as the incidence was relatively low, as compared to the 300 mg/kg/day group and the finding resolved prior to necropsy in three of the seven 100 mg/kg/day animals. In addition, no animals at the 30 and 100 mg/kg/day groups showed any signs of moderate to severe scaling. Scabbing was mainly observed at the

300 mg/kg/day dose level. Twenty 300 mg/kg/day females (77%) were noted with scabs at up to 25% of the test site. Over time, these the scabs progressed to include ever increasing areas of the test site, with one animal showing scabs covering most of the test site for the last week of dosing. The initial occurrence of scabbing varied considerably among the females, but there was a tendency for animals showing the greatest amount of scabs to have developed these lesions earlier. With the exception of one control animal, no other females were observed with any scabbing.

Overall, the dermal grading procedures revealed a treatment-related effect at the high-dose level of 300 mg/kg/day. The slight, transient scaling seen at lower dose levels was not considered to be toxicologically significant.

Body Weights/Body Weight Gains
There were no statistically identified differences in the mean gestation body weight for females at any dose level.

Feed consumption was increased at 100 mg/kg/day during GD 3-9 interval. These statistically identified parameters were not considered treatment related, but attributed to relatively low control values.

There were no statistically identified differences in mean terminal body weight, mean liver and kidney weights, or relative liver and kidney to body weight ratios in the treatment groups, as compared to the control. Gross pathological findings were spurious in nature and not attributed to treatment.

There were no significant treatment related effects on pregnancy rate, litter size, numbers of corpora lutea or implantations, percent preimplantation loss, fetal sex ratios, fetal body weights, or gravid uterine weights at any dose level. Mean percent postimplantation loss was statistically decreased in the 300 mg/kg/day group, but this was not considered to be test article-related, as the value was lower than seen in the control group. One female in the 30 mg/kg/day group was found to be non-pregnant, as confirmed by ammonium sulfide staining.

There were no statistically significant differences in the incidence of any fetal alteration (malformation or variation) in any of the treated groups, as compared to the control. There were no external fetal alternations observed. Any reported findings were sporadic in nature, and were not considered toxicologically relevant or treatment related.

Toxicokinetic Subgroup
Blood samples were obtained from four presumed pregnant females on the last day of dosing following approximately three hours of dermal application. The results indicate dermal absorption of AMP in a dose-responsive manner. The disproportionately higher mean blood concentration of AMP at 300 mg/kg/day may have been the result of a compromised skin barrier, as evidenced by significant dermal effects (scabbing and scaling) at the test site for that group. At this dose level, one female (animal # 7169) was noted with a blood value approximately three times that of the others. The increase in the blood value for this animal is likely the result of a greater degree of compromise to the skin barrier, as evidenced by a greater degree of scabbing (up to 50%) at the test site on the day of blood collection, as compared to the other animals in the group.

Conclusion : Dermal administration of 300 mg/kg/day of AMP produced significant effects at the test site, as evidenced by scabbing (77% affected) and moderate to severe scaling (35% affected). The dermal finding of slight scaling at 30 and 100 mg/kg/day was not considered adverse, as the observation was transient in nature and relatively low in incidence. There was no evidence of test article related systemic maternal or developmental

toxicity at any dose level tested. Under the conditions of this study, the NOAEL for maternal toxicity based on dermal effects was 100 mg/kg/day. The NOEL for developmental toxicity was 300 mg/kg/day, the highest dose level tested. Analyses of blood samples confirmed systemic exposure to AMP in a dose-responsive manner, although the study was not designed to quantify percent absorption.

Reliability : (1) valid without restriction
1 (GLP, guideline study)

Flag : Critical study for SIDS endpoint

07.09.2006 (48)

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	dermal
Exposure period	:	GD 6-20
Frequency of treatm.	:	daily
Duration of test	:	6 hours per day
Doses	:	0, 100, 300 or 400 mg/kg/day
Control group	:	yes, concurrent vehicle
Method	:	
Year	:	2005
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : The purpose of this study was to make a preliminary evaluation of the maternal toxicity and embryo/fetal lethality potential of 2-methyl-2-aminopropanol hydrochloride (AMP-HCl) in Crl:CD(SD) rats following repeated dermal administration. Groups of eight time-mated female rats received AMP-HCl by occluded dermal application, six hours/day at targeted dose levels of 0, 100, 300 or 400 mg/kg/day on gestation days (GD) 6-20. In-life parameters evaluated for all groups included clinical observations, body weight, body weight gain, feed consumption, and dermal grading of the test site. Blood samples were collected approximately three hours after dermal application, from 2-3 rats/group on GD 20 to evaluate dermal absorption of AMP-HCl. On GD 21, all surviving rats were euthanized and examined for gross pathologic alterations. Liver and kidney weights, the number of corpora lutea, implantations, resorptions, and live/dead fetuses were recorded.

Result : Dermal administration of AMP-HCl at dose levels of 100, 300 and 400 mg/kg/day resulted in no maternal toxicity and no indication of adverse embryo/fetal effects. Administration of the test material by the dermal route was well tolerated by all animals, without significant adverse dermal effects. Analyses of blood samples taken on the last day of dosing indicated a dose-responsive increase in systemic exposure, although the study was not designed to quantify percent absorption. Based on these results, the no-observed-effect level (NOEL) for maternal and embryo/fetal development was 400 mg/kg/day, which was the highest dose level tested. As no significant adverse dermal effects were noted in the developmental toxicity probe study, a two-week dermal follow-up study using male and non-pregnant female rats was initiated (Phase II). Solubility issues were encountered with AMP-HCl when it was prepared again using the hydrochloride salt. As a result, the base form of AMP was used in the prepration of the dose formulations for this study, and this alleviated the difficulties with solubility. AMP base (pH 9.5) at 300, 400 and 500 mg/kg/day was administered once daily, by the dermal route to the animals. Significant adverse dermal effects (scabbing, redness, tenderness to touch) were noted at the two highest dose levels (400 and 500 mg/kg/day) after a few days of dosing. This resulted in the immediate termination of treatment to these two dose groups. However, the low-dose of 300 mg/kg/day showed only slight dermal effects after 11 days of dosing and was, therefore, used as the high-dose level for the definitive

developmental toxicity study.

Based on the results obtained from this follow-up work and the developmental toxicity probe study, dose levels of 0, 30, 100, or 300 mg/kg/day of AMP base were selected for a definitive dermal developmental toxicity study in rats.

Reliability : (1) valid without restriction
1 (GLP study of acceptable quality with documentation sufficient for assessment)

07.09.2006 (49)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

Type : 2-Generation
In Vitro/In Vivo : In Vivo
Species : Rat
Sex : Male/Female
Strain : Sprague-Dawley
Route of admin. : oral gavage
Exposure period : 10 weeks prior to breeding and continuing through breeding (two weeks), gestation (three weeks) and lactation (three weeks) for each of two generations. F1 pups were exposed indirectly through milk and by gavage beginning at weaning.
Frequency of Treatm. : Once daily, 7 days/week
Duration of Test :
Doses : 0, 20,60, and 200 ppm
Control group : yes, concurrent vehicle
NOAEL : Endpoint: NOEL (Systemic Toxicity)
Generation: P
Sex: male/female
Effect Level: 20 mg/kg bw/day (actual dose received) (18 mg/kg bw/day as AMP)

Endpoint: NOEL (Reproductive Toxicity)
Generation: P
Sex: male/female
Effect Level: 60 mg/kg bw/day (actual dose received) (54 mg/kg bw/day as AMP)
Basis for effect Level/Remarks: Due to slighlty increased post-implantation loss at 200 mg/kg/day

Endpoint: NOEL (Systemic Toxicity)
Generation: F1
Sex: male/female
Effect Level: 20 mg/kg bw/day (actual dose received) (18 mg/kg bw/day as AMP)

Endpoint: NOEL (Reproductive Toxicity)
Generation: F1
Sex: male/female
Effect Level: 60 mg/kg bw/day (actual dose received) (54 mg/kg bw/day as AMP)
Basis for effect Level/Remarks: Due to slighlty increased post-implantation loss at 200 mg/kg/day
Result : Post-Implantation Loss
Method : OECD Guideline 416 (Two Generation Reproduction Toxicity Study)
Year : 2008
GLP : Yes
Test Substance : Test material: Biocide CS1135 breaks down in the gastric environment to produce formaldehyde and 2-amino-2-methylpropanol.
Result : **Details on results (parental animals)**

Clinical Signs And Mortality (Parental Animals)
Treatment-related clinical observations in the P1 animals included one high-dose male that was euthanized in moribund condition. Necropsy revealed marked stomach ulceration and inflammation in the nasal region as contributors to the moribund condition of this rat. Gastric irritation due to release of formaldehyde from the test material likely caused this treatment-related effect.

Test Substance Intake (Parental Animals)
Analyses of dose solutions for concentration verification were conducted on four different occasions during the study, representing all dose levels, sexes and major study phases. The mean concentrations of CS-1135 in the vehicle for all analyses ranged from 85.8 to 105.9% of the targeted concentrations. The overall mean concentration for the entire study ranged from 92.3 to 95.9% of the targeted concentrations. Analyses also confirmed that the test material was homogeneously distributed in the vehicle with relative standard deviations ranging from 0.5 to 4.7%.

Organ Weights (Parental Animals)
The absolute and relative liver weights of P1 males and females given 200 mg/kg/day were heavier than the controls, statistically identified, outside of the historical control range and were interpreted to be treatment-related. These higher liver weights were associated with microscopic altered staining of hepatocytes and vacuolization consistent with fatty change in males and females given 200 mg/kg/day. The absolute and relative liver weights of P1 males given 60 mg/kg/day were outside of the historical control data, but were not statistically identified. These higher liver weights were accompanied by treatment-related microscopic liver effects and were interpreted to be treatment-related. The liver weights of females given 20 or 60 mg/kg/day were not affected by treatment. The absolute and relative kidney weights of P1 males given 200 mg/kg/day were higher than the controls. Although only the increase in absolute kidney weight was statistically identified, both the absolute and relative kidney weights were outside of the historical control range and were interpreted to be treatment-related. These higher kidney weights were not associated with any microscopic treatment-related effects in the kidneys and may reflect an adaptive change associated with the excretion of the test material in the urine.

Gross Pathology (Parental Animals)
The only organ affected by treatment was the nonglandular stomach. The nonglandular stomach was thickened in the majority of males and females given 200 mg/kg/day. In addition, a few females in this group also had an erosion or ulcer in the glandular mucosa. These treatment-related effects were confined to rats in the 200 mg/kg/day group and were suggestive of an adaptive response to irritation associated with the presence of formaldehyde as a degradation product of the test material.

As was observed in the P1 adults, the only organ affected by treatment in the P2 adults was the stomach. The nonglandular stomach was thickened in the majority of the males and females given 200 mg/kg/day. Two males and one female in this dose group also had erosions or ulcers in glandular mucosa of the stomach.

Histopathology (Parental Animals)
The primary treatment-related effect was observed in the stomachs of males and females given 200 mg/kg/day, and to a lesser extent in females given 60 mg/kg/day. These stomach effects were likely due to point of contact irritation, associated with the presence of formaldehyde as a degradation product of the test material. In the nonglandular stomach the effects were primarily characterized by hyperkeratosis and hyperplasia of the mucosa. In females the nonglandular effects were more focal and

primarily affected the limiting ridge of the stomach. In addition, this minimal effect in the limiting ridge also affected several more rats in the 60 mg/kg/day rats than was observed in the low dose or control groups. This portion of the stomach is normally lined by squamous epithelium and this effect was interpreted to be an adaptive response to an irritant material. This was the region of the stomach with the grossly recognizable changes noted during necropsy.

The glandular stomachs of P1 males and females given 200 mg/kg/day were also significantly affected in a number of animals and interpreted to be reflective of contact with an irritant material. Hyperplasia of the glandular mucosa of the stomach was evident in the majority of P1 males and females given 200 mg/kg/day, with the effect being slightly more severe in females than males. In addition, some of the rats given 200 mg/kg/day had one or more mucosal erosions and ulcers with an associated inflammation and edema in the mucosa or submucosa of the glandular region of the stomach. In the 60 mg/kg/day group, a single female also had a mucosal erosion with edema in the submucosa of the glandular stomach. This rat also had acute, focal, glandular inflammation in the mucosa. Several more females given 60 mg/kg/day had subacute to chronic inflammation in the glandular submucosa, that was either focal or multifocal, and very slight in degree, when compared to the incidences in the control or 20 mg/kg/day group. The erosions, ulcers, edema, hyperplasia and inflammatory infiltrates primarily involved the fundic portion of the glandular stomach.

The microscopic findings in the liver of males and females given 60 or 200 mg/kg/day were considered treatment-related. The microscopic findings were characterized by 1) altered tinctorial properties; increased eosinophilia; hepatocyte; centrilobular of a very slight or slight degree; and 2) vacuolization consistent with fatty change, hepatocyte; individual cells; multifocal, very slight. All of the other microscopic findings in the remaining tissues were spontaneous occurrences or associated with a gavage incident.

The primary treatment-related effect was observed in the stomachs of P2 males and females at 200 mg/kg/day. As in the P1 generation, these stomach effects were likely due to point of contact irritation, associated with the presence of formaldehyde as a degradation product of the test material. In contrast to the minimal effect observed in the P1 60 mg/kg/day female stomach, this dose group was not affected in the P2 group. The responses in nonglandular and glandular portions of the stomach were identical to those in the P1 males and females with only slight numerical differences.

The microscopic findings in the liver which appeared to be treatment-related were the same in P2 males and females as in P1 rats. Vacuolization and altered tinctorial properties of the hepatocytes were the same. The changes in both were apparent in males at 60 and 200 mg/kg/day. Several more males had a slight grade of vacuolization in these groups in contrast to a very slight grade in P1 males. In the P1 females there were fewer control and 20 mg/kg/day rats with vacuolization of hepatocytes in contrast to the P2 groups. Therefore, the liver in P2 60 mg/kg/day females was unaffected. In the male P2 livers there was a slight increase in the number of rats with multifocal, and slight degree of aggregates of macrophages as histocytes. The increased kidney weights and adrenal weights were not associated with any microscopic findings due to the test chemical. All of the other microscopic findings observed in other tissues were considered normal spontaneous events or associated with changes secondary to gavage accidents. Histologic examination of the reproductive organs of animals with signs of reduced fertility did not reveal any effect of treatment

There were no treatment-related or statistically-identified differences in the mean number of small and growing ovarian follicles in females given 200

mg/kg/day as compared to the control females.

Other Findings (Parental Animals)
There were no effects of treatment at any dose level on mating, conception, fertility or gestation indices, time to mating, gestation length, or pup sex ratio in either generation.

There was a statistically identified increase in percent postimplantation loss (percentage of live born pups relative to total uterine implantation sites) in the mid- and high-dose group of the P1 litter, and the high-dose group of the P2 litter. The increase in postimplantation loss was considered treatment-related for the P1 and P2 high-dose litters because of the statistical significance and because the values were outside of the historical control ranges. The statistically identified increase in postimplantation loss of the mid-dose P1 litter was considered to be a spurious finding unrelated to treatment because the value was within the historical control range, was not present in the P2 litter at this dose level, and was largely driven by one dam with clear signs of maternal toxicity.

Details on results (offspring)

Viability (Offspring)
There were no effects of treatment at any dose level on mating, conception, fertility or gestation indices, time to mating, gestation length, or pup sex ratio in either generation.

In the P1 generation, there was a slight, but not-statistically identified, decrease in gestation survival index in the 200 and 60 mg/kg/day dose groups (96.0 and 95.5%, respectively) compared to controls (99.7%). Animals in the 200 mg/kg/day dose group also had slight, but not-statistically identified, decreases in PND 1 and 4 survival indices (96.5 and 94.8%) compared to controls (99.5 and 98.7). Because these values were not statistically significant, were near or just slightly outside the historical control range, and were not reproduced in the second generation, they were deemed spurious and unrelated to treatment.

There was a statistically identified increase in percent postimplantation loss (percentage of live born pups relative to total uterine implantation sites) in the mid- and high-dose group of the P1 litter, and the high-dose group of the P2 litter. The increase in postimplantation loss was considered treatment-related for the P1 and P2 high-dose litters because of the statistical significance and because the values were outside of the historical control ranges. The statistically identified increase in postimplantation loss of the mid-dose P1 litter was considered to be a spurious finding unrelated to treatment because the value was within the historical control range, was not present in the P2 litter at this dose level, and was largely driven by one dam (6158, see Clinical Observations section) with clear signs of maternal toxicity.

Organ Weights (Offspring)
There were no treatment-related alterations in organ weights of F1 or F2 weanlings at any dose level. See "Observations: parental animals" for results on organ weghts of adult P2 animals

Gross Pathology (Offspring)
There were no treatment-related gross pathologic observations. See "Observations: parental animals" for results on gross pathology of adult P2 animals

Histopathology (Offspring)
See "Observations: parental animals" for results on gross pathology of adult P2 animals.

Reliability	:	1 (reliable without restriction)
9.11.2010	:	(50)
Type	:	other: dietary toxicity
In vitro/in vivo	:	In vivo
Species	:	dog
Sex	:	male/female
Strain	:	Beagle
Route of admin.	:	oral feed
Exposure period	:	1 year
Frequency of treatm.	:	continuous
Duration of test	:	
Doses	:	0, 1.1, 11.0, 110.0 ppm
Control group	:	yes, concurrent vehicle
Result	:	No effect on reproductive organs
Method	:	
Year	:	1990
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Result :

There were no general in-life observations made for any treated or control animal. There is no statistically significant differences in body weights for any of the animals throughout the study.

There were no effects on organ weights or organ/body weight ratios that were attributed to test material administration by the authors. Reproductive organs that were examined included: testes, ovaries, and uteri.

At necropsy, evaluation of the tissues revealed isolated observations, none of which were attributed to the administration of the test material. There were no neoplastic observations in any of the dogs sacrificed at either 6 months or one year of administration in their diets.

Reliability	:	(2) valid with restrictions 2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)
01.06.2006		(36)
Type	:	other: dietary toxicity
In vitro/in vivo	:	In vivo
Species	:	dog
Sex	:	male/female
Strain	:	Beagle
Route of admin.	:	oral feed
Exposure period	:	90 days
Frequency of treatm.	:	continuous
Duration of test	:	90 days
Doses	:	
Control group	:	
Method	:	
Year	:	1981
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Result :

Physical Observations
Included emesis, loose stool, alopecia, and slight penile or vaginal discharge. These findings occurred sporadically in the control and/or treated animals and are not considered by the authors to be treatment-related.

Urinalysis

There is no evidence of a compound-related effect on any of the urinalysis parameters studied.

Reproductive organs evaluated: Testes (without epididimus), ovaries, and uterus.

Organ Weight/ Organ:Body Weight Ratios
Organ weight and organ/body weight differences were marginal and appear unrelated to the administration of the test material.

Pathology
There were no observations noted in any reproductive organ upon gross and microscopic evaluation.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

01.06.2006 (35)

Type : other: dietary toxicity
In vitro/in vivo : In vivo
Species : rat
Sex : male/female
Strain : other: CD
Route of admin. : oral feed
Exposure period : 8 weeks
Frequency of treatm. : continuous
Duration of test : 8 weeks
Doses : 1000, 2000, 4000, 8000, 16000 ppm
Control group : yes, concurrent vehicle
Method :
Year : 1976
GLP :
Test substance : as prescribed by 1.1 - 1.4

Result : Rats in the 16000ppm group gained slightly less weight as compared to control rats. Gains in the other dose groups were similar to the control. Food consumption patterns were similar for all animals.

At necropsy, all 16000ppm surviving rats, and all rats at the 8000ppm dose level were necropsied. Two males and two females from each of the other groups were also examined. Alopecia and focal skin erosions and ulcerations found in rats at the 16000ppm dose level were viewed as possibly compound related. Hepatocyte vacuolation descrived in rats from all the treated groups, with increasing severity at the higher dose levels, was considered compound related. Microscopic skin lesions found at the 16000ppm dose level, like the corresponding gross lesions, were viewed as possibly compound related.

There were no gross or microscopic observations noted in epididimus, uteri, ovaries, or testes.

Reliability : (2) valid with restrictions
2e

01.06.2006 (37)

5.8.4 DEVELOPMENTAL TOXICITY, OTHER STUDIES

Type : Range finding study
In vitro/in vivo : In Vivo
Species : Rat
Sex : Females
Strain : Sprague-Dawley
Route of admin. : Oral: gavage
Exposure period : Gestation days 6-15

Frequency of treatm.	:	Once per day 7 days/week on gestation days 6-15 inclusive
Duration of test	:	
Doses	:	0, 250, 500, 750, 1000, 1500 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL	:	Endpoint: NOEL Effect type: developmental toxicity Effect level: ca. 500 mg/kg bw/day (nominal) Basis for effect level / Remarks: Total maternal mortality in higher dose groups precluded assessment of uterine data at dose levels >500 mg/kg/day
Method	:	Dose range-finding study for definitive OECD 414
Year	:	1989
GLP	:	Yes
Test substance	:	Test material: Biocide CS-1135 This breaks down in the gastric environment to produce 10% formaldehyde and 90% 2-amino-2-methylpropanol
Method	:	According to OECD 414 (Prenatal Developmental Toxicity Study)
Result	:	**Details on maternal toxic effects** All animals given 750 mg/kg/day or higher died between GD 6 and GD 9. One animal given 500 mg/kg/day died on GD 14. Prior to death, the following observations were noted: tremors, prostration, lethargy, rales, shallow respiration, paleness, salivation, decreased body temperature, lacrimation, and dried perioral soiling. There were no adverse signs of toxicity noted in animals at 250 mg/kg/day. Dose-related mean body weight losses and decreased body weight gains occurred during the initial 3 days of dosing in the 250 and 500 mg/kg/day groups. Body weight gain following the treatment period (GD 16-20) was comparable to the control group at the 250 mg/kg/day dose level and was slightly decreased at the 500 mg/kg/day dose. Total mortality by GD 9 at dose levels of 750 mg/kg/day and higher precluded further assessment of body weight data. Necropsy findings of the stomach were the most prevalent in victims; reddened or thickened stomach mucosa was the most frequent, followed by, less frequently, gaseous stomach, reddened and enlarged adrenals, reddened lungs, reddened medullary area in the kidney, clear or gelatinous material in the stomach and hemorrhagic thymus. In survivors, there were three incidences of dilated renal pelvis (one at 250 mg/kg/day, and two at 500 mg/kg/day). One animal in the 250 mg/kg/day group had a distended ureter. **Details on embryotoxic / teratogenic effects** Mean numbers of viable fetuses, early and late resorptions, implantation sites, and corpora lutea in animals given 250 and 500 mg/kg/day were comparable to the controls. Total maternal mortality in higher dose groups precluded further assessment of uterine data. There was no indication of embryotoxicity at dose levels of 250 or 500 mg/kg/day **Embryotoxic / teratogenic effects** no effects
Reliability	:	(1) valid without restriction

11.09.10 (51)

Type	:	Other: OECD 414
Species	:	Rabbit
Sex	:	Female
Strain	:	New Zealand White
Route of Admin.	:	Oral: gavage
Exposure Period	:	Days 7-27 of gestation.
Frequency of Treatm.	:	Once per day on day 7 through 27 of gestation inclusive.
Duration of test	:	11 days

Doses	:	0, 4, 12, 40 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL	:	Endpoint: NOEL Effect type: maternal toxicity Effect level: 12 mg/kg bw/day (nominal) Basis for effect level / Remarks: decreased body weight gain, decreased feed consumption and increased incidence of watery contents of the cecum or gastrointestinal tract. Endpoint: NOAEL Effect type: developmental toxicity Effects level: 40 mg/kg bw/day (nominal) Basis for effect level/Remarks: statistically identified increase in paraovarian cysts (variation) in the fetuses of dams from 40 mg/kg/day group.
Method	:	OECD Guideline 414 (Prenatal Developmental Toxicity Study)
Year	:	2008
GLP	:	Yes
Test Substance	:	Test material: Biocide CS-1135 This breaks down in the gastric environment to produce 10% formaldehyde and 90% 2-amino-2-methylpropanol
Result	:	**Details on maternal toxic effects** Mean body weights for all treated groups were not significantly different from controls throughout the duration of the study. However, animals in the 40 mg/kg/day dose group had a clear, treatment-related, 19.3% decrease in mean body weight gain from GD 7-28, relative to controls. This effect was largely attributable to a 79% decrease in body weight gain from GD 24-28, which correlated with decreased food consumption during this period and was partly driven by body weight losses in two animals (1906 and 1916). There were no treatment-related effects on body weight gain in the 4 or 12 mg/kg/day dose groups. In the 40 mg/kg/day dose group, feed consumption during the last week of gestation, tended to be lower than controls, with the decreases being statistically identified GD 25-28. This decrease was concomitant with body weight loss and/or decreased body weight gain during this period. **Details on embryotoxic / teratogenic effects** There was also a treatment-related, statistically identified increase in paraovarian cysts (variation) in the fetuses of dams from this group. This finding was deemed to be of no toxicological significance due to the high prevalence in adult rabbits, the lack of any apparent parenchymal involvement, and the absence of any accompanying developmental effects. There were no treatment-related maternal or developmental effects any dose group. Conclusions Based on these findings the maternal no-observed-effect-level (NOEL) was considered to be 12 mg/kg/day, while the developmental toxicity no-observed-adverse-effect-level (NOAEL) was 40 mg/kg/day.
Reliability	:	**(1)** (1) valid without restriction
9.11.2010	:	**(52)**

5.9 SPECIFIC INVESTIGATIONS

Type	:	Similar to OECD 421 guideline study
Species	:	Rat
Sex	:	male and female
Strain	:	Crl:CD(SD)
Number of animals	:	Experiment 1: 6-7 females/group Experiment 2: 7-8 females/group
Route of Admin.	:	Dietary
Exposure Period	:	Experiment 1: Females were dosed for 14 days prior to mating, continuing

through mating (up to two weeks) and through GD 8, GD 10 or GD12. Male rats were not exposd to AMP-HCL except while co-housed with females during the breeding period.
Experiment 2: Two weeks prior to breeding, continuing through breeding (up to two weeks), and through GD 14.

Frequency of Treatm. : Continuous: dietary

Duration of test : Experiment 1: Two weeks prior to breeding, through breeding (2 weeks) and up to GD 8, GD 10 and GD 12.
Experiment 2: Two weeks prior to breeding, through breeding (2 weeks) and through GD 14.

Doses : 300 mg/kg/day

Control group : Control group: Test diet supplemented with 1800 ppm choline

Year : 2009

GLP : Yes

Test Substance : AMP-HCl

NOAEL :

Method : Experimental Design

Experiment 1: Establishment of the timing of AMP-HCl-induced postimplantation loss

Hypothesis: There is a critical gestational period between GD 6 – 14 in which AMP-HCl causes embryonic resorptions.This was a pilot study to identify the timing of embryonic resorptions and provide a preliminary evaluation of the changes in phospholipid precursors in the dam, visceral yolk sac (VYS), placenta and embryo following AMP-HCl exposure. Determination of the timing of embryonic resorptions was critical for all future studies (including Experiment 2) that necessitated evaluation of parameters in viable embryos and VYS. Three groups of six or seven females were fed diets containing 300 mg AMP-HCl/kg/day for 2 weeks prior to breeding, continuing through breeding (up to two weeks), and up to GD 8, 10 or 12. At necropsy, maternal plasma, maternal liver, the VYS and embryos (GD 10 and 12 only) were collected primarily for phospholipid precursor analytical methods development. Uterine implantation sites were counted and embryo viability was assessed. VYS blood circulation and vasculature development were also rated on GD 10 and 12. Any gross morphological abnormalities in the embryos or VYS were also noted.

Experiment 2: Phospholipid precursor involvement in AMP-HCl-induced postimplantation loss Hypothesis:

Depletion of choline and/or other phospholipid precursor levels in AMP-HCl treated dams causes embryonic resorptionsAll animals received the specified test diets for two weeks prior to breeding, continuing through breeding (up to two weeks), and through GD 14. At necropsy on GD 14, maternal plasma and liver samples were collected for analysis of choline and several phospholipids. Uterine implantation sites were recorded as either a live embryo or a resorption. If present, embryos were assessed for viability and presence of gross morphological abnormalities in the embryos or VYS were noted. Corpora lutea were also counted.

DIET PREPARATION-

Experiment 1: Animals were provided Lab Diet Certified Rodent Diet #5002(PMI Nutrition International, St. Louis, Missouri) in meal form. Calories in the 5002 diet, provided by PMI, were: protein (24.1%), fat (13.2%) and carbohydrate (62.7%).

Experiment 2: Animals were provided Mod TestDiet AIN-93M (PMI Nutrition International, St. Louis, Missouri) in meal form with the

concentration of choline chloride within the experimental group identified below.

Groups 1 and 3: Control and AMP-HCl treated animals were provided the Mod Test Diet AIN-93M in meal form, containing 1800 ppm choline chloride (product # 5T5J).

Group 2: Animals were provided the Mod Test Diet AIN-93M in meal form, containing 507 ppm choline chloride (product # 5T4D).

Group 4: Animals were provided the Mod TestDiet AIN-93M in meal form, containing 9000 ppm choline chloride (product # 5T4E).

Result : In-Life Observations

Cage side observations revealed no notable observations during the course of either experiment (data in study file). There were no clinical observations in Experiment 1 or 2 that bore any relation to treatment.

Body Weights/Body Weight Gains

Experiment 1: All rats gained weight during the experiment. Based upon the lack of a concurrent control group in this preliminary experiment, body weight and body weight gain could not be evaluated for treatment-related findings.

Experiment 2: There were no treatment-related effects on female mean body weight or body weight gain in any group during the pre-mating or gestation periods compared to controls.

Feed Consumption

Experiment 1: Based upon the lack of a concurrent control group in this experiment, food consumption could not be evaluated for treatment-related effects. In general, AMP-HCl treated animals consumed similar amounts of feed as control animals from Experiment 2.

Experiment 2: There were no treatment-related effects on feed consumption in any treated group during the pre-mating or gestation periods, when compared to controls.

Reproductive Parameters

Experiment 1: All study females had observable implantation sites (evidence of pregnancy) at the time of necropsy. The mean number of implantations per litter in GD 8, 10 and 12 dams was 18.8, 17.7 and 16.3, respectively. The mean percent postimplantation loss in GD 8, 10 and 12 dams was 75.9, 53.1 and 44.8%, respectively and demonstrates general concordance with values (67-70%) from previous studies. Variability in the data was most likely related to the small sample size (n=6) employed in this study and is not a meaningful difference. Four litters were completely resorbed (two litters in the GD 8 group, one litter each in the GD 10 and GD 12 groups). The remaining 15 litters exhibited varying degrees of partial loss, which were equally distributed across time points. The finding that mean percent postimplantation loss was highest on GD 8, and that the number of implantations/dam was not reduced compared to the Experiment 2 control rats, indicates that embryonic death and subsequent resorption occurred between GD 6 (day of implantation) and GD 8 in dams treated with 300 mg AMP-HCl/kg/day. Morphological assessment of surviving embryos on GD 10 and 12 indicated that the embryos appeared normal for gestational age and their associated visceral yolk sacs had normal blood circulation and vasculature development.

Experiment 2: Reproductive data are summarized in Table 10. Consistent with the results from Experiment 1, mean percent postimplantation loss was increased in dams exposed to 300 mg/kg/day of AMP-HCl (Group 3) compared to controls (Group 1) (66.3±41.2% versus 5.0±5.2%). Other reproductive parameters in Group 3 dams that highlight this effect include increased resorptions per litter (11.0 versus 0.8 in controls), and decreased embryo viability per litter (5.0 versus 14.2 in controls). Two litters from AMP-HCl treated dams (Group 3) were completely resorbed and the remaining four litters exhibited varying degrees of partial loss.

Administration of a choline-deficient diet (507 ppm choline chloride, Group 2) did not adversely affect embryonic survival, resulting in an outcome similar to controls for the following endpoints: mean percent postimplantation loss, mean number of resorptions per litter and mean number of viable embryos per litter. Mean percent preimplantation loss in this group was higher than controls (25.0±8.5 versus 11.6±5.6). This finding was deemed spurious and attributable to the summation of nonstatistically significant increases in the number of corpora lutea and viable embryos, as well as a decrease in the resorption rate in this group, relative to the other groups.

Supplementation with 9000 ppm dietary choline in AMP-HCl treated dams (Group 4) likely resulted in a partial amelioration of embryo viability compared to the AMP-HCl group (Group 3). Mean percent postimplantation loss was 27% lower than AMP-HCl in control diet (39.2 versus 66.3% in Group 3). Other parameters which highlight the partial amelioration effect of choline supplementation in AMP-HCl treated dams (Group 4) include: a 38% decrease in the mean number of resorptions per litter (6.8 versus 11.0 in Group 3 dams), a corresponding near two-fold higher mean number of viable embryos per litter (9.9 versus 5.0 in Group 3 dams), and a lack of complete litter loss (0% versus 28% in Group 3 dams). Despite the reduction in embryonic resorptions in the AMP-HCl choline rescue group (Group 4) compared to AMP-HCl treatment alone (Group 3), the numbers did not reach statistical significance. Although the sample size was small, these results indicate that postimplantation loss induced by AMP-HCl was not due to a simple choline deficiency; however, the ability of choline to partially ameliorate the postimplantation effect suggests that some other aspect of phospholipid biochemistry could be involved in the mode of action.

Plasma AMP Analysis

Analysis of plasma AMP concentrations was performed from blood collected at necropsy. Although there was variability (range 3.07-13.0 µg/g plasma) among animals in the AMP and AMP-choline rescue group, the mean values were similar (8.26± 2.60 and 7.49 ± 3.14 µg/g plasma). There was no correlation between plasma AMP concentration and degree of postimplantation loss; however, this lack of correlation may be confounded by the time delay between embryonic resorptions (between GD 6-8) and collection of plasma at necropsy (GD 14).

Phospholipid Analysis

This analysis was conducted on GD 14 maternal liver tissues from Experiment 2, thereby allowing these results to be directly comparable to previous CDP-Choline phospholipid precursor analysis following AMPHCl exposure. Due to the small mass of VYS and embryos at the gestational time points in Experiments 1 and 2, and high percentage of resorptions, sufficient tissue amounts were not available to conduct the phospholipid precursor analysis on these samples.

In the current study, AMP-HCl exposure (Group 3) resulted in increases of choline (1.8- fold) and glycerophosphocholine concentrations (1.9-fold), compared to controls (Group 1), the latter reaching statistical significance and deemed treatment-related. These data are similar to that of another study in 2006 in which increases of choline (1.3-fold) and glycerophosphocholine (2.7-fold) concentrations were observed, though neither reached statistical significance. In addition, phosphatidylcholine concentrations were similar between control and AMP-HCl groups in both studies. The current data, however, differ from the 2006 study with respect to phosphocholine concentrations, which were comparable to controls in the current study, but significantly decreased (22%) in the 2006 study.

Livers from animals given the choline deficient diet (507 ppm choline, Group 2) had phospholipid precursors levels comparable to the control group (Group 1). Supplementation of choline in AMP-HCl treated dams (Group 4) resulted in a partial normalization of maternal liver choline (19.9 ± 5.3 versus 14.0 ± 2.9 µg/g) and a complete normalization of glycerophosphocholine (31.2 ± 6.2 versus 28.2 ± 6.5 µg/g) concentrations similar to control dams. Phosphocholine and phosphatidylcholine concentrations in the choline supplemented group (Group 4) were similar to controls.

These phospholipid precursor analyses demonstrate that prolonged AMP-HCl exposure does not deplete maternal liver stores of components in the CDP-choline pathway. The toxicologic significance of the increases in maternal liver choline and glycerophosphocholine, after AMP-HCl exposure, with respect to postimplantation loss cannot be elucidated from this study because of the delay between embryonic death (determined to be GD 6-8 from Experiment 1) and tissue isolation at necropsy (GD 14) and, secondly, it remains unclear if components of the CDP-choline pathway are similarly altered between the liver and intrauterine environments.

CONCLUSIONS

In Experiment 1, groups of six to seven dams exposed via the diet to 300 mg/kg/day AMP-HCl starting two weeks prebreeding (PB -14), were evaluated for embryo viability following continuous exposure through GD 8, 10, or 12. Mean percent postimplantation loss was 75.9% in the GD 8 dams, which was similar to GD 14 data in previous studies (67-70%). These data indicated that postimplantation loss occurred at a time between implantation (GD 6) and GD 8. Postimplantation loss in the GD 10 and 12 dams was 53.1 and 44.8%, respectively. This lower postimplantation loss at GD 10 and 12 was attributed to the small sample size in this study and does not indicate a reduction in treatment effect at these time points.

The results of Experiment 2 indicated that postimplantation loss induced by AMP-HCl was not due to a simple choline deficiency; however, the ability of choline to partially ameliorate the postimplantation effect suggests that some other aspect of phospholipid biochemistry could be involved in the mode of action.

Reliability : 1 (reliable without restriction)
9.11.2010 : (53)

Type : In Vivo
Species : Rats
Sex : Males and females
Strain : Crl:CD(SD)
Concentration :
Dose : 0, 300 mg/kg bw/day
Rout e of admin. : Diet

No. of Animals	:	15 females/group
Vehicle	:	
Exposure Period	:	Two weeks prior to breeding, through breeding (two weeks), and through GD6.
Frequency of Treatm.	:	continuously in the diet
Year	:	2010
GLP	:	Yes
Test Substance	:	as prescribed by 1.1 - 1.4
Method	:	Groups of 15 female Crl:CD(SD) rats were administered 0 or 300 mg/kg/day AMP-HCl by diet for two weeks prior to breeding, through breeding (two weeks), and through GD 6. Male rats were not exposed to AMP-HCl except while co-housed with females during the breeding period. During the exposure period, female cage-side and clinical observations, body weight, body weight gain, and feed consumption were recorded. Data recorded from males were limited to daily cage-side observations. Animals were sent to necropsy on GD 6 where the uterus and liver were weighed. Histopathologic evaluation of the uterus was conducted. In addition, liver, uterus, and blood were collected and analyzed according to a tiered scheme. In the Tier I experiments, the phospholipid profile of the liver and blood was analyzed and blood was also used for clinical chemistry measurements and as serum for WEC. Tier II consisted of gene expression analysis of frozen liver and uterine tissue by microarrays, and histopathology of the uterus focused on the site of implantation. Whole embryo culture: Embryos (20/group) were explanted from untreated rats on GD 9 and cultured for approximately 48 hours in control or AMP-HCl exposed dam serum from Tier I of the study. After 24 hours, the embryos were visually inspected for any notable gross developmental or physical abnormalities, as well as verification of a heartbeat. On completion of the 48-hour exposure, the embryos were given a semi-quantitative assessment of the degree of growth and differentiation.
Result	:	Tier I Phospolipid Analysis There were no alterations in the amount of choline, phosphocholine, glycerophosphocholine, or phosphatidylcholine in the liver or plasma of AMP-HCl treated rats when compared to controls. Clinical Chemistry There were no alterations in the clinical chemistry parameters of AMP-HCl treated rats when compared to controls. Females from the 300 mg/kg/day group had slightly lower levels of cholesterol, which were statistically significant when compared to controls, but were not considered toxicologically significant. Whole Embryo Culture There were two control groups in the data tables, C/C and AMP/C, because not enough serum was available from the main study group control animals to culture the necessary number of embryos. Control serum from in-house stock animals was therefore used for one group of control embryos (group designated C/C). There were no treatment-related effects on embryo viability, visceral yolk sac diameter or circulation, allantois, crown-rump length, head length, crown-rump length:head length ratio, flexion, or on the number of somites or gross malformations when compared to controls. These data provided further evidence that the mode of action was maternally mediated and not

direct embryo toxicity to the test material or any metabolites that would be present in circulating blood.

Tier II
Tier II was triggered when the liver phospholipid analysis, clinical chemistry measurements, and WEC did not provide a strong suggestion for a testable mode of action for AMP-HCL-induced postimplantation loss.

Gene Expression
For the gene expression portion of the study, gene expression responses were analyzed from five decidual swellings from four control dams and thirteen decidual swellings from eight AMP-HCL-treated dams. Pre-filtering of the normalized microarray data sets, based on flags (present or absent) and expression levels followed by statistical analysis, was conducted in order to focus the downstream analysis and interpretation on the most reproducibly detectable and treatment-altered responses. An additional ±1.5 fold change relative to vehicle was also applied to further focus the generated gene lists. This combination of statistical and fold-change filters is consistent with previously evaluated approaches for identifying reproducible gene expression responses for biological interpretation.

Application of these criteria to the gene expression data identified 1749 probe-sets that were differentially expressed between the vehicle control and AMP treated samples. The dynamic range of the gene expression responses extended from a 9-fold repression through a 7-fold induction. These data were sent to a third party (Ingenuity Systems, CA) for functional and biological pathway assessment of the gene expression responses. The 1749 probe-set list was sent to Ingenuity for initial data processing after which it was determined to focus the pathway analysis using a 0.01 p-value statistical cut-off combined with a 2-fold change filter which further narrowed the gene list to 550 probe-sets. For functional annotation of these probe-sets, Ingenuity Systems used the Unigene mapping of the rat whole genome in order to provide the best coverage of the data. Annotation of these 550 probe-sets further narrowed the list to 281 annotated genes, of which 265 possessed functional annotation.

These data were analyzed by grouping of the top canonical pathways based on p-values generated by the Fisher Exact test (Figure 1), which resulted in “Tight Junction Signaling” identified as highly significantly different in treated animals ($p<0.0001$). Examination of the “Tight Junction Signaling” canonical pathway revealed four interrelated transmembrane genes, Crb, claudin, Jam, and occludin, that were downregulated in the dataset.

Following pathway analysis, the dataset was analyzed by grouping networks of genes together. Here again, genes involved in tight junction signaling (e.g., claudin family of genes) were identified. This network family was investigated further and thirteen “Tight Junction Signaling” pathway molecules were identified as all being significantly down-regulated. These included genes of the claudin family (e.g., claudins 1, 3, 4, 5, 7, 8, and 23), occludin, and E-cadherin.

These gene expression data are consistent with the reproductive toxicity profile of AMP-HCL-induced postimplantation loss. Occludin, claudin 1, and claudin 2 have been identified as integral tight junction membrane proteins that play a role in epithelial cell barrier maintanence and establishment of cell polarity. Immunohistochemistry of claudin 1 on rat uterine epithelium during early pregnancy has demonstrated localization of claudin 1 on the apical region of the lateral plasma membrane at all tested time points, namely GD 1, 3, 6, and 7. Interestingly, occludin staining in these experiments was only present in the early postimplantation period

(GD 6-7), which suggests that this gene, in concert with claudin and other tight junction genes, is important in successful implanatation. Additional immunohistochemical analysis showed that occludin and claudin 1 are expressed in decidualizing stroma of the primary decidual zone, which is responsible for creating a barrier between the embryo and maternal tissues. As part of this barrier function, the primary decidual zone confers an immune-privileged state protecting the embryo from deleterious molecules such as maternal immunoglobulins.

Taken together, the gene expression data demonstrate down-regulation of a number of tight junction regulatory genes which play a role in implantation of the rat embryo. In addition, the published localization of these down-regulated genes within the decidual swelling is consistent with histopathologic findings of vacuolation within the uterine epithelium lining the conceptus. These data support a maternally-mediated disruption of normal implantation as the primary mode-of-action of AMP-HCL-induced postimplantation loss.

Histopathology

Multiple paraffin embedded sections of one or more decidual swellings from GD 6 uteri were obtained from six control and seven AMP-HCL-exposed female rats and stained with hematoxylin and eosin for histological examination. There were a total of eight control and nine AMP-HCL-exposed GD 6 decidual swellings available for histologic examination.

Treatment-related effects consisted of very slight or slight multifocal vacuolation of the uterine luminal epithelium in all of the seven treated animals examined. This was a minimal change in most animals. In order to determine if these intracytoplasmic, clear, round vacuoles were lipid droplets, oil red O staining was conducted on four AMP-exposed uteri. Oil red O staining did not reveal the presence of lipid within these vacuoles and hence, the exact nature of these intracytoplasmic vacuoles is not clear. In general, the GD 6 embryos in AMP-HCL-exposed uteri were morphologically comparable to those of the controls. However, serial sections revealed increased amounts of karyorrhectic and pyknotic cellular debris in the uterine lumen of two treated females, in the near vicinity of the embryos. The source of the cellular debris may be from disintegrating luminal epithelium, or portions of resorbing embryo, or both. There were no treatment-related morphological changes in the decidual cells surrounding the embryos.

The examined GD 6 uteri represented a single time point in the development of the embryos exposed to AMP-HCL. Neither the pathogenesis of the vacuolar changes in the uterine luminal epithelium nor its relationship to subsequent early embryonic resorption of AMP-HCL exposed embryos are clear. Harvesting the uterus at precise time points beginning from GD 5 to 7 would likely shed more light into the progression of histopathologic events.

Overall summary

Results from Tier I experiments revealed no treatment-related effects on clinical observations, body weight, body weight gain, feed consumption, or organ weights. In addition, there were no treatment-related clinical chemistry findings nor any alterations in the phospholipid profile of the liver and blood. Culturing explanted control rat embryos with serum collected from dams treated with AMP-HCL resulted in no treatment-related effects on embryo growth or viability, suggesting a maternally mediated mode-of-action and not direct embryo toxicity to the test material or any metabolites that would be present in circulating blood. Based on

these findings, Tier II was triggered.

In Tier II testing, gene expression analysis of decidual swellings revealed significant down-regulation in genes of the tight junction signaling network such as occludin and members of the claudin family. The expression of these genes, in particular occludin and claudin 1, has been localized to rat uterine epithelial cells lining the implanting embryo and are involved in epithelial cell barrier maintanence and establishment of cell polarity. These gene expression data were consistent with treatment-related histopathologic findings of multifocal vacuolation of the uterine luminal epithelium lining the implanting embryo.

Taken together, this study provides additional data supporting a maternally-mediated mode-of-action for AMP-HCL-induced postimplantation loss, which may involve disruption of normal implantation through alterations in the tight junction signaling network.

Conclusions

Taken together, this study provides additional data supporting a maternally-mediated mode-of-action for AMP-HCL-induced postimplantation loss, which may involve disruption of normal implantation through alterations in the tight junction signaling network.

Executive summary

Groups of 15 female Crl:CD(SD) rats were administered 0 or 300 mg/kg body weight/day (mg/kg/day) AMP-HCl by diet for two weeks prior to breeding, through breeding (two weeks), and through gestation day (GD) 6 to provide a comprehensive assessment of maternal changes following gestational exposure and to reveal possible modes of action for the postimplantation loss (embryonic death) seen in previous studies. During the exposure period, female clinical observations, body weight, body weight gain, and feed consumption were evaluatedA necropsy was performed on GD 6 where the uterus and liver were removed and weighed. Histopathologic evaluation of the uterus was conducted. Liver, uterus, and blood were collected and analyzed according to a tiered scheme. In Tier I experiments, the phospholipid profile of the liver and blood was analyzed, and clinical chemistry measurements and whole embryo culture were evaluated. Tier II was triggered when the Tier I experiments did not provide a strong suggestion for a testable mode of action for AMP-HCL induced postimplantation loss. Tier II consisted of gene expression of frozen liver and uterine tissue analyzed by microarrays and histopathology of the uterus focusing on the site of implantation.

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(Furuseet al.,1998; Orchard and Murphy, 2002; Wanget al.,2004). These gene expression data were consistent with treatment-related histopathologic findings of multifocal vacuolation of the uterine luminal epithelium lining the implanting embryo.

Taken together, this study provides additional data supporting a maternally-mediated mode-of-action for AMP-HCL-induced postimplantation loss, which may involve disruption of normal implantation through alterations in the tight junction signaling network.

Reliability : 1 (reliable without exception)
9.15.2010 : (54)

:

Type : In Vivo
Species : Rat
Sex : male/female
Strain : Crl:CD(SD)
Species : Rat
Concentration : 99.7%
Dose : 300 mg/kg/day
Route of admin. : diet and gavage
No. of animals : 12
Vehicle : Dietary: Standard feed and gavage:water
Exposure period : Group 1 and 2: Two weeks prior to breeding, during breeding (2 weeks) and through GD 8
Group 3, 4, and 5 were dosed daily by gavage on GD 1-8, GD 6-8, and GD 9-11, respectively
Frequency of treatm. : Diet: Continuously Gavage: daily.
Method : Groups of 12 female Crl:CD(SD) rats were administered AMP-HCl by diet or gavage at a dose level of 300 mg/kg/day. Female rats in dose groups 1 and 2 were administered AMP-HCl by diet or gavage for approximately two weeks prior to breeding, through breeding (up to two weeks), and through GD 8. Female rats from dose groups 3, 4, and 5 were dosed daily by gavage on GD 1-8, 6-8, or 9-11, respectively. Male rats were not exposed to AMP-HCl via the dietary or oral gavage routes except during co-housing with dietary exposed females during the breeding period. During the exposure period, female cage-side and clinical observations, body weight, body weight gain and feed consumption were recorded. Data recorded on males were limited to daily cage-side observations. On GD 14, all surviving rats were euthanized and the number of corpora lutea, implantations, early resorptions, and live/dead fetuses were recorded.
Result : In-Life Observations

Body Weights/Body Weight Gains
There were no statistically identified differences in the pre-mating or gestation body weights and body weight gains of female rats given AMP-HCl in the diet (group 1) when compared to dams exposed via gavage (group 2) during the same dosing interval. addition, there were no statistically identified differences in the pre-mating or gestation body weights of females in groups 3-5 when compared to group 2 dams. However, gestation body weight gains in group 3-5 dams were significantly greater (~53-57%) for the GD 12-14 interval when compared to group 2 dams, which was likely the result of embryo resorptions in group 2. These data also correlate with an increase in overall gestation body weight gain in groups 3-5 when compared to group 2, although these comparisons were not statistically significant.

Feed Consumption
Mean pre-mating feed consumption for group 4 was statistically identified as increased when compared to group 2, however, this difference was spurious since there was no corresponding increase in body weight during

the same time interval. In addition, there was no difference in feed consumption among the dietary (group 1) and gavage treated rats (group 2) during the same dosing interval. Gestation feed consumption was similar amongst all groups and routes of exposure.

Anatomic Pathology

Reproductive Parameters
Because of ample historical experience, a negative control group was not included in this study. There were no significant effects on pregnancy rate or preimplantation loss in any group. As expected, the positive control group (group 1) produced mean postimplantation loss of 68.5%, which was remarkably consistent with previous studies utilizing a similar exposure duration by the dietary route.

In-Life Observations
No treatment-related effects on behavior were observed during the study period. One dam (730) in group 2 and two dams (732 and 739) in group 3 had no evidence of mating and were therefore euthanized with no further collection of data.

Body Weights/Body Weight Gains
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Anatomic Pathology

Reproductive Parameters:
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decreased mean postimplantation loss in group 2, only one of eleven dams had total litter loss compared to five of twelve in the dietary group. Overall, both the dietary and gavage routes of administration from PB -14 to GD 8 produced high levels of postimplantation loss; however, the severity of the effect was slightly lower following gavage administration. This difference could be due to variability, as the difference did not reach statistical significance. Alternatively, the difference might be explained by the higher 24-hour internal dose (AUC24hr) for dietary administration relative to gavage. Additionally, the greater effect from dietary administration might be due to concomitant intake of feed with test material, which could lead to interference of nutrient absorption within the gastrointestinal tract.

Group 3 (exposure from GD 1-8) had one dam with a totally resorbed litter; however, the remaining litters showed a low incidence of resorptions. Postimplantation loss for group 3 (14.2%) was higher than groups 4 and 5 and was outside of the laboratory historical control range for both dietary and gavage exposure studies of similar duration (Text Table 5). Inspection of the group 3 individual data indicated that the increase in postimplantation loss was largely driven by a single totally resorbed litter of 21 implantations. In fact, if the data from this dam (08A0741) were excluded from the analysis, mean percent postimplantation loss in group 3 would drop from 14.2 to 4.65%, which was well within the range of historical control data and similar to groups 4 and 5. Groups 4 (exposure from GD 6-8) and 5 (exposure from GD 9-11) had no dams with totally resorbed litters and a low incidence of resorptions/litter. Mean percent postimplantation loss (i.e., embryo resorption) and number of viable embryos/litter were within the historical control range in groups 4 and 5, and were statistically different from group 2 (gavage positive control) (Text Table 4). Taken together, the data from group 3 indicate a marginal treatment-related response possibly due to the slightly longer exposure period compared to groups 4 and 5.

The lack of an effect in groups 4 and 5 suggests that AMP-induced postimplantation loss is not the result of direct embryotoxicity. The much higher incidence of postimplantation loss with exposure starting prior to fertilization (PB -14 to GD 8) relative to gestation only (GD 1-8, 6-8 or 8-10) is suggestive of a maternallymediated mode of action, possibly associated with depletion of a factor necessary to support postimplantation embryo development.

The mean number of corpora lutea and implantations per dam (Text Table 6) observed on GD 14 (necropsy) in this study were above historical control data observed on GD 21 (Text Table 7) for all groups. The increased number of implantations per dam in groups 1 and 2 were related to difficulty in discerning individual implantation sites due to the high number of resorptions in these groups. Therefore, this finding is linked to resorption rate and considered treatment related. The higher than usual corpora lutea per dam was attributed to evaluation on GD 14 where the corpora lutea are smaller and more difficult to distinguish from other ovarian structures. The personnel in this laboratory usually perform these counts on GD 21. This observation was deemed unrelated to treatment.

Conclusions :

The results of this study indicate that repeated dose exposure to 300 mg/kg/day AMPHCl from PB -14 to GD 8 was sufficient to induce 68.5 and 44.4% postimplantation loss by the dietary or gavage route of administration, respectively. One-week exposure by gavage during GD 1-8 resulted in a marginal effect (14.2% postimplantation loss) that was limited to one dam with total litter loss. Two-day exposure during peri- (GD 6-8) or post- (GD 9-11) implantation phase resulted in 4.0 and 4.5% postimplantation loss, respectively, which was well within the range of

historical control. The much higher incidence of postimplantation loss with exposure starting prior to fertilization (PB -14 toGD 8) relative to gestation only (GD 1-8, 6-8, or 8-10) is suggestive of a maternallymediated mode of action, possibly associated with depletion of a factor necessary to support postimplantation embryo development.

Year	:	2009
GLP	:	Yes
Test substance	:	As prescribed by 1.1 – 1.4
Reliability	:	1 (reliable without restriction)
9.14.2010	:	(55)

Endpoint	:	Mechanistic Studies
Study descr. in chapter	:	
Reference	:	
Type	:	other: In vitro using Chinese hamster ovary cells
Species	:	
Sex	:	
Strain	:	
Route of admin.	:	
No. of animals	:	
Vehicle	:	
Exposure period	:	
Frequency of treatm.	:	
Doses	:	0.8, 4, 8, 16, 24, and 32 mM (100, 500, 1000, 2000, 3000, and 4000 mg/L, respectively) in the first Trial and 0.8, 4, 8, 16, 32, 48, and 80 mM (100, 500, 1000, 2000, 4000, 6000, and 10000 mg/L, respectively) in the second trial
Control group	:	
Observation period	:	
Result	:	
Method	:	
Year	:	2006
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The potential of AMP·HCl to inhibit the uptake of the essential nutrient choline in cultured mammalian cells was determined. Effects of AMP upon choline uptake by CHO cells were evaluated in actively growing cultures exposed to 3H-choline and a range of concentrations of AMP·HCl (0-32 mM; 0-4000 mg/L) for approximately 10 minutes. Concentrations of AMP·HCl used were based upon the results of a cytotoxicity assay and encompassed concentrations ranging from nontoxic to moderately toxic following 48 hours exposure. The pH of all dosing solutions was adjusted to that of the culture medium. Following treatment, cells were harvested, washed and lysed. The protein concentration of the lysate and 3H content were determined. Choline uptake as 3H activity was calculated on a per mg protein basis and treated and control data were compared statistically. In addition, the approximate effective concentration eliciting a 50% inhibition of uptake (EC50) was calculated and compared with that of several other alcohol amines. Descriptive statistics (means and standard deviations) for choline uptake were reported as total 3H counts per mg protein. Control and treated values for choline uptake were evaluated by Bartlett's test (alpha=0.01) for equality of variances. Exploratory data analysis was performed by a parametric analysis of variance (ANOVA) (alpha=0.05) and followed, if significant, by Dunnett's test (alpha=0.05; Winer, 1971). Dunnett's test corrects for multiple comparisons to the control and the experiment-wise alpha level was reported. Statistical outliers were identified by a

sequential test (alpha=0.02).

Result : A dose-response in cell viability versus concentration of AMP·HCl was observed in both toxicity trials. Survival relative to untreated controls ranged from 100+% to 61-68% over a concentration range of 0.04-32 mM. A 29% and 5.5% survival was noted at 48 and 80 mM, respectively, in trial II. The positive control consisting of 5% ethanol resulted in a survival of only 28-36% of untreated controls in both trials. No effects upon pH or osmolarity of the treated cultures were observed. Based upon these findings, a concentration range of 0.4 to 32 mM AMP·HCl was evaluated for effects upon 3H-choline uptake by CHO cells.

Uptake of 3H-Choline
There were no significant differences in protein content between treated and control cultures indicating no significant loss of cells in the 10-minute dosing period, including the high dose which resulted in cell numbers 61-68% of controls following 48 hours treatment. AMP·HCl was found to cause a dose-related decrease in 3H-choline uptake over the 40 to 80 fold concentration ranges examined; 0.8-32 mM in Choline Trial I and 0.4-32 mM in Choline Trial II. A statistically significant decrease in the uptake of 3H -choline was achieved at concentrations of 4 mM (65-81% of control) to 32 mM (30-36% of control) in both Trials. AMP·HCl appears to be a relative weak inhibitor of choline uptake in vitro with an approximate EC50 of 8.71-14.79 mM. It is approximately 10-17 fold less active than triethanolamine (EC50 ~0.89 mM), approximately 17-28 fold less active than diisopropanolamine (EC50 ~0.52 mM), and approximately 44-74 fold less active than diethanolamine (EC50 ~0.20 mM) in the same CHO cell based in vitro assay.

Conclusion : It was concluded that AMP·HCl has the potential to inhibit uptake of the essential nutrient choline in cultured CHO cells in the absence of significant toxicity. This activity, however, is relatively weak when compared to several other alcohol amines under similar assay conditions.

Reliability : (1) valid without restriction
1 (meets generally accepted scientific standards and is described in sufficient detail)

02.06.2006 (56)

Endpoint : Mechanistic Studies
Study descr. in chapter :
Reference :
Type : other: in vivo
Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : dietary
No. of animals :
Method :
Year : 2006
GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Method : The potential of dietary HCl salt of 2-amino-2-methyl-1-propanol (AMP-HCl) to depress hepatocellular levels of the essential nutrient choline in pregnant rats was evaluated. Two groups of 6 female Crl:CD(SD) rats each were fed diets containing 0 (control) or 300 mg/kg/day of AMP-HCl for approximately two weeks prior to breeding, continuing throughout breeding (up to two weeks), and two weeks into gestation (GD 0-13). Clinical observation, body weight, and feed consumption data were collected. On GD 14, the females were euthanized and samples of liver were excised and frozen in liquid nitrogen or saved in 10% formalin. Uteri were examined and the number of live implants and resorptions were determined. Formalin fixed liver underwent histopathological examination

following standard hematoxylin and eosin staining and osmium tetroxide treatment for identification of lipid. Frozen liver was analyzed for levels of choline (Cho), betaine, glycerophosphocholine (GPCho), phosphocholine (PCho), phosphatidyl choline (PtdCho), and sphingomyelin (SM).

Result : Dietary administration of 300 mg/kg/day of AMP-HCl resulted in no adverse clinical observations, mean body weight, or feed consumption effects. However, AMP-HCl ingestion caused an increased postimplantation loss (67.0% in treated versus 7.4% in control rats), and a slight increase in hepatic lipid vacuolation relative to controls. These changes to varying degrees, correlated with an approximately 20-25% statistically identified decrease in hepatic levels of PCho which is the primary storage pool of choline. Levels of GPCho, indicative of conversion of PtdCho to Cho, were elevated 2-3 fold relative to control dams. Hepatic levels of remaining metabolites were either unchanged or minimally changed relative to controls.

Conclusion : The results of this study, when combined with the known choline depression caused by pregnancy, suggest that previous findings of lipid accumulation and elevated resorptions in an OECD-421 study of AMP-HCl in rats (Carney et al., 2005) may be a consequence of, and secondary to, PCho deficiency, respectively.

Reliability : (1) valid without restriction
1 (GLP study, based in part on standard test methods, well-documented and acceptable for assessment)

16.10.2006 (57)

5.10 EXPOSURE EXPERIENCE

Type of experience : Human

Method : Skin prick tests and patch tests with the test material and common inhalant allergens were performed on all subjects. AMP-100 was dissolved in both water and ethyl alcohol separately at 0.1%, 0.5%, 1%, 2%, 5%, 10%, 20%. Patch tests were applied on the back and read at 48 and 72 hours. Positive results were recorded for erythema, oedema, and blisters.

Result : The prick and patch tests were all negative except for one positive reaction to Dermatophagoides pteronyssinus in a worker on a different production line. All patch tests carried out with substances used in the production line proved negative except for AMP-100 at 10% and 20% dilutions in water and ethyl alcohol. All positive results manifested as erythema with oedema without blistering.

Positive results at 10% and 20% may be considered as irritative responses. There was lack of response to the lower doses (that would have been considered allergenic responses) noted in both the asymptomatic and symptomatic subjects.

Test condition : Two cases of airborne contact dermititis were noted in a cosmetic company during the hair dye production using AMP-100. Subjects suffered for months from periorbital oedema and facial erythema which worsened during work hours and improved with time away from work. Eight additional asymptomatic workers from the same company were studied concurrently.

Conclusion : The authors conclude that under these conditions, AMP-100 causes airborne irritative contact dermatitis, favored in this study by elevated production temperatures and high ambient humidity.

Reliability : (2) valid with restrictions
2 (meets generally accepted scientific standards, well-documented, and acceptable for assessment)

01.06.2006 (58)

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT