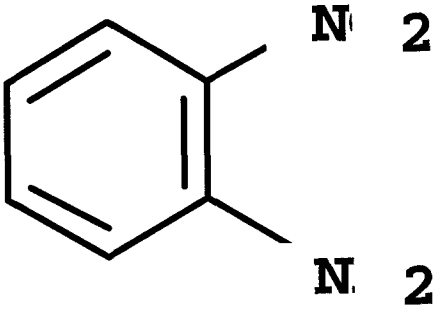
201-15173B1



2-Nitroaniline
CASNO 88-74-4

H P V / I U C L I D
Data Set

Existing Chemical	:	ID: 88-74-4
CAS No.	:	88-74-4
EINECS Name	:	2-nitroaniline
EC No.	:	201-855-4
TSCA Name	:	Benzenamine, 2-nitro-
Molecular Formula	:	C6H6N2O2
Producer related part		
Company	:	Toxicology and Regulatory Affairs
Creation date	:	08.03.2004
Substance related part		
Company	:	Toxicology and Regulatory Affairs
Creation date	:	08.03.2004
Status	:	
Memo	:	ONA
Printing date	:	30.03.2004
Revision date	:	
Date of last update	:	30.03.2004
Number of pages	:	25

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	manufacturer
Name	:	Solutia Inc.
Contact person	:	
Date	:	
Street	:	
Town	:	
Country	:	
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	
Remark	:	Revised by:

Toxicology and Regulatory Affairs
Freeburg IL, 62243
rauckman@toxicsolutions.com

29.03.2004

1.2 SYNONYMS AND TRADENAMES

2.1 MELTING POINT

Value	:	= 71.5 °C
Sublimation	:	
Method	:	other
Year	:	1989
GLP	:	no data
Test substance	:	no data
Test substance	:	Technical grade ONA had purity of > 99% and was likely the source used.
Reliability	:	(2) valid with restrictions Listed as Peer Reviewed reference in Hazardous Substances Data Bank (2002) for 2-nitroaniline.
Flag	:	Critical study for SIDS endpoint

24.10.2002 (4)

2.2 BOILING POINT

Value	:	= 284 °C at
Decomposition	:	
Method	:	other
Year	:	1989
GLP	:	no data
Test substance	:	no data
Reliability	:	(2) valid with restrictions Listed as Peer Reviewed reference in Hazardous Substances Data Bank (2002) for 2-nitroaniline.
Flag	:	Critical study for SIDS endpoint

09.03.2004 (4)

2.4 VAPOUR PRESSURE

Value	:	= .00115 hPa at 25 °C
Decomposition	:	no
Method	:	other (calculated)
Year	:	
GLP	:	no
Test substance	:	
Method	:	Ferro and Piacente (1985) took vapor pressure measurements of o-nitroaniline at approximately 50 different temperatures from 312° K to 385° K using a torsion effusion apparatus as described in the literature [V. Piacente and G. DeMaria, Ric. Sci., 39 (1969) 549]. The data were plotted as log P versus 1/T and the best straight line was determined for the liquid and solid form using the method of least squares.

The equation for the vapor-pressure temperature relationship was determined to be:

Log P (kPa) = 12.0 - 4750/T (Kelvin)*

* Ferro, D.; Piacente, V. Heat of Vaporization of o-, m-, p-Nitroaniline. Thermochimica Acta, 90: 387-9, 1985

This equation is used to extrapolate the vapor pressure at 25° C as follows:

log P (kPa) = 12.0 -4750/298

log P = -3.9396

P = 0.000115 kPa

Converting to hPa:

P = 0.00115 hPa (0.0086 mm Hg)

Result :

The extrapolated vapor pressure of ONA at 25° C is 0.00115 hPa

Test substance :

2-Nitroaniline, CASNO 88-74-4, Purity 99.99%

Reliability : (2) valid with restrictions

Calculated by an acceptable method from reliable data.

Flag : Critical study for SIDS endpoint

13.03.2004 (6)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : = 1.85 at °C
pH value :
Method : other (calculated)
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions

Listed as Peer Reviewed reference in Hazardous Substances Data Bank (2002) for 2-nitroaniline and listed as Recommended value in SRC CHEMFATE data base (2002).

Flag :

Critical study for SIDS endpoint

14.03.2004 (8)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= 1470 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other
Year	:	1991
GLP	:	no data
Test substance	:	no data
Reliability	:	(2) valid with restrictions Listed as Peer Reviewed reference in Hazardous Substances Data Bank (2002) for 2-nitroaniline and SRC CHEMFATE Data base (2002).
Flag	:	Critical study for SIDS endpoint

14.03.2004 (18)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	other
Light spectrum	:	> 290 nm
Relative intensity	:	based on intensity of sunlight
DIRECT PHOTOLYSIS		
Halflife t1/2	:	= 9.5 hour(s)
Degradation	:	= 16 % after 3 hour(s)
Quantum yield	:	
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	
Rate constant	:	= .000000000013 cm^3/(molecule*sec)
Degradation	:	% after
Deg. product	:	
Method	:	other (calculated)
Year	:	2002
GLP	:	no
Test substance	:	no data

Method :

Direct photodegradation measured using a medium-pressure mercury arc emitting > 290 mu; irridiations were conducted in triethylamine for 3 hrs; Additionally, a calculated value of 9.5 hr was derived by AOP Computer program v1.90. The program estimates the Atmospheric Oxidation Potential by estimating the rate constant for the atmosphere, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The methodology is based on the SAR methods developed by Atkinson et al, 1987, Intern. J. Chem. Kinet. 19: 799-828 and described by Meylan and Howard, 1993, Chemosphere 26:2293-2299.

Reliability : (2) valid with restrictions

Measurements published in a peer reviewed journal. Estimated value based on model recommended by US EPA.

Flag : Critical study for SIDS endpoint

29.03.2004 (1)

3.3.2 DISTRIBUTION

Media	:	other: water, air, soil, sediment
Method	:	Calculation according Mackay, Level III
Year	:	

Method :

Calculated according to Mackay, Level III. Assumed emission to water only as most likely industrial release. Physical parameters used measured values. Degradation values estimated from experimental determinations. Values shown in results section.

Result :

Level III Fugacity Model (Full-Output):

```
=====================================
  Chem Name   : 2-Nitroaniline
  SMILES      : Nc1ccccc1N(=O)(=O)
  Molecular Wt: 138.13
  Henry's LC  : 5.9e-008 atm-m3/mole (Henry database)
  Vapor Press : 0.0086 mm Hg  (user-entered)
  Liquid VP   : 0.0248 mm Hg  (super-cooled)
  Melting Pt  : 71.5 deg C (user-entered)
  Log Kow     : 1.85  (user-entered)
  Soil Koc    : 29  (calc by model)

          Concentration   Half-Life    Emissions
            (percent)        (hr)       (kg/hr)
   Air       0.000678        19           0
   Water     99.6            2e+003       1000
   Soil      0.0426          2e+003       0
   Sediment  0.326           7.5e+003     0

       Fugacity   Reaction Advection  Reaction  Advection
       (atm)      kg/hr)   (kg/hr)   (percent) (percent)
Air    8.93e-015  0.184    0.0505     0.0184    0.00505
Water  1.59e-012  257      742        25.7      74.2
Soil   7.56e-015  0.11     0          0.011     0
Sed    1.53e-012  0.224    0.0486     0.0224    0.00486

   Persistence Time: 745 hr
   Reaction Time:    2.89e+003 hr
   Advection Time:   1e+003 hr
   Percent Reacted:  25.8
   Percent Advected: 74.2

   Half-Lives (hr), (based upon user-entry):
      Air:      19
      Water:    2000
      Soil:     2000
      Sediment: 7500

   Advection Times (hr):
      Air:      100
      Water:    1000
      Sediment: 5e+004
```

Test substance :

2-Nitroaniline (CASNO 88-74-4)

Conclusion :

Material released to water expected to remain 99% in water with a small quantity distributing to sediment.

Reliability : (2) valid with restrictions

Calculated by an acceptable method using measured physical properties.

Flag : Critical study for SIDS endpoint

14.03.2004 (5)

3.5 BIODEGRADATION

Type : aerobic

Inoculum :

Concentration : 5 mg/l related to Test substance
related to

Contact time	:	24 hour(s)
Degradation	:	= 2 - 40 (±) % after 24 hour(s)
Result	:	other: little biodegradation observed under these conditions
Deg. product	:	
Method	:	other
Year	:	1975
GLP	:	no
Test substance	:	other TS

Method :

Semi-continuous activated sludge (SCAS) test was carried out over a 10-month period at a final addition rate of 5 mg ONA per 24-hr cycle. The methodology used was a standard procedure published in JAOCS 42:986 (1965) and used the modified feed techniques as described in JAOCS 46:432 (1969). ONA concentration was determined using UV spectrophotometry after extraction of the sludge with methylene chloride. Analysis was performed on one 24-hr cycle per week. Activated sludge obtained from local waste treatment facility.

Remark :

The conclusion that ONA is resistant to biodegradation is supported by the following literature reports:

Hallas LE, Alexander M; Appl Environ Microbiol 45 4: 1234-41 (1983).

Kitano M; Biodegradation and Bioaccumulation Test on Chemical Substances. OECD Tokyo Meeting. Ref Book TSU-No 3 (1978).

Alexander M, Lustigman BK; J Agric Food Chem 14: 410-3 (1966).

Malaney GW; J Water Pollut Control Fed 32: 1300-11 (1960).

Pitter P; Water Res 10: 231-5 (1976).

Urano K, Kato Z; J Hazardous Materials 13: 147-59 (1986).

Young JC, Affleck SB; Eng Bull Purdue Univ, Eng Ext Ser 1: 154-64 (1974).

Zeyer J, Kearney PC; J Agric Food Chem 31: 304-8 (1983).

Chemicals Inspection and Testing Institute Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan, Japan Chemical Industry Ecology - Toxicology and Information Center (1992).

Result :

No significant biodegradation occurred, as a mean (+/-95% CI) loss was 7 (+/-11) %. No evidence of any inhibition of the normal sludge growth rate was observed.

Test substance :

Used Technical grade ONA with purity > 99%.

Reliability : (2) valid with restrictions

Study was conducted prior to codification of GLPs but is considered well documented. The methodology used in this study has now been codified into internationally accepted

test guidance for biodegradability determination.

Flag : Critical study for SIDS endpoint
14.03.2004 (16)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	semistatic
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= 19.5
Limit test	:	
Analytical monitoring	:	yes
Method	:	Directive 84/449/EEC, C.1 "Acute toxicity for fish"
Year	:	1991
GLP	:	no data
Test substance	:	other TS

Method :

96 hr acute toxicity test was conducted in a semistatic system according to the OECD Guideline 202, as published in 1984. Zebrafish were approx. 3 mo. of age and weighed between 200-350 mg; both sexes were used. Fish were not fed 24h prior to testing and during the 96-h exposure period. A 12-h light;dark cycle was employed. The test water was charcoal-filtered, aerated tap water which was mixed with a stock solution of the chemical in distilled water and stirred at room temperature. The pH, dissolved oxygen and temperature of the water were 8.6+/-0.3, 85+/_15% and 26.5+/-1 degree C., respectively. Once a day the concentrations were checked photometrically and the test solutions were renewed if required. LC50 values were calculated using a computer program based on the method of Litchfield and Wilcoxon (1949).

Result :

The 96 hr LC50 was determined to be 19.5 mg/L with SE of +/- 1.7 mg/L.

Test substance :

Test sample purchased from a chemical supplier; Technical grade was typically > 99%.

Reliability : (1) valid without restriction

No information was reported in the article about conduct under GLPs; however, as this study was conducted specifically to meet OECD test guideline 202 it is reasonable to assume that it was conducted under GLPs as well.

Flag : Critical study for SIDS endpoint

29.03.2004 (19)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l

NOEC	:	= 12.5
EC50	:	= 14.5
Method	:	EPA OTS 797.1300
Year	:	
GLP	:	yes
Test substance	:	
Method	:	Test article dissolved in Dimethyl Formamide (0.5 ml/L) and introduced to glass jars filled with well water; DO, pH, alkalinity and hardness measured prior to and after testing. Three replicates run, using 10 Daphnia per dosage level per rep. Dosages evaluated: control, solvent control, 6.25, 12.5, 25, 50 and 100 mg/L.
Result	:	EC50 values (95% CI) of 18.7 (12.5-25) mg/L at 24 hr and 14.5 (12.5-25) mg/L. at 48-hr interval. The NOEC was 12.5 mg/L. Following was the % deaths observed: At 24 hr- Control (0%), solvent control (0 %), 6.25 mg/L (0 %), 12.5 (0 %), 25 (0 %), 50 (93.3%) and 100 mg/L (100%); At 48 hr - Control (0%), solvent control (0%), 6.25 mg/L (0 %), 12.5 (30%), 25, (100%), 50 (100%), and 100 mg/L (100%). pH and dissolved oxygen ranged from 7.0-8.4 and 7.8-9.3 mg/L, respectively. The mean temp. was 23.7 degrees C. Alkalinity ranged between 298-400 mg/L and water hardness ranged between 220-370 mg/L. Evidence of insolubility of test substance was seen at 100 mg/L.
Test substance	:	Used Technical grade ONA, with purity of > 99%.
Reliability	:	(1) valid without restriction Study conducted according to ASTM/EPA guidance, which is consistent with OECD test guidance.
Flag	:	Critical study for SIDS endpoint

29.03.2004 (15)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus sp. (Algae)
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	= 64.5
Limit test	:	
Analytical monitoring	:	no data
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS
Method	:	A 48-hr algae inhibition test following OECD test methods was conducted using S. obliquus as the test organism. Five concentration gradients were used, in concentration spacing of 0.2. pH of the culture medium was adjusted to 7.2+/-0.2. Two replicates of each concentration and untreated control

were run. The algae in the logarithmic growing period were inoculated into 250 ml Erlenmeyer flasks, and added to 60 ml of the culture media, compound and algae. The initial algae cell concentration was approx. 1 x 10E4 cells/ml. The culture was incubated under a continuous light by fluorescent bulb at 20+/-1 degree C and average illumination intensity of 4000 lux. Growth was monitored by electron microscope (400X). EC values were determined by one variable linear regression analysis.

Test substance :

Test sample purchased from chemical supplier; typical technical grade purity of ONA was 99%.

Reliability : (1) valid without restriction

No mention made regarding conduct under GLPs in article; however, as this study was conducted specifically to meet OECD guideline 201 it can reasonably be assumed that it also was conducted under GLPs.

Flag : Critical study for SIDS endpoint
29.03.2004 (7)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 2050 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	20
Vehicle	:	other
Doses	:	
Method	:	other
Year	:	
GLP	:	no
Test substance	:	

Method :

calc. method of deBeer, 1945, J. Pharmacol. Experimen. Ther. 85:1.

Test substance was Technical grade ONA with purity of > 99%; administered as 10% corn oil solution

Used 5 rats (mixed sex) /group. Four groups of rats were administered test article by gavage in increasing doses at increments of 0.1 fractional log intervals. Clinical signs recorded daily and body wts. recorded weekly. Animals observed for 14 days. Necropsies were performed on all animals. Food and water given ad libitum; humidity and temp. controlled.

Result :

OLD50=2050 mg/kg; 95% CI of 1760-2380; all deaths occurred within 24 hrs.; Deaths: 1260-0/5; 1580-1/5, 2000-2/5, 2510-5/5; Signs of toxicity: yellow colored urine, generalized weakness; Observations at autopsy for decedents-hemorrhagic lungs, liver hyperemia, abdominal cavity yellow stained, g.i. irritation; for survivors - viscera appeared normal.

Reliability : (2) valid with restrictions

Conducted using fewer animals than # 401; conduct consistent with but prior to enactment of GLP guidelines; This was a supplemental study to the HPV program in that an acute study by another route has been used to fullfill this HPV data endpoint.

Flag : Critical study for SIDS endpoint

29.03.2004 (17)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC0
Value	:	> 2529 mg/m³
Species	:	rat
Strain	:	Wistar
Sex	:	male/female
Number of animals	:	10
Vehicle	:	other
Doses	:	
Exposure time	:	4 hour(s)
Method	:	OECD Guide-line 403 "Acute Inhalation Toxicity"
Year	:	1996
GLP	:	yes
Test substance	:	other TS

Method :

Test article used was 65% aqueous solution of Technical grade ONA (typical purity of 99%). Groups of 5 male and 5 female rats were exposed to a single aerosol concentration of ONA solution in PEG (to facilitate nebulization) under nose only conditions; the chamber was operated under dynamic exposure conditions. Animals were observed daily for clinical signs; body wts recorded on days 3, 7 and 14. Clinical observations were consistent with a Functional Observational Battery set of indices; methemoglobin determinations were made following exposure. All rats underwent a gross necropsy at study term. Food and water were given ad libitum. Observation period was 14 days. A vehicle control group of rats was exposed similarly to polyethylene glycol/acetone. Analytical test levels determined by GC method; particle size determined using cascade impactor. Statistical evaluations performed on body weights and physiological data using ANOVA procedures.

Result :

Limit test
No deaths occurred at the maximum achievable level tested of 2,529 mg/m3 (analytical level); the MMAD was 2.1 um indicating particle sizes of a respirable range. Animals exposed at this level exhibited decrements in body weight gain, hypothermia, distinct discoloration of the urine, and bradypnea, all of which were attributed to test article. These observations persisted no longer than 1 day following exposure. No adverse effects were noted in reflex measurements. No macroscopic findings attributable to test article were observed.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

26.08.2002 (2)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD0
Value	:	> 7940 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	3
Vehicle	:	other
Doses	:	
Method	:	other
Year	:	
GLP	:	no
Test substance	:	
Method	:	Determination of Minimum Lethal Dose, thus used 1-2 animals /group; 24-hr occlusive dermal patch with 14-day observation period; necropsy at sacrifice, daily cage-side observations made for 2 weeks and weights recorded initially and after 7 and 14 days. Test article used was Technical grade ONA with purity > 99%; Administered as 40% solution-suspension in corn oil. Administered to clipped, intact skin of rabbits for 24-hr exposure under occluded conditions. Then removed and animals observed for 14 days.
Result	:	No deaths (0/1) at 5010 mg/kg or (0/2) at 7940 mg/kg; Observations: Yellow staining, reduced appetite and activi ty during first 3 days; all normal on day 14. No macroscopic necropsy findings.
Source	:	Solutia Inc. St. Louis
Conclusion	:	Considered sufficient to establish toxicity to rodents by dermal route
Reliability	:	(2) valid with restrictions Used a small no. animals; conducted consistent with but prior to enactment of US GLPs in 1979; this study was a Supplemental study to the HPV program since another study by a another route was chosen to fullfill this HPV Endpoint.

29.03.2004 (17)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-acute
Species	:	rat
Sex	:	male
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	6 hr/day
Frequency of treatm.	:	5 days/week for 4 weeks

Post exposure period	:	none
Doses	:	9.8 and 93 mg/m3 (analytically determined conc.)
Control group	:	yes, concurrent no treatment
NOAEL	:	= 9.8 mg/m³
LOAEL	:	= 93 mg/m³
Method	:	OECD Guide-line 412 "Repeated Dose Inhalation Toxicity: 28-day or 14-day Study"
Year	:	
GLP	:	yes
Test substance	:	

Method :

Test material used was Technical grade ONA with purity > 99%. Test article generation used preheated nitrogen which was passed over the test agent in a paraffin oil bath; thus, no solvent, like CELLOSOLV, as used in a previous 4-wk inhalation study (BD-81-322), was employed in this study.

This study was designed to determine whether ONA alone induced testicular effects observed in study BD-81-322, using CELLOSOLV solvent; Thus, each test group consisted of 10 male rats; daily observations, hematology (HGB, HCT, RBC, MET, retic, clot time, RBC morph and t/diff. leukocytes) evaluated on all animals prior to sacrifice; Brain and testicular wts were recorded while testes and epididymides were examined grossly and microscopically for all test animals. Body weight, hematology data and absolute and relative organ weights were treated for statistical differences. Parametric analysis was performed using ANOVA methods followed by Dunnet's test when mean differences were observed between dose groups; Kruskal Wallis test and Dunn's rank sum test were used for nonparametric analysis. Both 5% and 1% levels of significance were reported for each parameter.

Whole body exposure in stainless steel chamber; analytically determined doses were 9.8 and 93 mg/m3 respectively. Analysis done by UV 4x daily, particle size confirmed during week 1 and rechecked periodically using Cascade impactor.

Remark :

This study confirms that ONA produces no effects on testes following inhalation exposure and that results of a previous study (BD-81-322) were the result of use of CELLOSOLV as vehicle. These results, in conjuction with findings in the previous study cited earlier, are sufficient to meet all toxicity parameters established in OECD test guideline 412.

Result :

Mean testicular wts (absolute and relative) were comparable to controls in both ONA test groups; no gross or microscopic changes in testes/epididymides were observed; Minimal changes in some hematological parameters (increases in methemoglobin i.e. MET and HCT and decreased total leuk. and seg. neutrophils) were seen at 93 mg/m3

Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

29.03.2004 (11)

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	6 hrs/day
Frequency of treatm.	:	5 days/week for 4 weeks
Post exposure period	:	none
Doses	:	10, 30 and 73 mg/m3
Control group	:	yes, concurrent vehicle
NOAEL	:	= 30 mg/m³
LOAEL	:	= 73 mg/m³
Method	:	OECD Guide-line 412 "Repeated Dose Inhalation Toxicity: 28-day or 14-day Study"
Year	:	
GLP	:	yes
Test substance	:	

Method :

Test substance used was Technical grade ONA with purity of > 99% which was mixed with 2000 mg/m3 CELLOSOLVE (ethylene glycol monoethyl ether) as a concurrent vehicle; 10 rats/sex/group were exposed in 1 cub. meter steel/glass chambers via whole body exposure; Analytically determined (4X/d) concentration means were: 10, 27.5 and 73 mg/m3, respectively. Particle size means were all below 1 micron for each aerosol concentration. All animals were observed daily for toxic signs, weighed weekly, and underwent examination for clinical chemistries, hematology, ocular toxicity. Organ weights were taken at necropsy and microscopic exams were conducted on over 40 tissues for all high dose and control animals and target organs for all animals. Body weights, food consumption, hematology and clinical chemistry, absolute and relative organ weights were analyzed using ANOVA methods followed by Dunnet's test for parametric parameters while nonparametric parameters were subjected to Kruskal Wallis test followed by Dunn's rank sum test to determine statistical differences. Both 5% and 1% levels of significance were reported for each parameter.

Remark :

Ambiguous information on testicular effects were resolved with a follow up study (BD-82-270) which assessed the issue of testes effects and the confounding use of Cellosolv as the solvent in this study. Subsequent results confirmed cellosolv as the affective agent.

Result :

Treatment-related effects : 73 mg/m3 - Statistically decreased leukocytes in males, and significantly reduced hbg and rbc in females, increased polychromia, anisocytosis and poikilocytosis in males and females, increased rel. liver wts for females (no correponding histo), decreased absolute and relative testes wts corresponding with degeneration of the germinal epithelium seen microscopically.

Conclusion :

Study results involving effects on the testes are considered unreliable due to incorrect choice of vehicle control

(CELLOSOLVE, which was determined to be a testicular toxin but only after this study was conducted). The issue was resolved after conduct of a follow up study (BD-82-270). However, results in this study confirm that ONA, even in combination with CELLOSOLVE, did not affect measured clinical chemistry parameters, ophthalmology, organ weights, and gross and histopathology of a full set of tissues and organs which were not measured again in the second study (BD-82-270). For this reason, those portions of this study which were indicative of no discernable effect of ONA treatment , can be considered reliable.

Reliability : (2) valid with restrictions

Flag : Critical study for SIDS endpoint
30.03.2004 (10)

Type : Sub-acute
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : 14 days
Frequency of treatm. : daily gavage administration throughout test period
Post exposure period : none
Doses : 0, 1, 19, or 100 mg/kg bw
Control group : yes, concurrent vehicle
NOAEL : >= 100 mg/kg bw
Method : other
Year :
GLP : no data
Test substance :

Method :

Groups of 10 M/10 F rats administered test article in corn oil via gavage for 14 consecutive days. A comprehensive evaluation of biochemical, hematological and histopathological evaluations were made at study termination. All animals examined daily for clinical signs and body weights were recorded daily. All animals necropsied on d15 and weights recorded for the following organs: brain, heart, liver, kidney and spleen. Histopathological exams were conducted on approx. 30 tissues and organs, including the gonads. ANOV analyses and Duncan's Multiple Range test ($p<0.05$) used to determine group differences.

Result :

No treatment related findings in hematology, clinical chemistries, clinical observations,body and organ weights or macro- or microscopic findings attributable to treatment

Reliability : (2) valid with restrictions

This study was of insufficient duration to be used to meet HPV testing guidance. It study was provided as Supplemental information as the HPV requirement has been fullfilled with another Repeat Dose study.

30.03.2004 (9)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	S. typhimurium strains TA98, TA100, TA1535 and TA1537 w & w/o S9
Test concentration	:	1.5, 3, 6, 7, 15, 30, 40, 150, 225, 450, 600, and 1500 ug/plate
Cycotoxic concentr.	:	3000 ug/plate (no background lawn) using TA100; 1000 ug/plate tolerated w & w/o S9
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	
GLP	:	no
Test substance	:	

Method :

Statistical test used: after data transformation - 1-sided t-test; $p<0.01$

Test material used was Technical grade ONA with purity of > 99%; Appropriate positive controls were employed to validate this test methodology.

Result :

Negative response seen in spot test at maximum conc. of 10000 ug/plate with and without S9

No significant mutagenic activity seen in any of the 4 tester strains; all positive controls validated adequacy of method used.

Reliability : (2) valid with restrictions

Study conducted consistent with but prior to development of US GLP's in 6/79; study results are confirmed in numerous other published articles.

Flag : Critical study for SIDS endpoint

30.03.2004 (12)

Type	:	Chromosomal aberration test
System of testing	:	CHO cells maintained in Eagle MEM media
Test concentration	:	1 - 10 mM
Cycotoxic concentr.	:	no information provided
Metabolic activation	:	with and without
Result	:	ambiguous
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	

Method :

After overnight incubation in complete medium, the medium was replaced with either serum-free complete medium or an exogenous metabolic activation medium, each containing test material. Cells were treated for 1 h, followed by washing (3X) and incubated in complete medium for either 10h or 16 hr. Colcemid was added for the final 2h of incubation. 100 metaphase cells scored from each of 2 cultures for each

treatment level. Negative control group was used. Positive controls included MMS and CP. Statistical package used was EPA's Chromosomal aberration assay data management and analysis system.

Remark :

This study is Supplemental information as a fully acceptable micronucleus test has been used to fullfill this HPV endpoint.

Result :

Test material induced a significant ($p<0.01$) increase in chromosomal aberrations measured 10h after pretreatment both in the presence and absence (1 of 2 trials) of S9. A statistically significant increase in aberrations was also detected after 16h, but only with S9. A dose-response trend was evident in all cases, but only strong responses were observed at the very highest (10 mM) dose tested.

The primary aberration observed was a large isochromatid discontinuity seen only in the long arm of the X chromosome. Image enhancement revealed presence of material in the affected region and the alignment of the dislocated segment, making classification of this lesion uncertain. In a separate experiment, all X-chromosome isochromatid anomalies were screened to perform the analysis with and without discontinuity. When excluded, there was no increase in aberrations observed. The cause of this isochromatid discontinuity is uncertain.

Source : Solutia Inc. St. Louis

Conclusion :

The authors state that "It is not clear whether this phenomenon represents a legitimate chromosomal aberration."

Reliability : (3) invalid
30.03.2004 (3)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : CD-1
Route of admin. : i.p.
Exposure period : Single doses given twice, 24 hrs apart
Doses : 0, 50, 250, and 500 mg/kg
Result : negative
Method : OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year :
GLP : yes
Test substance :

Method :

Dosages administered in corn oil (10 ml/kg). In a preliminary study, the IP LD50 in mice was determined to be 723 mg/kg; further, the PCD/total erythrocyte ratio was evaluated to determine bone marrow cytotoxicity potential. After completion of dosing, bone marrow was taken from both femors and pooled for slide preparation. Slides were stained

with Wright-Giemsa stain pak and scoring was conducted by 2 independent readers. The no. of micronuclear polychromatic erythrocytes (PCEs) per 1000 PCEs and the no. of PCEs and normochromatic erythrocytes/1000 erythrocytes were evaluated for each animal. The individual animal was used as the statistical unit and the Student's T (1-sided) test used to compare treatment and control group means. A level of p <0.05 was used for all parameters to determine statistical significance.

Highest dosage used was approx. 70% of calc. IP LD50 of 730 mg/kg, as determined in intralaboratory range-find study with mice

Technical grade ONA with purity of > 99% used in this test. Cyclophosphamide (40 mg/kg) used a positive control.

Result :

No increases in micronuclei observed at any ONA dose level; positive control verified the method. Signs of listlessness and unresponsive behavior seen in both sexes at 500 and 250 mg/kg and females at 50 mg/kg ONA; statistically lower body weights observed in females at 500 mg/kg after 48 hr dosing.

Source : Solutia Inc. St. Louis
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
30.03.2004 (14)

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : C57BL
Route of admin. : i.p.
Exposure period : Treated twice with 24 h between each treatment
Doses : 0, 246, 492 and 738 mg/kg
Result : ambiguous
Method : OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year : 1994
GLP : no data
Test substance : no data

Method : Test article administered IP in olive oil to groups of 5M and 5F mice; controls received only olive oil.High dose reportly was estimated to be 75% of LD50 as determined in a preliminary experiment. After 36 h following the second treament, mice were sacrificed and bone marrow removed, a cell suspension made and slides prepared. 500 polychromatic erythrocytes from each animal were scored for the presence of micronuclei. The ratio of PEs to normochromatic cells was also determined to assess cytotoxicity. Data were analyzed using EPA's micronucleus assay data management and analysis system (p<0.05)

Result : No statistically significant increase in PE ratios; thus, no indication of cytotoxicity. A small 1.2+/- 0.08 vs. 2.8+/- 1.50, but statistically (p<0.05) significant increase in micronuclei was observed at the highest dose tested of 738 mg/kg only in male mice. This effect was observed only in males, not females at this dose level; no effects were seen

in either males or females at lower dose levels.

Source : Solutia Inc. St. Louis
Reliability : (3) invalid
Considered ambiguous, as the effect noted was small, seen only at one dose level and observed in only one sex.
Provided as Supplemental information.

07.11.2002 (3)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	Days 6-15 of gestation
Frequency of treatm.	:	Daily throughout exposure period
Duration of test	:	Treated on gestation days 6-15, sacrificed on gestation day 21 for fetal exams
Doses	:	0, 100, 300, 600 mg/kg/day in corn oil
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 100 mg/kg bw
NOAEL teratogen.	:	= 600 mg/kg bw
NOAEL Embryotoxicity	:	= 600 mg/kg bw
NOAEL Fetotoxicity	:	= 600 mg/kg bw
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	
GLP	:	yes
Test substance	:	

Method :

25 pregnant females/group; daily gavage in corn oil at constant volume of 10 ml/kg/d from gestation days 6-15. Dosing solutions were analyzed (GC) for test material concentration and stability periodically throughout the study. Nidation data collected at sacrifice, live fetuses examined externally and by Wilson sections and skeletal exam techniques were used to detect any variations or abnormalities. Body weights and food consumption were collected on gestation days 0, 6, 10, 13, 16 and 21 (day of termination). Daily clinical signs of toxicity recorded on gestation days 6-21. Statistical methods used: body wts. analyzed using Dunnett's test; Counted data (corpora lutea, implants, resorption, live/dead pups) analyzed using Mann-whitney U test; response data (eg. pregnancy rates, litters with postimplantation loss, etc.) assessed with Fischer's exact test and Chi square test.

Result :

Maternal toxicity was evidenced by reduced body weight gains, reduced food consumption and piloerection in the 300 and 600 mg/kg groups.

Marginal reduction in food consumption (94% of control) was reported for 100 mg/kg dams that was only statistically significant in the overall time period of day 6 to 21 of gestation but not significant at any other time interval. In contrast 300 mg/kg-dams showed statistically significant reduction in food consumption on days 6-10 of gestation as well as for the day 6-21 period. Only the high-dose group of dams showed a reduction in food consumption (87% of control) that appeared biologically significant. Body weight gains were 94, 88 and 85% of controls for low, mid and high-dose dams, respectively. Both mid and high-dose dams lost body weight between gestation day 6 and 10; however, the statistical analysis of body weights only showed significant differences for the high-dose group. Since there was a slight loss of body weight in the mid-dose group between gd 6 and 10; this is considered an indication of maternal toxicity and the maternal LOAEL is considered to be 300 mg/kg with a Maternal NOAEL of 100 mg/kg. This is supported by the clinical observations where mid and high-dose dams were reported to demonstrate piloerection while low-dose dams did not.
No effects on pregnancy rates, mean no. live and dead pups, resorptions, nidations, c. lutea; Mean fetal wts were slightly, but not statistically lower than control in 600 mg/kg group. No differences seen in no. litters, fetuses or malformations. One malformation (situs inversus syndrome) was seen in single fetuses from two litters at the 600 mg/kg level; this incidence and lack of correlation to similar findings associated with other mononitroanilines supports the conclusion that this was a spurious finding.

Test substance :

Technical grade of ONA used with purity of > 99%.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
29.03.2004 (13)

(1) Barltrop, JA and Bunce, NJ. 1968. Organic photochemistry. Part VIII. The photochemical reduction of nitro-compounds. J. Chem. Soc. (C) 1968:1467-1474.

(2) Bayer Corp. 1996. Study on acute inhalation toxicity in rats according to OECD No. 403 by T. Martins. Bayer Study no. T3044113. [EPA Document No. 86960000565; Fiche no. OTS0558766]

(3) Blakey, DH, Maus, KL, Bell,R, Bayley, J, Douglas, GR, and Nestmann, ER. 1994. Mutation Research 320:273-283.

(4) Budavari, S. (ed.) 1989. The Merck Index- an encyclopedia of chemicals, drugs and biologicals. Whitehouse Station, NJ. p. 1042.

(5) Calculated by Toxicology and Regulatory Affairs, March 2004, using EPIWIN 3.05.

(6) Calculation by Toxicology and Regulatory Affairs, Freeburg IL, 2004

(7) G.-H. Lu, Yuan, X, and Zhao, Y-H. 2001. Chemosphere 44:437-440

(8) Hansch, C and Leo, L, 1985. Medchem Project. Claremont, CA. Pomona College Issue 26.

(9) Komsta, E, Secours, VE, Chu, I, Valli, VE, Morris, R, Harrison, J, Baranowski, E and Villeneuve, DC. 1989. Bull. Environ. Contam.Toxicol. 43:87-94.

(10) Solutia study no. BD-81-322. Four week inhalation toxicity study of O-Nitroaniline in the rat. [EPA 878214205; Fiche no. OTS0206486]

(11) Solutia study no. BD-82-270. Four week inhalation study of Ortho-Nitroaniline in male rats [EPA Document no. 878214205; Fiche no. OTS0206486]

(12) Solutia study no. LF-78-144. Salmonella mutagenicity assay of O-Nitroaniline (Technical). [EPA Document no. 878211039; Fiche no. OTS0206222].

(13) Solutia study no. ML-82-89. Orthonitroaniline: A teratology study in rats. [EPA Document no. 868600002; Fiches no. OTS0510153]

(14) Solutia study no. ML-89-7. Micronucleus assay with o-nitroaniline.

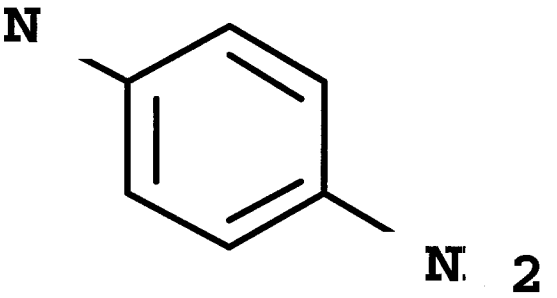
(15) Solutia study no. MO1983X083. Acute toxicity of o-Nitroaniline for Daphnia magna.

(16) Solutia study no. MO20020140. Biodegradation testing of o-nitroaniline (ONA) and p-nitroaniline (PNA).

(17) Solutia study no. Y-76-438 Toxicological investigation: O-Nitroaniline [EPA Document No. 878211634; Fiche no. OTS0206222].

(18) Suzuki, T.1991. J. Computer-Aided Molecular Design 5:149-166.

(19) Zok, S, Goerge, G, Kalsch, W and Nagel, R. 1991. Sci. Total Environ. 109/110:411-421.



4-Nitroaniline
CASNO 100-01-6

H P V / I U C L I D
Data Set

Existing Chemical	:	ID: 100-01-6
CAS No.	:	100-01-6
EINECS Name	:	4-nitroaniline
EC No.	:	202-810-1
TSCA Name	:	Benzenamine, 4-nitro-
Molecular Formula	:	C6H6N2O2

Producer related part

Company	:	Toxicology and Regulatory Affairs
Creation date	:	07.03.2004

Substance related part

Company	:	Toxicology and Regulatory Affairs
Creation date	:	07.03.2004
Status	:	
Memo	:	Revised
Printing date	:	30.03.2004
Revision date	:	
Date of last update	:	30.03.2004
Number of pages	:	28

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	manufacturer
Name	:	Solutia Inc.
Contact person	:	
Date	:	
Street	:	
Town	:	
Country	:	
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

Remark : Revised by:

Toxicology and Regulatory Affairs
Freeburg IL, 62243
rauckman@toxicsolutions.com

29.03.2004

Source : Solutia Inc. St. Louis
24.10.2002

1.2 SYNONYMS AND TRADENAMES

2.1 MELTING POINT

Value	:	= 146 °C
Sublimation	:	
Method	:	other
Year	:	1989
GLP	:	no data
Test substance	:	other TS
Reliability	:	(2) valid with restrictions Reference cited as Peer reviewed in Hazardous Substance Data Bank for p-Nitroaniline (2002) and as Recommended value in SRC CHEMFATE data base (2002).
Flag	:	Critical study for SIDS endpoint

07.11.2002 (2)

2.2 BOILING POINT

Value	:	= 332 °C at
Decomposition	:	
Method	:	other
Year	:	1989
GLP	:	no data
Test substance	:	other TS
Reliability	:	(2) valid with restrictions Reference cited as Peer Reviewed in Hazardous Substances Data Band for p-Nitroaniline (2002) and cited as SRC Recommended value in CHEMFATE data base (2002)
Flag	:	Critical study for SIDS endpoint

07.11.2002 (2)

2.3 DENSITY

2.4 VAPOUR PRESSURE

Value	:	= .0000042 hPa at 25 °C
Decomposition	:	no
Method	:	other (calculated): extrapolated
Year	:	
GLP	:	no
Test substance	:	
Method	:	Ferro and Piacente (1985) took vapor pressure measurements of p-nitroaniline at approximately 50 different temperatures from 357° K to 420° K using a torsion effusion apparatus as described in the literature [V. Piacente and G. DeMaria, Ric. Sci., 39 (1969) 549]. The data were plotted

as log P versus 1/T and the best straight line was determined for the liquid and solid form using the method of least squares.

The equation for the vapor-pressure temperature relationship was determined to be:

Log P (kPa) = 12.4 - 5595/T (Kelvin)*

* Ferro, D.; Piacente, V. Heat of Vaporization of o-, m-, p-Nitroaniline. Thermochimica Acta, 90: 387-9, 1985

This equation is used to extrapolate the vapor pressure at 25° C as follows:

log P (kPa) = 12.4 -5595/298

log P = -6.3752

P = 0.00000042 kPa

Converting to hPa:

P = 0.0000042 hPa (0.0000032 mm Hg)

Result :

The extrapolated vapor pressure of PNA at 25° C is 0.0000042 hPa

Test substance :

4-Nitroaniline, CASNO 100-01-6, Purity 99.99%

Reliability : (2) valid with restrictions

Calculated by an acceptable method from reliable data.

Flag : Critical study for SIDS endpoint

13.03.2004 (4)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : = 1.39 at °C
pH value :
Method : other (calculated)
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Recommended value in CHEMFATE data base (2002)

Flag : Critical study for SIDS endpoint

14.03.2004 (8)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= 724 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other
Year	:	1991
GLP	:	no data
Test substance	:	other TS
Reliability	:	(2) valid with restrictions Cited as a Peer Reviewed reference in Hazardous Substance Data Bank for p-nitroaniline (2002).
Flag	:	Critical study for SIDS endpoint

14.03.2003 (22)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	other
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	
Rate constant	:	= .00000000001345366 cm^3/(molecule*sec)
Degradation	:	= 50 % after 9.5 hour(s)
Deg. product	:	not measured
Method	:	other (calculated)
Year	:	2002
GLP	:	no
Test substance	:	no data

Method :

Calculated by AOP Computer Program, Vers. 1.90, Syracuse Research Corp. which estimates the Atmospheric Oxidation Potential. This program estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The model is based on SAR methods developed by Atkinson et al, 1987, Intern. J. Chem. Kinet. 19:799 and described in Meylan and Howard, 1993, Chemosphere 26: 2293-2299.

Reliability : (2) valid with restrictions

Estimated value based on model recommended by EPA

Flag : Critical study for SIDS endpoint

29.03.2004 (6)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

Media	:	other: water, air, soil, sediment
Method	:	Calculation according Mackay, Level III
Year	:	

Method :

Calculated according to Mackay, Level III. Assumed emission to water only as most likely industrial release. Physical parameters used measured values. Degradation values estimated from experimental determinations. Values shown in results section.

Result :

Level III Fugacity Model (Full-Output):

```
=======================================
  Chem Name   : 4-Nitroaniline
  SMILES      : Nc1ccc(N(=O)(=O))cc1
  Molecular Wt: 138.13
  Henry's LC  : 1.26e-009 atm-m3/mole (Henry database)
  Vapor Press : 3.2e-006 mm Hg  (user-entered)
  Liquid VP   : 5.03e-005 mm Hg  (super-cooled)
  Melting Pt  : 146 deg C (user-entered)
  Log Kow     : 1.39  (user-entered)
  Soil Koc    : 10.1  (calc by model)

          Concentration   Half-Life    Emissions
            (percent)        (hr)       (kg/hr)
   Air       6.59e-007       19           0
   Water     99.8            1e+003       1000
   Soil      0.000979        1e+003       0
   Sediment  0.229           4e+003       0

        Fugacity   Reaction  Advection Reaction   Advection
          (atm)     (kg/hr)  (kg/hr)  (percent)  (percent)
Air    6.77e-018   0.00014  3.9e-005 1.42e-005  3.9e-006
Water  2.69e-014   409       591     40.9       59.1
Soil   5.42e-018   0.00401   0       0.000401   0
Sed    2.49e-014   0.235     0.0271  0.0235     0.00271

   Persistence Time: 592 hr
   Reaction Time:    1.45e+003 hr
   Advection Time:   1e+003 hr
   Percent Reacted:  40.9
   Percent Advected: 59.1

   Half-Lives (hr), (based upon user-entry):
      Air:      19
      Water:    1000
      Soil:     1000
      Sediment: 4000

   Advection Times (hr):
      Air:      100
      Water:    1000
      Sediment: 5e+004
```

Test substance :

4-Nitroaniline (CASNO 100-01-6)

Conclusion :

Material released to water expected to remain 99% in water with a small quantity distributing to sediment.

Reliability : (2) valid with restrictions

Calculated by an acceptable method using measured physical properties.

Flag : Critical study for SIDS endpoint

14.03.2004 (3)

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	
Concentration	:	5 mg/l related to Test substance related to
Contact time	:	24 hour(s)
Degradation	:	= 10 - 100 (±) % after 24 hour(s)
Result	:	other
Deg. product	:	
Method	:	other
Year	:	1975
GLP	:	no
Test substance	:	other TS

Method :

Semi-continuous activated sludge (SCAS) testing was carried out over a 10-month period at an addition rate of 5 mg per 24-hr cycle. The standardized test method used was published in JAOCS 42:986 (1965) and used the modified feed technique (JAOCS 46:432, 1969). Sludge was obtained from a local waste disposal site. Disappearance was measured after one 24-hr cycle per week using UV spectrophotometry to analyze the methylene chloride extract of the mixed liquor samples taken at that time.

Remark :

Support for this conclusion comes from several published studies using high concentrations of PNA that have indicated poor biodegradation* and from the report by Young et al (Eng Bull Purdue Univ, Eng Ext Ser 1: 154-64 (1974)) that PNA at a concentration of 50 mg/L was biodegraded by aerobic sewage bacteria after a lag period of about 20 days. In addition, the observation that the half-life of PNA (at about 1 mg/L) in the water column of the Rhine River has been estimated to be 3.8 days based on water monitoring data collected in the Netherlands (Zoeteman BCJ et al; Chemosphere 9: 231-49 (1980)) lends support to the biodegradable at low concentration theory. Finally, the report by Zeyer and Kearney [Zeyer J, Kearney PC; J Agric Food Chem 31 (2): 304-8 (1983)] who were able to isolate a strain of Pseudomonas from soil that grew slowly on PNA as a sole source of carbon (but not on ONA), provides supporting evidence for the biodegradability of PNA under optimal conditions.

*

Malaney GW; J Water Pollut Control Fed 32: 1300-11 (1960)

Kitano M; Biodegradation and Bioaccumulation Test on Chemical Substances; OECD Tokyo Meeting, Reference Book TSU-No. 3 (1978)

Pitter P; Water Res 10: 231-5 (1976)

Alexander M, Lustigman BK; J Agric Food Chem 14: 410-3 (1960)

Urano K, Kato Z; J Hazard Materials 13: 147-59 (1986)

Chambers CW et al; J Water Pollut Control Fed 35: 1517-28 (1963)

Chemical Inspection and Testing Institute; Biodegradation and

Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan, Japan Chemical Industry Ecology - Toxicology and Information Center (1992)

Klopman G et al; J Chem Inf Comput Sci 32: 474-482 (1992)

Result :

24-Hour removal of PNA from the culture varied from about 10% to 100%. The first fourteen weeks of the testing with PNA showed about a 30% removal. From week 16 to week 33, the loss of PNA was high with a mean 24-hour removal of 82% and six of the weeks the removal was greater than 95%. After week 33, the apparent ability of the culture to remove PNA declined to a mean of 19% removal per 24-hour cycle. The reason for this decline is not known.

It is speculated that the decline is due to bacterial inhibition by PNA or by a toxic degradation product. PNA is known to be inhibitory to Pseudomonas species with a threshold of inhibition in the area of 10 mg/L (ECB IUDLID 2000, PNA). There may have also been build up of some unknown bacterial inhibitor that eventually caused the inhibition. This is supported by the observation that substantial inhibition of the normal sludge growth rate was reported during the study.

Test substance : Technical grade PNA with purity > 99%.

Conclusion :

PNA appears to be biodegradable by treatment-plant bacteria under optimal conditions.

Reliability : (2) valid with restrictions

Study conducted prior to codification of GLPs but considered well documented. Methodology used has subsequently been incorporated into a standardized international test guideline for this study type.

Flag : Critical study for SIDS endpoint

14.03.2004 (19)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 10
LC50	:	= 45
Limit test	:	
Analytical monitoring	:	no
Method	:	other
Year	:	1980
GLP	:	yes
Test substance	:	other TS

Method :

Followed study design adopted by US EPA Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975; design consistent with OECD 203. Groups of 10 fingerling (mean wt of 0.83 g/fish and length of 38 mm) were exposed to varying test concentrations in 15 liters of soft reconstituted water with a dissolved oxygen level of 8.6 mg/l, a pH of 7.4, total hardness of 45 mg/L CaCO3 and total alkalinity of 35 mg/l CaCO3. These vessels were kept in a water bath at 12 degrees C. Fish acclimated to the dilution were held without food for 48 hours prior to testing. Based on preliminary testing, each group of fish was exposed to one of six test concentrations ranging in a logarithmic series from 5.6 to 100 mg/L. Fish were added to the test chambers within 30 min. of the addition of the test article. Test concentrations were prepared in acetone (0.5 ml), based on total compound as the test article was > 99% pure and the dose solution was then added to each respective test chamber. Mortality rates, fish behavior and water quality data (temp, pH, ammonia levels) were monitored after 24, 48 and 96 hrs of treatment. Antimycin A was similarly tested as a concurrent positive control. Calculation of the LD50 and confidence limits was performed using a computerized program developed by Stephan, Busch, Smith, Burke and Andrew, 1978 from the US EPA Duluth, Minn Aquatic Laboratory.

Result :

LC50 and (Confidence Limits): 96-hr=46(32-56) mg/L; 48-hr= 45 (32-56) mg/L; 24-hr = 47 (32-100) mg/L. No deaths were seen at any test concentration up to 32 mg/l through 96 hrs of testing. At 56 mg/l, mortality reached 80% after 24 hrs and 90% after 48 and at 96 hrs. 100% mortality occurred at all three time points at 100 mg/l. A yellow precipitate was observed at all test levels. Dissolved oxygen concentration ranged between 60-100% saturation and was considered adequate for testing. The pH values remained consistent throughout the test and the ammonia concentrations were below the toxic limit. The positive control responded as expected.

Test substance :

Technical grade PNA (CASNO 100-01-6) with purity > 99%.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
29.03.2004 (11)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : = 10
EC50 : = 20
Analytical monitoring : no
Method : other
Year :
GLP : yes
Test substance : other TS

Method :

Followed study design outlined by the US EPA Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975, and consistent with OECD Guideline # 202.The study was conducted in 250 ml glass beakers containing 200 ml well water with specified chemical characteristics and kept at 20 degrees C. The photoperiod was controlled to give 16 hr daylight. After an inital range-find study, groups of 10 D. magna (first instar less than 24 hr old) were added to one of 5 beakers containing a range of test material between 3.2 and 32 mg/L, spaced logarithmically. The test article was originally prepared in 0.5 mL acetone solutions (0.5 ml) prior to charging the beakers. Each concentration was run in duplicate. Fish mortality and behavior and water quality parameters (dissolved oxygen levels, pH and temperature) were measured at the beginning of the test and after 24 hr (mortality and behavior only) and 48 hrs. Predicted LC50 values and 95% confidence limits were calculated using the computerized program developed by Stephan, Busch, Smith, Burke and Andrew, 1978 from the US EPA Duluth, Minn Aquatic Laboratory.

Result :

48 hr LC50 (CI) =20 (18-23) mg/L. All water quality parameters (20-12 deg. C; 8.8-9,0 mg/L DO, pH of 8.1-7.9 and water hardness of 255 ppm CaCO3) were found to be acceptable.

Test substance :

Technical grade PNA with purity > 99%.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
29.03.2004 (12)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Scenedesmus sp. (Algae)
Endpoint : other: Turbidity
Exposure period : 4 day(s)
Unit : mg/l

Method :

Green algae (Scenedesmus species) were grown in 300 ml Erlenmeyer flasks containing 100 ml algae media. One week before the test started, fresh flasks were inoculated to use as inoculums for the tests. The test material dissolved in algal media was inoculated with 10 ml algal suspension (formula given in the publication) and maintained at 24 deg C for four days using Osram HNI and HNT 40 W light-bulbs. At the end of the 4-day incubation period turbidity was determined. As multiple chemicals of various toxicities to algae were tested, individual concentrations were not reported for each compound in this publication

Result :

p-Nitroaniline showed a threshold of inhibition of 20 mg/L after 4 days of incubation.

Test substance :

p-Nitranilin, NOS

Reliability : (2) valid with restrictions

Published articles are assigned a score of 2.

Flag : Critical study for SIDS endpoint
29.03.2004 (1)

Species : Scenedesmus sp. (Algae)
Endpoint : growth rate
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : = 54.9
Limit test :
Analytical monitoring : no data
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 2001
GLP : no data
Test substance : other TS

Method :

48-hr algae growth inhibition test following OECD guideline 201. Organism used was S. obliquus. pH of the culture medium was adjusted to 7.2+/-0.2. Five concentrations were used at log intervals of 0.2. Two replicates of each concentration plus a negative control were tested. The algae in the logarithmic growing period were inoculated into 250 ml Erlenmeyer flasks containing approx 60 ml of media, test article and algae. The initial algae cell concentration was 1x10E4 cells/ml. The culture was incubated under a continuous light at 20+/-1 degrees C while flourescent lamp and the average illumination intensity was about 4000 lux. Growth was monitored by an electron microscope (400X). The EC value was determined using a one variable linear regression analysis.

Source :

Solutia Inc. St. Louis

Test substance :

Test material purchased from chemical supplier; typical technical grade purity of PNA was 99%.

Reliability : (1) valid without restriction

No mention was made regarding conduct under GLPs in the literature article; however, as this study was conducted specifically to meet OECD Guideline 201, it can reasonably be assumed that it also was conducted under GLPs.

29.03.2004 (9)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 1400 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	25
Vehicle	:	other
Doses	:	
Method	:	other
Year	:	
GLP	:	no
Test substance	:	

Method :

Consistent with # 401,but fewer animals, ie. 5 rats of mixed sex/group were given test article in 5 increasing doses at increments of 0.1 fractional log intervals; animals observed daily for 14 days for clinical signs and weighed weekly. Food and water provided ad libitum and temp./humidity controlled. Necropsies performed on all animals that died and on survivors after 14d.

Technical grade PNA used, with purity > 99%. Administered as 20% solution-suspension in corn oil

Result :

OLD50 = 1400 mg/kg; Confidence Limits of 1230-1590 mg/kg; used method of deBeer, J.Pharmacol. Experimen. Ther. 85:1; Deaths - mg/kg: 794 (0/5), 1000 (1/5), 1260 (1/5), 1580 (4/5), 2000 (5/5), occurred within 7 days of dosing; Signs of toxicity: ocular discharge, tremors and convulsions; necrospy (decedents) - hemorrhagic areas of lung, liver discoloration and gi inflammation; all survivors had normal vicera after 14 days observation

Source : Solutia Inc. St. Louis

Conclusion :

Sufficiently robust to provide degree of acute toxicity in rodents; numerous additional literature citations for this endpoint also available.

Reliability : (2) valid with restrictions

Conducted prior to, but consistent with, US GLPs which were enacted 6/79. Results are consistent with data in ECB IUCLID-PNA, 2002 for this endpoint, which had 5 values between 920-3250 mg/kg and 1 value as low as 750 mg/kg.

Flag : Critical study for SIDS endpoint
29.03.2004 (20)

Source : Solutia Inc. St. Louis
04.04.2002

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD0
Value	:	> 7940 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	3
Vehicle	:	other
Doses	:	
Method	:	other
Year	:	
GLP	:	no
Test substance	:	

Method :

Test article administered as 40% solution-suspension in corn oil; applied occluded for 24 hrs to intact, clipped skin of rabbits, animals observed clinically for 14 days. Body weights were recorded weekly; all animals were necropsied after d14. Food and water available ad libitum and temp./humidity was controlled.

Result :

Determination of Minimum Lethal Dose: Two dosages tested, 5010 mg/kg (0/1 deaths) and 7940 mg/kg (0/2 deaths); no significant untoward toxic signs were observed during the study, all viscera normal at necropsy

Test substance :

Used Technical grade PNA, with purity of > 99%.

Conclusion :

Sufficiently robust study to evaluate the minimum lethal dose; as this dose proved to be of a low toxicity, there would appear to be no reason to test at higher levels to define an LD50 by this route.

Reliability : (2) valid with restrictions

This is provided as supplemental information since an acute oral toxicity study has been used to fulfill this HPV endpoint. Small, but sufficient no. animals to characterize toxicity; study conducted prior to, but consistent with, US GLPs enacted in 6/79.

29.03.2004 (20)

Source : Solutia Inc. St. Louis
04.04.2002

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	90 days
Frequency of treatm.	:	daily consecutive
Post exposure period	:	none
Doses	:	0, 3, 10, 30 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL	:	< 3 mg/kg bw
LOAEL	:	= 3 mg/kg bw
Method	:	OECD Guide-line 408 "Subchronic Oral Toxicity - Rodent: 90-day Study"
Year	:	
GLP	:	yes
Test substance	:	

Method :

Corn oil vehicle used and dosing occurred at a constant volume of 0.2 ml/100 g bdy wt; 20 rats/sex/group used; Clinical signs recorded daily, individual body weights and food consumption measured weekly, serum chemistries (SGPT, SAP, BUN, T. Bili., GLU, T. Prot., K, Na), urinalysis (Prot, microscop. elements, pH, Spec. grav., blood, Glu, ketones, urobilinogen, vol.) and hematology parameters (Hgn, HCT, WBC, RBC, MCV, MCHC, retics, red cell fragility and methemoglobin) examined after 44 and 88 days. All animals necropsied at study term and organ weights (brain, adrenals, kidneys, liver, spleen, pituitary, testis) weighed. Histopathologic exams were conducted on approx. 40 tissues and organs from all high dose and control rats and the spleens of all lower dose rats. Specifically, gonads were examined for all HD and C animals. Statistical analysis performed using: Bartlett's test ($p<0.01$), ANOVA, Dunnets' test, Mann-whitney U with Bonferroni Inequality test, and Kolmogorov-Smiranov 1 tail test (all at $p<0.05$ and $p<0.01$)

Result :

30 mg/kg: Pale appearance around ears, statistically significant increase in urinary urobilinogen and methemoglobin levels, statistical increases in RBC counts and hemoglobin levels of both sexes. All animals had discolored spleens at necropsy, statistically increased spleen weights and splenomegaly and microscopic evidence of excessive splenic hemosiderin. 10 mg/kg: Statistically increased methemoglobin and decreased RBC counts and hemoglobin conc. (females only), all animals had splenomegly, elevated splenic wts, discolored spleens and microscopic pathology associated with excessive hemosiderin; 3 mg/kg: statistically elevated methemoglobin (both sexes) and microscopic findings in spleen

Test substance :

Used Technical grade PNA with purity > 99%.

Conclusion :

No effects observed on gonads.

Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

29.03.2004 (17)

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	6 hours per day, 5 days per week
Frequency of treatm.	:	4 weeks
Post exposure period	:	none
Doses	:	0, 10, 32, 80 mg/m3 (analytical)
Control group	:	yes, concurrent vehicle
NOAEL	:	< 10 mg/m³
LOAEL	:	= 10 mg/m³
Method	:	OECD Guide-line 407 "Repeated Dose Oral Toxicity - Rodent: 28-day or 14-d Study"
Year	:	1984
GLP	:	yes
Test substance	:	other TS

Method :

Aerosol derived by passing air over PNA dissolved in isopropanol and warmed. Groups of 10 rats/sex/group were housed in stainless steel and glass chamber and exposed under whole body conditions to one of three levels of test material. A vehicle control group was exposed to isopropanol in a similar fashion and treated similarly for evaluation. Chamber atmospheres and particle size were analytically determined. Dosing occurred 6h/d, 5d/wk for 4 consecutive weeks; animals were observed daily for clinical signs, weighed weekly, food and water given ad libitum , serum chemistry (BUN, SGPT, SAP, GLU, ALB, T.Protein, Glob., Na, K, P, Ca, Cl) and hematology (Hgb, HCT, RBC, Methem., clot time, T/Differ. Leuko, red cell morph) parameters collected on day 0 and 28. Ophthalmoscopic exams conducted on day 0 and 28. Organ weights (gonads, hrt, kid, lvr, lu, pit, spln, brain) recorded at termination; all animals necropsied at term; microscopic evaluation of approx. 40 tissues and organs (including gonads) for all high dose and control rats; spleens examined for all lower dose animals. Statistical methods used included: Bartlett's test ($p<0.01$), and ANOVA, Krusal-Wallis, Dunn's Summed rank test - all ($p<0.05$ and $p<0.01$)

Result :

80 mg/m3:non-statistical decreases in hemoglobin and hematocrit seen in males and females, statistical increase in methemoglobin in males and females, higher incidence of polychromasia and anisocytosis (females only), statistically elevated absolute and relative spleen wts for both sexes, histopthological exams revealed elevated iron deposition within splenic macrophages, extramedullary hematopoiesis in spleen (male and female) and liver (females only); 32 mg/kg: non-statistical decrease in hemoglobin in males, statistically elevated methemoglobin in males and females, higher incidence of polychromasia (both sexes) and

anisocytosis (females only), relative spleen wts increased statistically (males only), histopathology - increased iron deposition and extramedullary hematopoiesis in both males and females; 10 mg/m3: non-significant elevation in blood methemoglobin, significant increases in mean spleen weight (both sexes), iron deposition and extramedullary hematopoiesis seen in spleens (both sexes)

Test substance :

Technical grade PNA with purity > 99%.

Reliability : (1) valid without restriction

Supplemental HPV study since a fully acceptable Subchronic study (see earlier entry in this Section) fulfills the Repeated Dose HPV Endpoint.

29.03.2004 (10)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : S. thyphimurium test strains TA98, TA100, TA1535, TA1537 w & w/o S9
Test concentration : 0.01, 0.04, 0.2, 1, 1.5, 3, 4, 5, and 10 mg/plate
Cycotoxic concentr. : no significant microbial toxicity observed up to 10 mg/plate with TA100
Metabolic activation : with and without
Result : positive
Method : OECD Guide-line 471
Year :
GLP : yes
Test substance :

Method :

Conducted both Spot test and Plate Incorporation Assay. Used DMSO as solvent, S9 was commerically available rat and mouse liver preparations. Appropriate positive (2-AA, 9-AA, B(a)P, 2-NF, NaNo2) controls run to validate method. All assays run in triplicate. Bartletts' test for homogeneity of variance and group-wise comparisons made within levels of pooled variance, 1-sided t-test applied, $p<0.05$. For positives, Grubb's test run to determine outliers and regression analysis and t-test of transformed data to determine dose response.

Result :

Negative in all 4 test strains, with and without activation, up to max. conc. of 25 mg/spot in Spot test.

Positive finding only with TA98 (statistically elevated without activation and elevated, but not statistically with activation) in plate incorporation assay; all other strains were negative with and without activation

Test substance :

Technical grade PNA with purity of > 99%.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
30.03.2004 (15)

Type	:	Cytogenetic assay
System of testing	:	Chinese Hamster Ovary cell culture
Test concentration	:	50 to 5000 ug/mL
Cycotoxic concentr.	:	Laboratory 1 - 1600 ug/ml and higher; laboratory 2- none up to 5000 ug/ml
Metabolic activation	:	with and without
Result	:	ambiguous
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	

Method :

NTP study design, exposing cells for 8-12 hr nomally and for 2hr in presence of S9; 100 cells per dose group were scored, all types of aberrations were recorded; Dunnett's adjusted P value ($p<0.05$) was used for statistical assessment.

Result :

Two separate testing labs used, each giving nonconfirmatory results. Positive results reported with S9 in studies at laboratory 1, and weak positive without S9 at Lab 2, Effects only seen at very highest test levels, with no evaluation of influence of pH or osmolarity. Cytotoxicity observed at Lab 1 but not reported at lab 2.

Test substance :

4-Nitroaniline, CASNO 100-02-6, reportedly commercially available material; i.e. technical grade of > 99%

Reliability : (3) invalid

Results considered ambiguous. Inconsistency of positive findings renders results inconclusive; additional concerns regarding inconsistency in cytotoxicity seen within lab trials and between labs. No effort made to determine affect, if any, of pH or osmolarity changes on study outcome. Supplemental HPV study since a fully acceptable in vivo micronucleus test fulfills this HPV Endpoint.

30.03.2004 (7)

Type	:	Cytogenetic assay
System of testing	:	CHO-K1 (Chinese Hamster Ovary) cells
Test concentration	:	173, 345, 690, and 1035 ug/ml
Cycotoxic concentr.	:	none observed
Metabolic activation	:	without
Result	:	ambiguous
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	

Method :

Unique, research methodology performed. Used established cell line without incorporation of S9 fraction as data included in this paper considered PNA as a weak, direct acting mutagen in an Ames/Salmonella test. After incubation for 2 hrs with test compound dissolved in DMSO, cells were washed twice with PBS and incubated for another 20 hr in

fresh medium. After colchicine addition, and three further hrs of incubation, metaphase cells were harvested by mitotic shake-off and resuspended. Cells were fixed, stained and selected for analysis. At least 100 metaphases per flask were scored for each dose for individual types of aberrations, including breaks, deletions, exchanges and dicentrics. Both the percentage of aberrant cells and the frequency of aberrations were calculated. The tests were repeated three times in total such that at least 300 metaphases were scored for each dose. A positive response was determined based on the percentage of cells with aberrations showing a dose-response trend and at least a four-fold increase over that of the negative controls at one or more doage levels. Both Eagles' basal medium and DMSO were tested as negative controls. TEM served as a positive control.

Result :

The results obtained are considered ambiguous since specified criterion for determination of a positive response (4X % aberrant cells over negative control-in this case DMSO) were not met. Neither the positive control (0.25 ug/ml TEM) nor any of the PNA dose levels exhibited a 4X increase from the negative DMSO control; the positive control and all PNA dose levels did exhibit a 4X increase in aberrant cells over the Eagle's medium negative control. The % aberrant cells reported were: Eagle's medium (3), DMSO (6), TEM (22), 173 ug/ml PNA (13), 345ug/ml PNA (19), 690 ug/ml PNA (20), and 1035 ug/ml PNA (20).

Test substance :

Obtained commercially (Sigma Chem.), and thus technical grade of > 99%.

Reliability : (3) invalid

Supplemental HPV study since a fully acceptable in vivo micronucleus test is available to fullfill this endpoint; also ambiguous outcome of this study renders it unuseable.

30.03.2004 (5)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male/female
Strain	:	CD-1
Route of admin.	:	i.p.
Exposure period	:	two doses, 24-hours apart
Doses	:	80, 400 and 800 mg/kg
Result	:	negative
Method	:	OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year	:	
GLP	:	yes
Test substance	:	

Method :

High dose considered to be 80% of IP LD50, as determined by preliminary study using probit method; corn oil used as

vehicle (10 ml/kg); 12 mice/sex were used for the 800 mg/kg test group, 5/sex at 400 and 80 mg/kg and 10/sex for the untreated control group; Doses were administered by IP twice with 24 hr separating each dose. Bone marrow was taken after 24 and 48 hr following last treatment from HD and C mice and after 24 h from mid and low dose animals; all mice were observed daily for clinical signs. Micronuclei recorded after assessment of 1000 PCEs/mouse at all test levels; cyclophosphamide (40 mg/kg, twice) used as positive control. Statistical significance was determined by Student's t-test (1-sided), $p<0.05$.

Result :

No increases were seen in micronucleated PCE frequency in any PNA test group; toxicity to the cell population observed at 800 mg/kg @ 48h interval; elevated incidence of micronuclei with the positive control confirmed validity of method.

One death and clear signs (unresponsiveness and tremors up to 4 hr after dosing) of toxicity were noted at 800 mg/kg; at 400 mg/kg - listlessness and some tremors seen occasionally after dosing; 80 mg/kg - listlessness immediately after dosing; No effects on body weight were observed at any test level.

Test substance :

Technical grade PNA with purity > 99%.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
30.03.2004 (18)

04.04.2002

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type : Two generation study
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : F0 & F1 Adults-premating through litter weaning(F0) and postweaning (F1)
Frequency of treatm. : daily (7d/wk) gavage
Premating exposure period
Male : F0- 14 weeks; F1 - 18 weeks
Female : F0- 14 weeks; F1 - 18 weeks
Duration of test : F0 M/F -167d; F1 M/F - 216d
No. of generation studies :
Doses : 0, 0.25, 1.5 and 9 mg/kg/d
Control group : yes, concurrent vehicle
NOAEL parental : = 1.5 mg/kg bw

NOAEL F1 offspring	:	= 9 mg/kg bw
Method	:	OECD Guide-line 416 "Two-generation Reproduction Toxicity Study"
Year	:	
GLP	:	yes
Test substance	:	

Method :

Test material was given to groups of 15M and 30F rats (vehicle control group also included) to F0 and F1 generations during a premating (14 wks for F0 and 18 wks for F1) growth period, and through the ensuing mating, gestation and lactation intervals (1 litter/generation). F1 rats continued on treatment during a post-weaning period of 30d. Dosing concentrations were confirmed for accuracy Body weights were recorded weekly for F0 and F1M. For F0 and F1 F wts were recorded weekly through the growth period and up to mating, then resumed after mating until sacrifice. Food consumption was recorded weekly for F0 and F1 M from study start up to mating, then resumed after mating through study term. Food consumption for adult females F0 and F1 was recorded weekly through the growth period and again after weaning of litters. Cageside observations were made weekly, as well as daily observations of clinical signs. Temperature, humidity and light-dark cycles were controlled. F0 adults were sacrificed following weaning of the F1 litters and given a gross postmortem examination; reproductive tissues (testes, epididymides, seminal vesicles) were evaluated histopathologically for all control and high dose males. Adult M and F rats were sacrificed
following completion of a post-weaning treatment interval, given a gross necropsy, and full histopathological examination of over 40 tissues and organs (including gonads) performed on 10 randomly selected animals/sex/group. Pups delivered to F0 and F1 females were evaluated for growth, survival and external irregularities during lactation days 0, 4, 14 and 21. F1 pups not selected for the adult generation were sacrificed and given a gross postmortem
exam. Tissues were evaluated histopathologically (~40 tissues/organs) from 5/sex/group of F1 pups.

Remark :

Data from the 90-day gavage study is supporting and given in a seperate robust summary in this section.

Result :

No adverse effects observed in either F0 or F1 adults in mortality, body weights or food consumption or physical in-life evaluations. Mating indices were comparable to controls for both F0 and F1. A statistically significant reduction in pregnancy rate was observed in the 9 mg/kg F0 group vs concurrent control value, and just outside of laboratory historical control range. The male fertility index was slightly, but not statistically, lower at 9 mg/kg dose in F0. Both male and female fertility indices in F1 generation were comparable to control group at all test levels. No adverse effects were observed in mean length of gestation, no. live and dead pups at monitored time points, pup weights during lactation, pup and litter survival. No compound-related gross postmortem changes were observed during examination of any F0 or F1 adults or offspring. No microcopic changes were noted with respect to gonads evaluated on F0 adults or F1 offspring.

As the most sensitive endpoints for PNA toxicity reported in the 90-day

gavage study were met-Hb and spleen pathology. These parameters were evaluated to determine the correspondence of the 2-generation study with the 90-day gavage study. Met-Hb was not reported in the 2-generation study. The animals in the 2-generation study that has the longest exposure to test substance was the F1 generation, which was exposed by gavage from weaning to the time their offspring were weaned (roughly 22 weeks). Spleens from all dose levels were examined and minimal effects were observed at the high dose where 1/10 males and 3/10 females showed evidence of "brown pigment" in the spleen. Although the original study authors did not flag this effect as being compound related, it appears that 9 mg/kg-day can be considered a LOAEL for systemic toxicity for the females with the effect being marginal in the males.

Test substance :

Technical grade PNA (CASNO 100-01-6) with purity > 99%.

Conclusion :

The reduction in female fertility index seen in F0 adults is considered unrelated to treatment for the following reasons: No similar findings occurred in F1 Females, even though they were exposed for a substantially longer period (both in utero and during premating phase) than their F0 counterparts and there was no evidence of histological changes in gonads which could account for this finding; Similarly, no treatment-related effects were observed on the gonads of rats exposed for up to 2 years by the same dosage (9 mg/kg/d) by the same exposure route (gavage) (Nair et al FAAT 15:607-621)

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
30.03.2004 (13)

Type : other: Supporting 90-day gavage
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : 90 days
Frequency of treatm. : daily
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 0, 3, 10, 30 mg/kg/day
Control group :
NOAEL parental : < 3 mg/kg bw
Method : other: OECD 408
Year :
GLP :
Test substance :

Method :

The base methods for this study are described in the repeated-dose section of this dossier.

At the conclusion of the 90-day study the following organs related to reproductive function were examined from all high-dose and control

animals:

Pituitary
Thyroid
Prostate
Testis
Uterus
Ovary

Result :

Findings of the histopathologic examination of high-dose and control animals revealed only the following lesions in organs from the above list:

Pituitary	MALES	FEMALES
Control	autolysis 1/20	
High-dose	cyst 2/20	
Thyroid		
Control	no findings	cyst 4/19
High-dose	cyst 1/20	cyst 1/20, congestion 1/20
Prostate		
Control	inflammation 1/20	
High-dose	no findings	
Testis		
Control	no findings	
High-dose	no findings	
Uterus		
Control		no findings
High-dose		no findings
Ovary		
Control		cyst 1/20; congestion 1/20
High-dose		no findings

Spleens of all dosed groups were affected microscopically.

Test substance :

Technical grade PNA (CASNO 100-01-6) with purity > 99%.

Conclusion :

Overall NOAEL < 3 mg/kg
Reproductive organ NOAEL = 30 mg/kg

Reliability : (1) valid without restriction

Fully acceptable study (see earlier entry in this Section)

28.03.2004 (16)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : gestation days 6 through 19
Frequency of treatm. : once per day, gestation days 6-19

Duration of test	:	dosing during gestation days 6-19, sacrificed on day 20
Doses	:	25, 85, 250 mg/kg
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 25 mg/kg bw
NOAEL teratogen.	:	= 85 mg/kg bw
NOAEL Embryotoxicity	:	= 85 mg/kg bw
NOAEL Fetotoxicity	:	= 25 mg/kg bw
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	
GLP	:	yes
Test substance	:	

Method :

24 pregnant female rats per group; dosing occurred during days 6-19; vehicle used was corn oil (10 ml/kg constant volume), Corn oil vehicle control also included. Nidation data collected at sacarifice; live fetuses examined externally and by Wilson sections and skeletal exam techniques used to detect any variations or abnormalities. Body weights collected on gestation days 3, 6, 8, 13, 15, 17 and 20. Statistical methods used: body wts analyzed using Dunnett's test, Counted data (corpora lutea, implants, resorptions, live/dead pups) were analyzed using Mann-whitney U test; Response data (eg. pregnancy rates, litters with postimplantation loss, etc.) assessed with Fischer's exact test and Chi square test. ($p<0.05$ and $p<0.01$),

Result :

250 mg/kg: Reduced maternal wt gain between d6-d20, observations - pale eye coloration and occasional convulsions after dosing, significant increase in mean no. resoprtions and % resorptions, significant increase in maternal mean spleen wts (abs. and rel), significantly lower mean fetal wts (both sexes), significant increase in no. fetuses with ossif. variations and fetuses with external, soft tissue or skeletal malformations (predominantly kinked or shortened tail, absence of kidneys or ureter and fused ribs); 85 mg/kg - Significant increase in mean maternal spleen wts, significantly lower mean fetal wts (both sexes); no increases in variations or malformations; 25 mg/kg - no effects on maternal, embryo- or fetotoxicity and no increase in malformations; 25 mg/kg - no treatment-related effects on maternal, embryotoxicity, fetotoxicity or terata.

Test substance :

Technical grade p-nitroaniline (CASNO 100-01-6) with purity > 99%.

Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

29.03.2004 (21)

Species	:	rabbit
Sex	:	female
Strain	:	New Zealand white
Route of admin.	:	gavage
Exposure period	:	gestation days 7 through 19
Frequency of treatm.	:	daily

Duration of test	:	dosed from gestation day 7 through 19, sacrificed on g. day 30
Doses	:	0, 15, 75, 125 mg/kg
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 75 mg/kg bw
NOAEL teratogen.	:	= 125 mg/kg bw
NOAEL Embryotoxicity	:	= 125 mg/kg bw
NOAEL Fetotoxicity	:	= 125 mg/kg bw
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	
GLP	:	yes

Method :

18 mated females used per dose group; vehicle used was corn oil. Treated and control groups (corn oil) were dosed at constant volume of 2 ml/kg; Observations made for signs of toxicity on gestation days 0, 7, 10, 15, 19, 25 and 30; Body weights recorded on gestation days 0, 7, 19 and 30. Nidation data collected at sacrifice (gestation day 30). live fetuses examined externally and by Wilson sections and skeletal exam techniques to detect any variations or abnormalities. Statistical methods used: Bartlett's and ANOVA, Dunnett's test , Mann-whitney U test, Dunn's Rank Sum, Fischer's exact test and Jonckheere's test; $p<0.05$ and $p<0.01$.

Remark :

Supplemental information for HPV program as an adequate 2-generation study is available on PNA to fulfill the Reproductive Toxicity Endpoint.

Result :

125 mg/kg - 7/18 deaths between gestation days 14 and 20, observations - grayish appearing eyes; overall body wt gain similar to controls but higher no. of animals which lost wt during dosing observed at this test level; no increase in absol or rel spleen wt; incidence of spontaneous abortions was 4 (vs 2 for controls), however, this incidence level was frequently seen with rabbits at the test facility and thus could not be attributed to test article; no significant differences observed in mean no. implantations, resorptions or viable fetues or mean fetal wts between treated and control group; incidence and types of ossification variations in fetuses , soft tissue anomalies and external malformations were similar between treated and control groups; a slightly higher (not statistically significant) incidence in skeletal malformations was observed in treated groups vs. controls but was not considered treatment related as there was no dose response relationship for individual malformations identified in this study and they have been observed as spontaneous lesions in this rabbit strain; 75 mg/kg: observations - grayish eyes, otherwise no effects on other measured maternal, embryo, or fetal parameters. No evidence of treatment-related effect on variations or malformations; 15 mg/kg - no treatment related study findings

Test substance :

Technical grade PNA with purity of > 99%.

Reliability : (1) valid without restriction

30.03.2004 (14)

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(16) Solutia Study no. ML-79-11. Ninety-day study of p-Nitroaniline administered to male and female Sprague-Dawley rats via gavage.

(17) Solutia Study no. ML-79-11. Ninety-day study of p-Nitroaniline administered to male and female Sprague-Dawley rats via gavage; also Houser, TAP 3:128

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