Chemical
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Corporation

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October 31, 2006

Associate Director for Communications
Office of the Director
National Institutes of Health
Building 1, Room 344
9000 Rockville Pike
Bethesda, MD 20892

Subject: Chemical Products Corporation's Request for Correction of NTP Technical Report 494 - Additional information relating to the critical factual error documented in our July 17, 2006 addendum letter

Dear Madam or Sir;

Chemical Products Corporation (CPC) submits this letter in support of our Request for Correction of NTP Technical Report 494 (TR494). CPC has requested that TR494 be withdrawn because it does not meet the information quality standards required by the NIH Information Quality Guidelines. Information provided in this letter from the European IUCLID dataset strongly suggests that the negative mutagenicity assay on the TR494 test article incorporated into the third and final draft TR494 in late 2004, cannot be representative of an Anthraquinone sample containing about 0.1% 9-nitroanthracene contaminant. The TR494 test article has been acknowledged by NIEHS to have been contaminated with “about 0.1%” of 9-nitroanthracene, a mutagen.

This letter presents mutagenicity assay data provided by Zenica Specialties for inclusion in the IUCLID dataset. IUCLID, the **I**nternational **U**niform **C**hemica**L**

**I**nformation **D**atabase, contains data reported by European Industry within the framework of the European existing chemicals risk assessment program. The mutagenicity assay data in the IUCLID dataset is inconsisent with the mutagenicity assay data generated by NTP on an aliquot of TR494 test article submitted for assay in June 2004 and incorporated into the third and final draft TR494 presented on December 9, 2004 to the Technical Reports Review Subcommittee as the basis for vacating the February 18, 2004 draft TR494 and the directives of the February 18, 2004 peer review panel.

The mutagenicity test results presented on page 35 of the IUCLID dataset demonstrate the high probability that the aliquot of the TR494 test article submitted for Ames mutagenicity assay by NTP in June 2004, which had been stored at room temperature under air for at least 7 years prior to testing, was found to be non-mutagenic because of decomposition of the mutagenic impurities present in the test article when it was administered to animals in the mid-1990's.

NTP submitted an aliquot of TR494 test article for mutageinicity assay in June 2004. This aliquot is reported in TR494 to be non-mutagenic in Salmonella typhimurium strains TA98, TA100, and TA1537 both without and with metabolic activation.

The NTP Technical Report 494 (TR494) test article has been acknowledged by NIEHS to have contained “about 0.1%” of the mutagenic contaminant 9-nitroanthracene when it was analyzed by NTP years before submission of the TR494 aliquot for mutagenicity assay in June, 2004. The mutagenicity assay data on page 35 of the enclosed IUCLID dataset, and presented below, states that “equivocal” mutagenicity was observed in TA1537 for an AQ sample containing only 0.032% 9-nitroanthracene contamination, while no mutagenicity was observed in TA1537 for an AQ sample containing 0.005% 9-nitroanthracene contamination.

Ames test

Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100

Metabolic Activation: with

Result: positive

Sample known to contain 0.032 % 9-Nitroanthracene positive in strains TA 1535, TA 1538, equivocal in strain TA 1537

Zeneca Specialties, Manchester
Bayer AG Leverkusen

(60)

Ames test

Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100

Metabolic Activation: with

Result: negative

Sample known to contain 0.005 % 9-Nitroanthracene

Zeneca Specialties, Manchester
Bayer AG Leverkusen

(61)

TR494 reports that no mutagenicity was observed when an aliquot of TR494 test article was tested in TA1537 with metabolic activation.

NIEHS Deputy Director Samuel H. Wilson stated in a letter to CPC dated September 8, 2003 that he had reviewed data and ongoing tests with the staff of NIEHS' Environmental Toxicology Program who were responsible for the TR494 studies and the first draft TR494 which had been peer reviewed in May,1999. He further stated that he had been assisted with his review by staff from the NIEHS Office of Policy, Planning and Evaluation. As a result of his review, Dr. Wilson concluded that the TR494 test article “was contaminated with 9-nitroanthracene at

a level of about 0.1%" and that "the presence of this contaminant raises doubt as to the effect(s) of anthraquinone itself, or its metabolites, and confounds interpretation of the NTP studies referenced in draft TR-494". He withdrew the 1999 draft TR494. A copy of Dr. Wilson's letter is enclosed.

An extensively revised second draft TR494 underwent peer review on February 18, 2004. This second draft TR494 acknowledged that the TR494 test article had been found to be mutagenic in Salmonella typhimurium strains TA98 and TA100 without metabolic activation and in TA98 with S9 metabolic activation; this observed mutagenicity in the TR494 test article was attributed to 9-nitroanthracene contamination in the TR494 test article. A comment letter submitted by Orn Adalsteinsson on February 2, 2004, and enclosed with this letter, presents analytical data showing that the TR494 test article contained other significant impurities in addition to the "about 0.1%" 9-nitroanthracene acknowledged to be present by NIEHS. Adalsteinsson's analysis determined the TR494 test article to be 99.4% pure AQ. An analysis conducted for NTP on an aliquot of the TR494 test article on November 19 and 20, 1998 - obtained by CPC under a Freedom of Information Act request - also states, "99.4% relative purity" although this analysis is not disclosed in TR494 (see the attachment to our July 13, 2006 letter and attachments 1d and 2 to our July 17, 2006 letter submitted as parts of this Request for Correction of TR494).

CPC has previously submitted NTP records, obtained by CPC under a Freedom of Information Act request, demonstrating that the TR494 aliquot submitted for mutagenicity assay in June 2004 had been stored at room temperature under air since animal testing had ended in 1996 or early 1997. The mutagenicity assay data from the IUCLID dataset indicates that the 9-nitroanthracene contaminant level would have to have been well below about 0.03% in the TR494 test article for no mutagenic response to have been detected in TA1537. TR494 reports no increase in reventants observed in TA1537 with 10%S9 activation – 7 at 0 µg/plate and 7 at 10,000 µg/plate in propylene glycol, and 10 at 0 µg/plate and 11 at 10,000 µg/plate in dimethylsulfoxide. Thus, decomposition of more that 70% of the mutagenic

contaminant 9-nitroanthracene acknowledged by NIEHS to have been present in the TR494 test material appears likely to have occurred prior to the June 2004 mutagenicity assay conducted by NTP.

In the draft TR494 submitted for peer review on February 18, 2004, NTP argued that the 9-nitroanthracene contamination had not confounded the interpretation of the TR494 studies and, thus, that the conclusions presented in the 1999 draft TR494 and presented unchanged in this second draft TR494 should be accepted as written. The NTP Board of Scientific Counselors Technical Reports Review Subcommittee did not find these arguments persuasive. The February 18, 2004 peer review panel directed that TR494 unequivocally restrict the conclusions presented in TR494 to "anthracene-derived anthraquinone". Anthracene-derived anthraquinone (produced by the oxidation of anthracene process) has been replaced completely in commerce in the United States with Anthraquinone produced by two manufacturing processes which do not involve anthracene - the Friedel-Crafts process and the Diels-Alder process.

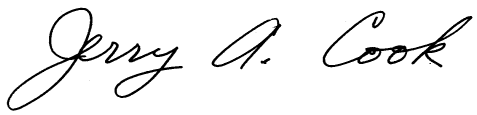
In the fall of 2004, a third draft TR494 was prepared based upon new data indicating that the TR494 test article was not mutagenic. This third draft TR494 was submitted for peer review on December 9, 2004. CPC has previously submitted documentation of a critical factual error contained in TR494 – the peer review panel was told the aliquot of TR494 test article submitted for mutagenicity assay in June 2004 had been stored "frozen under argon" during the 7-plus years between the time the test article was administered to animals and the time that aliquot was submitted for mutagenicity assay, so decomposition of impurities in the aliquot was "unlikely". Documents obtained by CPC under the Freedom of Information Act, and submitted earlier with our Request for Correction, demonstrate that the aliquot submitted for mutagenicity assay in June 2004 had been stored at room temperature under air.

The negative finding in the June 2004 mutagenicity assay of the TR494 test article was the sole basis for vacating the second draft TR494 peer reviewed on February 18, 2004 and the restrictions placed on the TR494 conclusions by the February 18, 2004 peer review panel. The February 18, 2004 peer review panel concluded that contamination of the TR494 test article with at least one mutagenic contaminant had confounded interpretation of the TR494 studies to such an extent that the conclusions presented in TR494 should be applicable only to “anthracene-derived anthraquinone”. The TR494 test article was produced by the oxidation of anthracene and the peer review panel apparently assumed that all anthracene-derived anthraquinone would be likely to be contaminated with mutagens.

In summary, the TR494 test article has been acknowledged by NIEHS to contain “about 1%” of mutagenic 9-nitroanthracene. The aliquot of TR494 test article submitted for mutagenicity assay in June 2004 was reported to be non-mutagenic in TA98, TA100, and TA1537. Zenica Specialties, the source for NTP's TR494 test article, submitted data to the IUCLID dataset showing that Anthraquinone contaminated with only 0.032% 9-nitroanthracene was mutagenic to Salmonella typhimurium TA 1535 and TA1539 and showed equivocal mutagenicity in TA1537. CPC submitted an aliquot of TR494 test article for preincubation mutageinicity assay in 1999, it was found to be mutagenic in TA98 and TA100. Butterworth et al. submitted another aliquot of TR494 test article for mutagenicity assay in 2000; it was found to be mutagenic in TA98 and TA100. The December 9, 2004 peer review panel approved the conclusions in TR494 based upon NTP's assertion that the TR494 test article was non-mutagenic when it was administered to animals in the mid-1990's. The information contained in the IUCLID dataset and included with this letter is additional evidence that the critical factual error concerning the storage of the aliquot of TR494 test article submitted by NTP for mutagenicity assay in June 2004 is sufficient to justify withdrawal of TR494 because it fails to meet the requirements of NIH's Information Quality Guidelines.

Thank you for your attention to this matter. If I can answer any questions concerning this letter or CPC's Request for Correction of TR494, please telephone me at 770-382-2144 Ext. 272 or 770-714-3806 (cell), or email me at jcook@cpc-us.com.

Sincerely,



Jerry A. Cook, Technical Director
Chemical Products Corporation

Enclosures

IUCLID dataset for Anthraquinone
Letter from NIEHS Deputy Director Samuel H. Wilson dated Sept. 8, 2003
Comments submitted to NTP by Orn Adalsteinsson dated February 2, 2004

# I U C L I D

# D a t a s e t

| | |
|---|---|
| **Existing Chemical** | Substance ID: 84-65-1 |
| **CAS No.** | 84-65-1 |
| **EINECS Name** | anthraquinone |
| **EINECS No.** | 201-549-0 |
| **Molecular Formula** | C14H8O2 |

**Dataset created by:** EUROPEAN COMMISSION - European Chemicals Bureau

**This dossier is a compilation based on data reported by the European Chemicals Industry following 'Council Regulation (EEC) No. 793/93 on the Evaluation and Control of the Risks of Existing Substances'. All (non-confidential) information from the single datasets, submitted in the IUCLID/HEDSET format by individual companies, was integrated to create this document.**

**The data have not undergone any evaluation by the European Commission.**

**Creation date:** 19-FEB-2000

**Number of Pages:** **59**

**Chapters:** **all**

**Edition:** **Year 2000 CD-ROM edition**

**Flags:** **non-confidential**

## 1.0.1 OECD and Company Information

**Name:** ACNA C.O.
**Town:** 17010 Cengio (SV)
**Country:** Italy

**Name:** B.V. CONSOLCO
**Street:** De Ruyterkade 44
**Town:** 1012 AA Amsterdam
**Country:** Netherlands
**Phone:** 020-6221444
**Telefax:** 020-6254449
**Telex:** 12458

**Name:** BASF AG
**Street:** Karl-Bosch-Str
**Town:** 67056 Ludwigshafen
**Country:** Germany

**Name:** Bayer AG
**Town:** 51368 Leverkusen
**Country:** Germany

**Name:** Organic Chemicals srl / ACNA Chimica Organica
**Street:** Piazza della Vittoria, 10
**Town:** 17010 Cengio (Savona)
**Country:** Italy
**Phone:** 019-5561
**Telefax:** 019-555049
**Telex:** 273876

**Name:** RÜTGERS VFT Handel GmbH
**Street:** Varziner Straße 49
**Town:** D-47138 Duisburg
**Country:** Germany
**Phone:** 0049(0)203/4296-01
**Telefax:** 0049(0)203/4296-328

**Name:** ZENECA Specialties
**Street:** PO Box 42
**Town:** M9 3DA Manchester
**Country:** United Kingdom

## 1.0.2 Location of Production Site

-

## 1.0.3 Identity of Recipients

-

## 1.1 General Substance Information

**Substance type:** organic
**Physical status:** solid

### 1.1.1 Spectra

-

## 1.2 Synonyms

9,10-ANTHRACENDION
**Source:** Bayer AG Leverkusen

9,10-Anthracenedione
**Source:** B.V. CONSOLCO Amsterdam
RÜTGERS VFT Handel GmbH Duisburg

9,10-ANTHRACENEDIONE
**Source:** Bayer AG Leverkusen

9,10-Anthracenedione (9CI)
**Source:** BASF AG Ludwigshafen

9,10-ANTHRAQUINONE
**Source:** ACNA C.O. Cengio (SV)
Organic Chemicals srl / ACNA Chimica Organica Cengio (Savona)

9,10-Anthraquinone
**Source:** BASF AG Ludwigshafen

9,10-DIOXOANTHRACEN
**Source:** Bayer AG Leverkusen

9,10-DIOXOANTHRACENE
**Source:** ACNA C.O. Cengio (SV)
Organic Chemicals srl / ACNA Chimica Organica Cengio (Savona)

ANTHRACEN-9,10-CHINON
**Source:** Bayer AG Leverkusen

ANTHRACENE, 9,10-DIHYDRO-9,10-DIOXO-
**Source:** ACNA C.O. Cengio (SV)
Organic Chemicals srl / ACNA Chimica Organica Cengio (Savona)

Anthracene-9,10-quinone
**Source:** BASF AG Ludwigshafen

ANTHRACHINON
**Source:** Bayer AG Leverkusen

ANTHRADIONE
**Source:** ACNA C.O. Cengio (SV) Organic Chemicals srl / ACNA Chimica Organica Cengio (Savona)

Anthradione
**Source:** BASF AG Ludwigshafen

ANTHRAQUINONE
**Source:** ACNA C.O. Cengio (SV) Organic Chemicals srl / ACNA Chimica Organica Cengio (Savona)

Anthraquinone
**Source:** ZENECA Specialties Manchester

Anthraquinone (8CI)
**Source:** BASF AG Ludwigshafen

CORBIT
**Source:** ACNA C.O. Cengio (SV) Organic Chemicals srl / ACNA Chimica Organica Cengio (Savona)

DIPHENYLENKETON
**Source:** Bayer AG Leverkusen

HOELITE
**Source:** ACNA C.O. Cengio (SV) Organic Chemicals srl / ACNA Chimica Organica Cengio (Savona)

Hoelite
**Source:** BASF AG Ludwigshafen

MORKIT
**Source:** ACNA C.O. Cengio (SV) Organic Chemicals srl / ACNA Chimica Organica Cengio (Savona)

Morkit
**Source:** BASF AG Ludwigshafen

**Source:** ZENECA Specialties Manchester

## 1.3 Impurities

-

## 1.4 Additives

-

## 1.5 Quantity

**Quantity** 10 000 - 50 000 tonnes

## 1.6.1 Labelling

-

## 1.6.2 Classification

-

## 1.7 Use Pattern

**Type:** type
**Category:** Non dispersive use

**Type:** type
**Category:** Use in closed system

**Type:** type
**Category:** Wide dispersive use

**Type:** industrial
**Category:** Agricultural industry

**Type:** industrial
**Category:** Chemical industry: used in synthesis

**Type:** industrial
**Category:** Paper, pulp and board industry

**Type:** industrial
**Category:** other

**Type:** use
**Category:** Colouring agents

**Type:** use
**Category:** Intermediates

**Type:** use
**Category:** Pesticides

**Type:** use
**Category:** Process regulators

**Type:** use
**Category:** other: in ACNA C.O. / Organic Chemicals Srl, il 9,10-anthracenedione, veniva utilizzato come materia prima, nella sintesi dell'1-anthracenesulfonic acid-9,10-dihydro-9,10-dioxo-, ammonium salt (n°CAS: 55812-59-4; n°EINECS: 2598361).

**Type:** use
**Category:** other: in ACNA C.O. viene utilizzato come prodotto intermedio nella sintesi dell'acido antrachinon-1-solfonico, sale ammonico (1-Anthracene Sulfonic Acid-9,10-Dihydro-9,10-Dioxo Ammonium Salt; n° CAS 55812-59-4 e n° EINECS 2598361).

**Type:** use
**Category:** other: intermedio per coloranti e antiparassitari; come ausiliario nella stampa dei tessuti.

**Type:** use
**Category:** other: prodotto di partenza o intermedio chimico nella preparazione di alcuni coloranti, pigmenti e composti organici; in agricoltura nelle semine come repellente per gli uccelli.

**Type:** use
**Category:** other

## 1.7.1 Technology Production/Use

-

## 1.8 Occupational Exposure Limit Values

**Type of limit:** other
**Limit value:**
**Remark:** Opmerking: andere = onbekend
**Source:** B.V. CONSOLCO Amsterdam

**Type of limit:** other
**Limit value:**
**Remark:** no limit value
**Source:** RÜTGERS VFT Handel GmbH Duisburg

**Type of limit:** other: MAC (Unione Sovietica)
**Limit value:** 5 mg/m3
**Remark:** Grandezza MAC (Unione Sovietica): 5 mg/m3
Stato prevalente di aggregazione nell'aria all'atto della fabbricazione: a (aerosol)
Classe di pericolosita': sostanza nociva moderatamente pericolosa.
**Source:** ACNA C.O. Cengio (SV)
Organic Chemicals srl / ACNA Chimica Organica Cengio (Savona)

(1)

**Type of limit:** other: TLV (ACNA C.O. / Organic Chemicals Srl)
**Limit value:** 1 mg/m3
**Remark:** Per il 9,10-anthracenedione, in assenza di un limite di esposizione professionale proposto dall' ACGIH, veniva preso come limite di riferimento cautelativo interno ACNA C.O. / Organic Chemicals Srl., il valore TLV/TWA = 1 mg/m3 (x 8 ore lavorative).
**Source:** Organic Chemicals srl / ACNA Chimica Organica Cengio (Savona)

(2)

**Type of limit:** other: TLV (ACNA C.O.)
**Limit value:** 1 mg/m3
**Remark:** Non sono noti i limiti di esposizione professionale per la sostanza proposti dall'ACGIH.

In ACNA C.O. viene preso come limite di riferimento interno il valore TLV/TWA = 1 mg/mc.

Monitoraggio Ambientale (dal 1988 al 1993).

Nel ambito del impianto sono state individuate le aree di lavoro e i fattori di rischio pertinenti e, all'interno di ciascuna area, sono state individuate posizioni di campionamento rappresentative della stessa tenendo conto: . della natura e dello stato fisico della sostanza in esame; . delle lavorazioni svolte e della dislocazione delle apparecchiature e delle possibili sorgenti di emissione; . delle caratteristiche strutturali dei locali.

Il valore medio e la punta massima (concentrazione in mg/mc) calcolati sul totale delle misure effettuate nell'impianto di produzione, sono pari a:

Reparto: Acidi Lettera/Sale ALfa
Sostanza: antrachinone
TLV-TWA: 1 mg/mc
Metodica: DC-FGS * 1 l/min * HPLC

. numero aree di lavoro indagate: 2
. numero totale misure effettuate: 17
. valore medio: 0.131 mg/mc
. valore massimo: 0.774 mg/mc
**Source:** ACNA C.O. Cengio (SV)
**Test substance:** Come ai punti 1.1-1.4.

(3)

## 1.9 Source of Exposure

**Remark:** La concentrazione massima teorica calcolata a livello del suolo, all'interno dello stabilimento, considerando le emissioni e le successive ricadute, e' pari a 1.4 g/mc; la concentrazione reale riscontrata e' inferiore a 1 g/mc.
**Source:** ACNA C.O. Cengio (SV)
**Test substance:** Come ai punti 1.1-1-4.

(4)

**Source:** Organic Chemicals srl / ACNA Chimica Organica Cengio (Savona)

**Remark:** no data available due to imported and commercial product
**Source:** RÜTGERS VFT Handel GmbH Duisburg

## 1.10.1 Recommendations/Precautionary Measures

-

## 1.10.2 Emergency Measures

-

## 1.11 Packaging

-

## 1.12 Possib. of Rendering Subst. Harmless

-

## 1.13 Statements Concerning Waste

-

## 1.14.1 Water Pollution

**Classified by:** other: Bayer AG
**Labelled by:** other: Bayer AG
**Class of danger:** 1 (weakly water polluting)
**Source:** Bayer AG Leverkusen

## 1.14.2 Major Accident Hazards

**Legislation:**
**Substance listed:** no
**Source:** Bayer AG Leverkusen

### 1.14.3 Air Pollution

**Classified by:** other: Bayer AG
**Labelled by:** other: Bayer AG
**Number:** 3.1.7 (organic substances)
**Class of danger:** III
**Remark:** Dust 3.1.3
**Source:** Bayer AG Leverkusen

### 1.15 Additional Remarks

**Remark:** no data available due to imported and commercial product
**Source:** RÜTGERS VFT Handel GmbH Duisburg

### 1.16 Last Literature Search

-

### 1.17 Reviews

-

### 1.18 Listings e.g. Chemical Inventories

-

## 2.1 Melting Point

**Value:** 284 degree C
**Source:** Bayer AG Leverkusen
(5)

**Value:** 287 degree C
**Remark:** Pour point
**Source:** Bayer AG Leverkusen
(6)

## 2.2 Boiling Point

**Value:** 377 degree C at 1013 hPa
**Source:** Bayer AG Leverkusen
(5)

**Value:** 379.8 degree C
**Source:** Bayer AG Leverkusen
(6)

## 2.3 Density

**Type:** density
**Value:** 1.44 g/cm3 at 20 degree C
**Source:** Bayer AG Leverkusen
(5)

**Type:** bulk density
**Value:** 500 - 700 kg/m3 at 20 degree C
**Source:** Bayer AG Leverkusen
(5)

## 2.3.1 Granulometry

-

## 2.4 Vapour Pressure

**Value:** .000013 hPa at 68.8 degree C
**Source:** Bayer AG Leverkusen
(6)

**Value:** 1.3 hPa at 190 degree C
**Source:** Bayer AG Leverkusen
(5)

## 2.5 Partition Coefficient

**log Pow:** 2.7
**Method:** other (calculated): Leo, A.: CLOGP-3.54 MedChem Software 1989. Daylight, Chemical Information Systems, Claremont, CA 91711, USA
**Year:**
**Source:** Bayer AG Leverkusen

(7)

**log Pow:** 3.39
**Method:**
**Year:**
**Remark:** experimentally determined
**Source:** Bayer AG Leverkusen

(8)

## 2.6.1 Water Solubility

**Value:** .125 mg/l at 22 degree C
**Source:** Bayer AG Leverkusen

(5)

## 2.6.2 Surface Tension

-

## 2.7 Flash Point

**Value:** 185 degree C
**Type:**
**Method:** other: Open cup
**Year:**
**Source:** Bayer AG Leverkusen

(9)

## 2.8 Auto Flammability

**Value:**
**Remark:** ignition temperature: 650 degree C
**Source:** Bayer AG Leverkusen

(6)

## 2.9 Flammability

-

## 2.10 Explosive Properties

-

## 2.11 Oxidizing Properties

-

## 2.12 Additional Remarks

**Remark:** Saturation concentration: 0.0001 g/cm3 (68.8 degree C)
**Source:** Bayer AG Leverkusen

(6)

### 3.1.1 Photodegradation

-

### 3.1.2 Stability in Water

-

### 3.1.3 Stability in Soil

-

### 3.2 Monitoring Data (Environment)

-

### 3.3.1 Transport between Environmental Compartments

-

### 3.3.2 Distribution

-

### 3.4 Mode of Degradation in Actual Use

-

### 3.5 Biodegradation

**Type:** aerobic
**Inoculum:** activated sludge, domestic
**Concentration:** 20 mg/l related to Test substance
**Degradation:** 75 % after 24 day
**Method:** OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
**Year:** **GLP:** no
**Test substance:**
**Remark:** Related to CO2-evolution
**Source:** Bayer AG Leverkusen

(10)

**Type:** aerobic
**Inoculum:** predominantly domestic sewage
**Concentration:** 100 mg/l related to Test substance
**Degradation:** 93 % after 25 day
**Method:** OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
**Year:** **GLP:** no
**Test substance:**
**Remark:** Related to O2-demand
**Source:** Bayer AG Leverkusen

(10)

**Type:** aerobic
**Inoculum:** predominantly domestic sewage, adapted
**Concentration:** .8 mg/l related to Test substance
**Degradation:** > 70 % after 20 day
**Method:** OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
**Year:** 1977 **GLP:** no
**Test substance:**
**Remark:** Related to BOD
**Source:** Bayer AG Leverkusen

(11)

**Type:** aerobic
**Inoculum:**
**Concentration:** 100 mg/l
**Degradation:** 42 % after 28 day
**Method:** other: UK-MITI test (manometric respirometry) for biodegradability testing of poorly soluble compounds
**Year:** **GLP:**
**Test substance:**
**Remark:** sonification in the test flask; related to ThOD
**Source:** Bayer AG Leverkusen

(12)

**Type:** aerobic
**Inoculum:**
**Concentration:** 100 mg/l
**Degradation:** 46 % after 28 day
**Method:** other: UK-MITI test (manometric respirometry) for biodegradability testing of poorly soluble compounds
**Year:** **GLP:**
**Test substance:**
**Remark:** direct addition; related to ThOD
**Source:** Bayer AG Leverkusen

(12)

**Type:**
**Inoculum:** predominantly domestic sewage
**Concentration:** related to DOC (Dissolved Organic Carbon)
**Degradation:** 70 % after 14 day
**Method:** other: modif. Repetitive Die Away (EG DG11/400/84 Rev. 1)
**Year:** **GLP:** no
**Test substance:**
**Source:** Bayer AG Leverkusen

(10)

## 3.6 BOD5, COD or BOD5/COD Ratio

**Remark:** COD 2310/2300 mg/l
**Source:** Bayer AG Leverkusen

(13)

## 3.7 Bioaccumulation

-

## 3.8 Additional Remarks

-

## AQUATIC ORGANISMS

### 4.1 Acute/Prolonged Toxicity to Fish

**Type:** flow through
**Species:** Pimephales promelas (Fish, fresh water)
**Exposure period:** 96 hour(s)
**Unit:** mg/l **Analytical monitoring:** yes
**LC0:** .24
**Method:**
**Year:** **GLP:**
**Test substance:** other TS: 97 %
**Remark:** length 18.5 mm, weight 0.086 g, age 29 d,
only conc. tested (96% saturated solution)
analytic monitoring: HPLC
**Source:** Bayer AG Leverkusen

(14)

**Type:** static
**Species:** Lepomis macrochirus (Fish, fresh water)
**Exposure period:** 24 hour(s)
**Unit:** mg/l **Analytical monitoring:**
**LC0:** >= 5
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** Fingerlings >= 10 cm, only conc. tested
**Source:** Bayer AG Leverkusen

(15)

**Type:** static
**Species:** Oncorhynchus kisutch (Fish, fresh water, marine)
**Exposure period:**
**Unit:** mg/l **Analytical monitoring:**
**LC100:** 10
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** length 5-10 cm; exposure period 5-9 h
**Source:** Bayer AG Leverkusen

(16)

**Type:** static
**Species:** Oncorhynchus mykiss (Fish, fresh water)
**Exposure period:** 24 hour(s)
**Unit:** mg/l **Analytical monitoring:**
**LC0:** >= 5
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** Fingerlings >= 10 cm, only conc. tested
**Source:** Bayer AG Leverkusen

(15)

**Type:** static
**Species:** Oncorhynchus tschawytscha (Fish, fresh water, marine)
**Exposure period:**
**Unit:** mg/l **Analytical monitoring:**
**LC100:** 10
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** length 5-10 cm; exposure period 5-9 h
only conc. tested
**Source:** Bayer AG Leverkusen

(16)

**Type:** static
**Species:** Petromyzon marinus
**Exposure period:** 24 hour(s)
**Unit:** mg/l **Analytical monitoring:**
**LC0:** >= 5
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** larvae 8-13 cm, only conc. tested
**Source:** Bayer AG Leverkusen

(15)

**Type:** static
**Species:** Ptychocheilus oregonensis (Fish, fresh water)
**Exposure period:**
**Unit:** mg/l **Analytical monitoring:**
**LC100:** 10
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** length 5-10 cm; exposure period 9-13 h
lost of equilibrium in 5-9 h; only conc. tested
**Source:** Bayer AG Leverkusen

(16)

**Type:**
**Species:** Pimephales promelas (Fish, fresh water)
**Exposure period:** 96 hour(s)
**Unit:** mg/l **Analytical monitoring:**
**LC50:** 2650
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Bayer AG Leverkusen

(17)

## 4.2 Acute Toxicity to Aquatic Invertebrates

**Species:** Daphnia magna (Crustacea)
**Exposure period:** 30 day
**Unit:** mg/l **Analytical monitoring:**
**EC0:** 1
**EC100:** 5
**Method:** other: semi-static, 17.5-20 degree Celsius
**Year:** **GLP:** no
**Test substance:**
**Remark:** survival, reproduction, physiolog. parameters
**Source:** Bayer AG Leverkusen

(18)

**Species:** other aquatic arthropod: Chydorus
**Exposure period:** 30 day
**Unit:** mg/l **Analytical monitoring:**
**EC0:** 1
**Method:** other: semi-static, 20-25 degree Celsius
**Year:** **GLP:** no
**Test substance:**
**Remark:** survival, reproduction
**Source:** Bayer AG Leverkusen

(18)

**Species:** other aquatic arthropod: Daphnia longispina
**Exposure period:** 30 day
**Unit:** mg/l **Analytical monitoring:**
**EC0:** 1
**Method:** other: semi-static, 18-21 degree Celsius
**Year:** **GLP:** no
**Test substance:**
**Remark:** survival, reproduction
**Source:** Bayer AG Leverkusen

(18)

**Species:** other aquatic arthropod: Daphnia longispina
**Exposure period:** 16 day
**Unit:** mg/l **Analytical monitoring:**
**EC0:** 1
**Method:** other: semi-static, 20-23 degree Celsius
**Year:** **GLP:** no
**Test substance:**
**Remark:** survival, reproduction
**Source:** Bayer AG Leverkusen

(18)

## 4.3 Toxicity to Aquatic Plants e.g. Algae

-

## 4.4 Toxicity to Microorganisms e.g. Bacteria

**Type:** aquatic
**Species:** activated sludge
**Exposure period:** 3 hour(s)
**Unit:** mg/l **Analytical monitoring:** no
**EC50:** 7264
**Method:** ISO 8192 "Test for inhibition of oxygen consumption by activated sludge"
**Year:** 1988 **GLP:** no
**Test substance:** other TS: techn.grade 99.5 %
**Source:** Bayer AG Leverkusen

(11)

**Type:** aquatic
**Species:** Pseudomonas fluorescens (Bacteria)
**Exposure period:** 24 hour(s)
**Unit:** mg/l **Analytical monitoring:** no
**EC0:** 5000
**Method:** other: Bestimmung der biologischen Schadwirkung toxischer Abwaesser gegen Bakterien. DEV, L 8 (1968) modifiziert.
**Year:** 1976 **GLP:** no
**Test substance:**
**Source:** Bayer AG Leverkusen

(11)

## 4.5 Chronic Toxicity to Aquatic Organisms

### 4.5.1 Chronic Toxicity to Fish

-

### 4.5.2 Chronic Toxicity to Aquatic Invertebrates

-

## TERRESTRIAL ORGANISMS

### 4.6.1 Toxicity to Soil Dwelling Organisms

-

### 4.6.2 Toxicity to Terrestrial Plants

-

### 4.6.3 Toxicity to other Non–Mamm. Terrestrial Species

-

### 4.7 Biological Effects Monitoring

-

### 4.8 Biotransformation and Kinetics

-

### 4.9 Additional Remarks

**Remark:** Agelaius phoeniceus (red-winged blackbird)
LD 50: 100-300 mg/kg
**Source:** Bayer AG Leverkusen
(19)

**Remark:** Anthraquinone has an effect as repellent on Pyrrhula pyrrhula (bullfinch)(field test in fruit plantages)
**Source:** Bayer AG Leverkusen
(20)

## 5.1 Acute Toxicity

### 5.1.1 Acute Oral Toxicity

**Type:** LD50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** > 5000 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** no deaths
**Source:** Bayer AG Leverkusen
(21) (22) (23) (24)

**Type:** LD50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** > 5000 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** mortality after administration of 5000 mg/kg bw: 1/10
**Source:** Bayer AG Leverkusen
(25)

**Type:** LD50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** > 5000 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** mortality after administration of 5000 mg/kg bw: 0/15 in male rats and 2/15 in female rats
**Source:** Bayer AG Leverkusen
(26)

**Type:** LD50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** > 10000 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** no deaths
**Source:** Bayer AG Leverkusen
(27)

**Type:** LD50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** > 20000 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Bayer AG Leverkusen
(28)

**Type:** LDLo
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** = 15000 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Bayer AG Leverkusen
(29)

**Type:** LD50
**Species:** mouse
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** > 5000 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** no deaths
**Source:** Bayer AG Leverkusen
(26)

**Type:** LD50
**Species:** sheep
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** 150 - 300 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** 1 male and 1 female test animal were used per dose level after administration of 150 mg/kg bw no deaths occurred, after administration of 300 mg/kg bw the female test animal died after 6 d and the male animal was sacrificed 8 d after administration of the test substance
**Source:** Bayer AG Leverkusen

(30)

## 5.1.2 Acute Inhalation Toxicity

**Type:** LC50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Exposure time:** 4 hour(s)
**Value:** > 1.327 mg/l
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** no deaths
**Source:** Bayer AG Leverkusen

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**Type:** LC50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Exposure time:** 4 hour(s)
**Value:** > .244 mg/l
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** no deaths
**Source:** Bayer AG Leverkusen

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## 5.1.3 Acute Dermal Toxicity

**Type:** LD50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** > 1000 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** exposure time: 7 d
no deaths
**Source:** Bayer AG Leverkusen
(33)

**Type:** LD50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** > 500 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** no deaths
**Source:** Bayer AG Leverkusen
(26)

**Type:** LD50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** > 5000 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** no deaths
**Source:** Bayer AG Leverkusen
(31)

**Type:** other: LD50
**Species:** rabbit
**Sex:**
**Number of Animals:**
**Vehicle:**
**Value:** > 3000 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** no deaths
**Source:** Bayer AG Leverkusen

(34)

## 5.1.4 Acute Toxicity, other Routes

**Type:** LD50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Route of admin.:** i.p.
**Value:** > 5000 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** mortality after administration of 5000 mg/kg bw: 1/15 in male rats and 3/15 in female rats
**Source:** Bayer AG Leverkusen

(26)

**Type:** LD50
**Species:** rat
**Sex:**
**Number of Animals:**
**Vehicle:**
**Route of admin.:** i.p.
**Value:** = 3500 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Bayer AG Leverkusen

(29) (35) (36)

## 5.2 Corrosiveness and Irritation

## 5.2.1 Skin Irritation

**Species:** rabbit
**Concentration:**

**Exposure:**
**Exposure Time:**
**Number of Animals:**
**PDII:**
**Result:** not irritating
**EC classificat.:**
**Method:** other: see remarks
**Year:** **GLP:**
**Test substance:** other TS: dispersion of 9.10-anthraquinone
**Remark:** method: exposure time: 24 h, ear, dose: ca. 500 ul/animal, semi-occlusive, observation period: 7 d
**Source:** Bayer AG Leverkusen

(37)

**Species:** rabbit
**Concentration:**

**Exposure:**
**Exposure Time:**
**Number of Animals:**
**PDII:**
**Result:** not irritating
**EC classificat.:**
**Method:** other: see remarks
**Year:** **GLP:**
**Test substance:**
**Remark:** method: exposure time: 24 h, ear, semi-occlusive, observation period: 7 d
**Source:** Bayer AG Leverkusen

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**Species:** rabbit
**Concentration:**

**Exposure:**
**Exposure Time:**
**Number of Animals:**
**PDII:**
**Result:** not irritating
**EC classificat.:**
**Method:** other: see remarks
**Year:** **GLP:**
**Test substance:**
**Remark:** method: exposure time: 24 h, flank, dose: 0.5 g/animal, semi-occlusive, observation period: 3 d
**Source:** Bayer AG Leverkusen

(31)

**Species:** rabbit
**Concentration:**

**Exposure:**
**Exposure Time:**
**Number of Animals:**
**PDII:**
**Result:** not irritating
**EC classificat.:**
**Method:** OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
**Year:** **GLP:**
**Test substance:**
**Source:** Zeneca Specialties, Manchester
Bayer AG Leverkusen

(39)

**Species:** human
**Concentration:**

**Exposure:**
**Exposure Time:**
**Number of Animals:**
**PDII:**
**Result:**
**EC classificat.:**
**Method:** other: see remarks
**Year:** **GLP:**
**Test substance:**
**Remark:** 8 male probands were tested; none of them showed signs of skin irritation
method: exposure time: 24 h, upper arm, semi-occlusive, observation period: 7 d
**Source:** Bayer AG Leverkusen

(38)

**Species:** other: no data
**Concentration:**

**Exposure:**
**Exposure Time:**
**Number of Animals:**
**PDII:**
**Result:**
**EC classificat.:**
**Method:** other: see remarks
**Year:** **GLP:**
**Test substance:**
**Remark:** method: a 15 % solution of the test substance was applied 30 times to the skin (no details)
no effects observable
**Source:** Bayer AG Leverkusen

(35)

## 5.2.2 Eye Irritation

**Species:** rabbit
**Concentration:**
**Dose:**
**Exposure Time:**
**Comment:**
**Number of Animals:**
**Result:** not irritating
**EC classificat.:**
**Method:** other: see remarks
**Year:** **GLP:**
**Test substance:** other TS: dispersion of 9.10-anthraquinone
**Remark:** method: dose: ca. 100 ul/animal, observation period: 7 d
**Source:** Bayer AG Leverkusen

(37)

**Species:** rabbit
**Concentration:**
**Dose:**
**Exposure Time:**
**Comment:**
**Number of Animals:**
**Result:** not irritating
**EC classificat.:**
**Method:** other: see remarks
**Year:** **GLP:**
**Test substance:**
**Remark:** method: eyes rinsed with water 1 h after application of the test substance, observation period: 7 d
**Source:** Bayer AG Leverkusen

(38)

**Species:** rabbit
**Concentration:**
**Dose:**
**Exposure Time:**
**Comment:**
**Number of Animals:**
**Result:** not irritating
**EC classificat.:**
**Method:** other: see remarks
**Year:** **GLP:**
**Test substance:**
**Remark:** method: dose: ca. 0.1 g/animal, observation period: 14 d
**Source:** Bayer AG Leverkusen

(31)

**Species:** rabbit
**Concentration:**
**Dose:**
**Exposure Time:**
**Comment:**
**Number of Animals:**
**Result:**
**EC classificat.:**
**Method:** other: see remarks
**Year:** **GLP:**
**Test substance:**
**Remark:** method: single application to the conjunctival sac
(no details)
no effects observable
**Source:** Bayer AG Leverkusen

(35)

**Species:** rabbit
**Concentration:**
**Dose:**
**Exposure Time:**
**Comment:**
**Number of Animals:**
**Result:**
**EC classificat.:**
**Method:** other: see remarks
**Year:** **GLP:**
**Test substance:**
**Remark:** effects: only immediate sensory and inflammatory reaction (discomfort, blepharospasm, and conjunctival congestion) that disappears very soon; the eyes appear normal in a few hours (the authors conclusion is that the test substance produces these reactions by the mechanical contact of the powder, which is almost insoluble in the eye secretion)
method: the drug was applied to the eyes as a dry powder and held there for one-half minute; this was daily repeated for several times (no details)
**Source:** Bayer AG Leverkusen

(40)

**Species:** rabbit
**Concentration:**
**Dose:**
**Exposure Time:**
**Comment:**
**Number of Animals:**
**Result:** slightly irritating
**EC classificat.:**
**Method:** OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
**Year:** **GLP:**
**Test substance:**
**Source:** Zeneca Specialties, Manchester
Bayer AG Leverkusen

(41)

## 5.3 Sensitization

**Type:** Intracutaneus test
**Species:** guinea pig
**Number of Animals:**
**Vehicle:**
**Result:** not sensitizing
**Classification:**
**Method:** other: see remarks
**Year:** **GLP:**
**Test substance:**
**Remark:** method: according to: "Appraisal of the safety of chemicals in foods, drugs and cosmetics", Assoc. of Food and Drug Officials of the United States, 1959

**Source:** Bayer AG Leverkusen

(33)

**Type:** Patch-Test
**Species:** human
**Number of Animals:**
**Vehicle:**
**Result:**
**Classification:**
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** the case of a 40-year-old man with sub-acute dermatitis in face, neck and dorsum of the hands is reported: he was patch tested with anthraquinone (10 % pet.) and showed no reaction
**Source:** Bayer AG Leverkusen

(42)

## 5.4 Repeated Dose Toxicity

**Species:** rat **Sex:** no data
**Strain:** no data
**Route of admin.:** inhalation
**Exposure period:** 4 months
**Frequency of treatment:** 5-6 h/d
**Post. obs. period:** 1 month
**Doses:** 0.0052 or 0.0122 mg/l
**Control Group:** yes
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** dynamic inhalation
**Result:** 0.0052 mg/l: no toxic effects
0.0122 mg/l: body weight loss, changes of the bloodpicture (lowered level of hemoglobin, erythrocytopenia, relative reticulopenia); histopathological findings in the lungs: emphysema, atelectasis, cellular proliferation, in particular perivascular hyperemia of the capillaries and exsudation in the alveolar lumen (blood picture normalised during the experimental period, changes of the lung regenerated within the first month after termination of the experiment)
**Source:** Bayer AG Leverkusen

(35)

**Species:** rat **Sex:** male/female
**Strain:** Wistar
**Route of admin.:** oral feed
**Exposure period:** 3 months
**Frequency of treatment:** daily
**Post. obs. period:** no
**Doses:** 15, 150 or 1500 ppm (= ca. 1, 10 or 100 mg/kg bw/d)
**Control Group:** yes
**NOAEL:** ca. 1 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Result:** all dose groups: no deaths
15 ppm (= ca. 1 mg/kg bw/d): no symptoms of toxicity
150 and 1500 ppm (= ca. 10 and 100 mg/kg bw/d): decreased food intake, increased absolute weights of the liver
1500 ppm (= ca. 100 mg/kg bw/d): decreased body weight gain, enlargement of the centrilobular hepatocytes; clinical chemistry: increased cholesterol levels at the end of the test period mainly in the females
**Source:** Bayer AG Leverkusen

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**Species:** rat **Sex:** male/female
**Strain:** Wistar
**Route of admin.:** gavage
**Exposure period:** 28 d
**Frequency of treatment:** daily
**Post. obs. period:** no
**Doses:** 2, 10, 20, 50 or 250 mg/kg bw/d
**Control Group:** yes
**NOAEL:** 2 mg/kg bw
**Method:**
**Year:** **GLP:**
**Test substance:**
**Result:** all dose groups: no deaths
2 mg/kg bw/d: no signs of toxicity
10, 20, 50 and 250 mg/kg bw/d: impairment of the general condition, black-coloured spleen, splenic congestion, increased relative weights of the liver and the spleen
10 mg/kg bw/d: hepatocyte enlargement
10, 50 and 250 mg/kg bw/d: increased relative renal weights in the females
20, 50 and 250 mg/kg bw/d: decreased body weight gain in the females, erythropenia
50 and 250 mg/kg bw/d: decreased body weight gain in the males, increased relative weights of the thyroid, the heart, the testes and the kidneys in the males, hepatocyte enlargement 250 mg/kg bw/d: decreased relative weights of the ovaries, clinical chemistry: slightly increased concentrations of glutamatepyruvate transaminase and of glutamate oxalo-acetate transaminase
**Source:** Bayer AG Leverkusen

(44)

**Species:** rat **Sex:** male
**Strain:** other: Wistar Alpk = AP FSD
**Route of admin.:** gavage
**Exposure period:** 10 d
**Frequency of treatment:** daily
**Post. obs. period:** none
**Doses:** 50, 250, 1000 mg/kg/day in corn oil
**Control Group:** yes
**Method:** other: animals were subjected to microscopic post mortem examination, selected organs weighed and examined histologically
**Year:** **GLP:**
**Test substance:**
**Result:** No chemical or bodyweight effects in animals dosed with 50 or 250 mg/kg/day. Signs of slight systemic toxicity was seen in animals dosed at 1000 mg/kg/day. There was a dose related increase in liver to bodyweight ratio which was associated with slight macrocytic anaemia.
**Source:** Zeneca Specialties, Manchester
Bayer AG Leverkusen

(45)

**Species:** rat **Sex:** no data
**Strain:** no data
**Route of admin.:** oral unspecified
**Exposure period:** 7 d
**Frequency of treatment:** daily
**Post. obs. period:** no data
**Doses:** 50 mg/kg bw/d
**Control Group:** no data specified
**Method:**
**Year:** **GLP:**
**Test substance:**
**Result:** inhibition of the absorptive and excretory functions of the liver (no further data)
**Source:** Bayer AG Leverkusen

(46)

**Species:** other: not clearly described **Sex:** no data
**Strain:** no data
**Route of admin.:** oral unspecified
**Exposure period:** 30 administrations
**Frequency of treatment:**
**Post. obs. period:** no data
**Doses:** no data
**Control Group:** yes
**Method:**
**Year:** **GLP:**
**Test substance:**
**Result:** body weight loss, changes of the bloodpicture (lowered level of haemoglobin, erythrocytopenia), disturbed function of liver, spleen and kidneys, function of the central nervous system not adversely affected
**Source:** Bayer AG Leverkusen

(35)

## 5.5 Genetic Toxicity 'in Vitro'

**Type:** Ames test
**System of testing:** S. typhimurium TA 98, TA 100, TA 1535, TA 1537
**Concentration:**
**Metabolic activation:** with and without
**Result:** negative
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Bayer AG Leverkusen

(47) (48) (49)

**Type:** Ames test
**System of testing:** S. typhimurium TA 98, TA 100, TA 1535, TA 1538
**Concentration:**
**Metabolic activation:** with
**Result:** negative
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Bayer AG Leverkusen

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**Type:** Ames test
**System of testing:** S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
**Concentration:**
**Metabolic activation:** with and without
**Result:** negative
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Bayer AG Leverkusen

(51) (52) (53)

**Type:** Ames test
**System of testing:** S. typhimurium TA 98, TA 1535, TA 1538
**Concentration:**
**Metabolic activation:** without
**Result:** negative
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** Before being tested, 9,10-anthraquinone was subjected to 60Co gamma radiation in air; testing was conducted with the bacterial strains alone, thus not fortified with liver-microsomal enzymes or other metabolizing systems
**Source:** Bayer AG Leverkusen

(54)

**Type:** Ames test
**System of testing:** S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
**Concentration:**
**Metabolic activation:** with and without
**Result:** positive
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** anthraquinone was shown to be mutagenic only for strains TA 1537, TA 1538, and TA 98 in the absence of rat liver homogenate
**Source:** Bayer AG Leverkusen

(55)

**Type:** Ames test
**System of testing:** S. typhimurium TA 97, TA 98, TA 100
**Concentration:**
**Metabolic activation:** with and without
**Result:** negative
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Bayer AG Leverkusen

(56)

**Type:** Ames test
**System of testing:** S. typhimurium TA 98, TA 100, TA 2637
**Concentration:**
**Metabolic activation:** with and without
**Result:** negative
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Bayer AG Leverkusen

(57)

**Type:** Ames test
**System of testing:** S. typhimurium TA 98, TA 100
**Concentration:**
**Metabolic activation:** with and without
**Result:** positive
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Bayer AG Leverkusen

(58)

**Type:** Ames test
**System of testing:** S. typhimurium TA 98, TA 100, TA 102, TA 1537
**Concentration:**
**Metabolic activation:** with and without
**Result:** negative
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** the strains TA 98, TA 100 and TA 1537 were tested with and without metabolic activation, the strain TA 102 was tested only with metabolic activation
**Source:** Bayer AG Leverkusen

(59)

**Type:** Ames test
**System of testing:** Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
**Concentration:**
**Metabolic activation:** with
**Result:** positive
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** Sample known to contain 0.032 % 9-Nitroanthracene positive in strains TA 1535, TA 1538, equivocal in strain TA 1537
**Source:** Zeneca Specialties, Manchester
Bayer AG Leverkusen

(60)

**Type:** Ames test
**System of testing:** Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
**Concentration:**
**Metabolic activation:** with
**Result:** negative
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** Sample known to contain 0.005 % 9-Nitroanthracene
**Source:** Zeneca Specialties, Manchester
Bayer AG Leverkusen

(61)

**Type:** Ames test
**System of testing:** Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
**Concentration:**
**Metabolic activation:** with and without
**Result:** positive
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Zeneca Specialties, Manchester
Bayer AG Leverkusen

(62)

**Type:** Ames test
**System of testing:** Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
**Concentration:**
**Metabolic activation:** with
**Result:** negative
**Method:**
**Year:** **GLP:**
**Test substance:**
**Source:** Zeneca Specialties, Manchester
Bayer AG Leverkusen

(63)

**Type:** Ames test
**System of testing:** Salmonella typhimurium TA 1535, TA 1537, TA 98, TA 100, E. coli WP2P and WP2P uvrA
**Concentration:**
**Metabolic activation:** with and without
**Result:** positive
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** negative in both strains of E. coli
**Source:** Zeneca Specialties, Manchester
Bayer AG Leverkusen

(64)

**Type:** Ames test
**System of testing:** Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
**Concentration:**
**Metabolic activation:** with
**Result:** positive
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** Sample known to contain 0.26 % 9-Nitroanthracene
**Source:** Zeneca Specialties, Manchester
Bayer AG Leverkusen

(65)

**Type:** Bacterial gene mutation assay
**System of testing:** S. typhimurium TM677
**Concentration:**
**Metabolic activation:** with
**Result:** negative
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** type: quantitative bacterial assay for forward mutation in Salmonella typhimurium, using resistance to the purine analog 8-azaguanine as a genetic marker
**Source:** Bayer AG Leverkusen

(66)

**Type:** DNA damage and repair assay
**System of testing:** S. typhimurium TA 1535/pSK1002
**Concentration:**
**Metabolic activation:** with and without
**Result:** negative
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** type: umu-test, which can detect the induction of DNA repair
**Source:** Bayer AG Leverkusen

(67)

## 5.6 Genetic Toxicity 'in Vivo'

**Type:** Cytogenetic assay
**Species:** other: Hordeum vulgare and Secale cereale **Sex:**
**Strain:**
**Route of admin.:** other
**Exposure period:** single application
**Doses:** 0.2 g/100 g of the seeds
**Result:**
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** chromosomal aberration test: the capacity of the test substance to polyploidize the root tip cell chromosomes was investigated
the seeds were treated directly with the test substance
**Result:** negative
**Source:** Bayer AG Leverkusen

(68)

**Type:** other: DNA damage assay: single-strand DNA-breaks in liver and kidney
**Species:** mouse **Sex:** male
**Strain:**
**Route of admin.:** i.p.
**Exposure period:** single application
**Doses:** 250 mg/kg bw
**Result:**
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** DNA damage was evaluated by the alkaline elution technique coupled with a microfluorometric method for DNA assay
**Result:** effects: an increased elution rate in alkali of DNA from liver and kidney was obtained
**Source:** Bayer AG Leverkusen

(69)

## 5.7 Carcinogenicity

**Species:** mouse **Sex:** male/female
**Strain:** no data
**Route of admin.:** dermal
**Exposure period:** no data
**Frequency of treatment:** no data
**Post. obs. period:** no data
**Doses:** no data
**Result:**
**Control Group:** yes
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** 15 male and 15 female animals used in the study
**Result:** no increase in tumor incidence
**Source:** Bayer AG Leverkusen

(70)

**Species:** mouse **Sex:** no data
**Strain:** no data
**Route of admin.:** dermal
**Exposure period:** see remarks
**Frequency of treatment:** see remarks
**Post. obs. period:** no data
**Doses:** 0.1 or 0.25 % solution (no further data)
**Result:**
**Control Group:** yes
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** data concerning the exposure period: the longest period of survival recorded was 1159 d (no further data)
frequency of treatment: the painting was repeated every day or every other day (no further data)
in the study the results on the 38 mice surviving over 200 d were considered
the test substance was dissolved in benzene
**Result:** production of a single papilloma in 1/38 mice surviving over 200 d, no skin cancer occurred, 2/38 mice showed lung cancer (1 mouse showed a papilloma before the 200 d period; the test group in which the findings were observed, is not specified; in the control group (benzene alone) a papilloma was found in 1/46 mice, no skin cancer occurred, 1/46 control animals showed lung cancer)
**Source:** Bayer AG Leverkusen

(71)

**Species:** mouse **Sex:** male/female
**Strain:** other: other (see remarks)
**Route of admin.:** oral unspecified
**Exposure period:** 18 months
**Frequency of treatment:** daily
**Post. obs. period:** no
**Doses:** see remarks
**Result:**
**Control Group:** yes
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** 18 mice of each sex of each strain were used
experimental design: 464 mg/kg bw/d (the maximal tolerated dose) was given daily by stomach tube, beginning when the mice were 7 d of age, until the mice were 4 w old; the dose was not readjusted according to weight gain during this period; after the mice were weaned at 4 w of age, anthraquinone was given with the diet ad libitum at a concentration of 1206 ppm
strains used: two F1 hybrid stocks, namely (C57BL/6 x C3H/Anf)F1 and (C57BL/6 x AKR)F1
the postmortem procedure included an external examination and a thorough examination of thoracic and

abdominal cavities, with histologic examination of major organs and of all grossly visible lesions; the cranium was not dissected; the entire carcass and all internal organs were fixed and have been saved; blood smears were made on all mice before they were killed

**Result:** no significant increase in tumor incidences compared to the controls

**Source:** Bayer AG Leverkusen

(72)

**Species:** mouse **Sex:** male/female
**Strain:** other: other (see remarks)
**Route of admin.:** s.c.
**Exposure period:** single application
**Frequency of treatment:**
**Post. obs. period:** 18 months
**Doses:** 1000 mg/kg bw
**Result:**
**Control Group:** yes
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** 18 mice of each sex of each strain were used
a blood smear was taken the day before killing; the total chest contents, liver, spleen, kidneys with adrenal glands, stomach, intestines and genital organs of male and female mice were dissected and selected tissues were taken for histological processing; it was also looked for tumors at the site of application (in the nape of the neck)
experimental design: a single s.c. injection was given in the nape of the neck to weanling mice on approximately the 28th day of age
strains used: two F1 hybrid stocks, namely (C57BL/6 x C3H/Anf)F1 and (C57BL/6 x AKR)F1

**Result:** no significant increase in tumor incidences compared to the controls

**Source:** Bayer AG Leverkusen

(73)

**Species:** mouse **Sex:** male/female
**Strain:** Swiss
**Route of admin.:** s.c.
**Exposure period:** 3 months
**Frequency of treatment:**
**Post. obs. period:** no
**Doses:** 0.02 mmol (= 4.164 mg)/implant
**Result:**
**Control Group:** yes
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** discs (physical properties considered to be non-tumorigenic, 13 mm diameter, pore size 0.22 um) containing the test substance were implanted s.c. into the dorso-lumbar region of the animals; 3 months after implantation the surviving mice were killed and the skin and implant site tissue were removed
the animals were examined at daily intervals
the appearance of the tissue surrounding the test implants was assessed histopathologically relative to that seen with control implants
**Result:** no tumors appearing at the site of implant (20 animals, 10 of each sex, were used)
**Source:** Bayer AG Leverkusen

(74)

**Species:** mouse **Sex:** male/female
**Strain:** no data
**Route of admin.:** s.c.
**Exposure period:** no data
**Frequency of treatment:** no data
**Post. obs. period:** no data
**Doses:** no data
**Result:**
**Control Group:** yes
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** 15 male and 15 female animals used in the study
**Result:** no increase in tumor incidence
**Source:** Bayer AG Leverkusen

(70)

**Species:** **Sex:**
**Strain:**
**Route of admin.:**
**Exposure period:**
**Frequency of treatment:**
**Post. obs. period:**
**Doses:**
**Result:**
**Control Group:**
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** mammalian cell transformation in vitro: Chang (human liver) cells and BHK-21 C13 (baby Syrian hamster kidney) cells were incubated separately with solutions of anthraquinone, with and without metabolic activation by rat liver postmitochondrial supernatant (concentrations of anthraquinone: 250, 50, 10, 2, 0.4, 0.08 ug/ml of the cell suspension)
**Result:** anthraquinone was negative in the cell transformation test, i.e., it did not induce malignant transformation at frequencies significantly different from the spontaneous transformation frequencies
**Source:** Bayer AG Leverkusen

(75)

## 5.8 Toxicity to Reproduction

**Type:** other: In vitro teratogenesis assay which utilizes Drosophila embryonic cultures
**Species:** other: Drosophila **Sex:**
**Strain:**
**Route of admin.:**
**Exposure Period:**
**Frequency of treatment:**
**Duration of test:**
**Doses:**
**Control Group:** yes
**Method:**
**Year:** **GLP:**
**Test substance:**
**Remark:** study design: Drosophila embryonic cell cultures were allowed to differentiate at 26 degrees centigrade in the presence of anthraquinone (concentration: 1000 uM) for 24 h. Cultures were stained and numbers of differentiated myotubes and ganglia were counted
**Result:** the test substance did not result in a statistically significant reduction in the total number of myotubes and ganglia when compared to controls, thus it did not show interference with normal cell differentiation
**Source:** Bayer AG Leverkusen

(76)

## 5.9 Developmental Toxicity/Teratogenicity

-

## 5.10 Other Relevant Information

**Type:**

**Remark:** following a single oral administration of anthraquinone (labelled with 14C in the 9,10-positions) at dose levels of 0.1, 1.0, 3.0 mg/kg bw (male rats) or of 1.0 mg/kg bw (female rats), the radioactivity resulting from anthraquinone was nearly completely absorbed, the absorption commencing after a short lag period of ca. 2-3 minutes; after dosing male or female rats with 1.0 mg/kg bw, the absorption could not be desribed by a unique half-life; following administration of 0.1 mg/kg bw to males, the absorption period was best to characterize by a half-life of roughly 40 minutes, the maximum plasma level of P = 0.75 was reached after 2.5 h; following oral administration of 1.0 mg/kg bw to males or females, the plasma concentration peaked after 5 h (P = 0.46) and 12 h (P = 0.43), respectively; the radioactivity was slowly eliminated from the body: 2 d after oral intubation on average ca. 5 % of the administered dose could be measured in the body excluding the gastrointestinal tract, within 2 d after oral administration less than 0.01 % of the recovered radioactivity were excreted with the expired air; within the test interval of 2 d ca. 95 % of the retrieved radioactivity were excreted with urine and feces after oral administration, the ratio of the amounts excreted via both routes was ca. 1.6 (feces:urine); at sacrifice of the male rats 48 h after administration of 1.0 mg/kg bw, a relative concentration of P = 0.052 was determined in the body excluding the gastrointestinal tract; in the kidney and in the liver these values were ca. 7 times higher and in the brain they were ca. 10 times lower as compared with the sum of all organs tissues; at sacrifice of the females a relative concentration of P = 0.063 was determined in the body excluding the gastrointestinal tract and in the kidney and in the liver these values were ca. 8 times higher and in the fat and in the brain the relative concentrations were 4 times and 8 times, respectively, lower (results representing the sum of the unchanged substance and its labelled metabolites. P = relative concentration = activity measured/grams of plasma : activity administered/grams of bw)

**Source:** Bayer AG Leverkusen

(77)

**Type:**

**Remark:** after a single intravenous administration of anthraquinone (labelled with 14C in the 9,10-positions) at a dose level of 1.0 mg/kg bw to male rats, the distribution of the radioactivity to the tissues at first proceeded quickly: 10 minutes p. appl. the relative concentration in the plasma amounted to ca. P = 0.5, but the concentration remained at about this level for roughly 8 h, then it was eliminated

with a constant rate; the radioactivity was slowly eliminated from the body: 2 d after i. v. injection roughly 3 % of the administered dose could be measured in the body excluding the gastrointestinal tract; within the test interval of 2 d ca. 95 % of the retrieved radioactivity were excreted with urine and feces after intravenous administration, the ratio of the amounts excreted via both routes was ca. 1.6 (feces:urine); at sacrifice (48 h p. appl.) a relative concentration of P = 0.039 was determined in the body excluding the gastrointestinal tract; in the kidney these values were ca. 6 times and in the liver ca. 8 times higher; the relative concentrations in the fat were ca. 3 times and in the brain ca. 7 times lower as compared with the sum of all organs and tissues (results representing the sum of the unchanged substance and its labelled metabolites. P = relative concentration = activity measured/grams of plasma : activity administered/grams of bw)

**Source:** Bayer AG Leverkusen

(77)

**Type:**

**Remark:** following a single intraduodenal administration of anthraquinone (labelled with 14C in the 9,10-positions) at a dose level of 0.1 mg/kg bw to male rats (with bile fistulae), about two third of the retrieved dose were excreted within 2 d via the bile fluid, more than 50 % thereof within the first 4 h and more than 90 % within 18 h p. appl.; most of the biliarily excreted radioactivity underwent an extensive enterohepatic circulation (results representing the sum of the unchanged substance and its labelled metabolites)

**Source:** Bayer AG Leverkusen

(77)

**Type:**

**Remark:** anthraquinone (labelled with 14C in the 9,10-positions) was administered orally in a dose of 5 mg/kg bw to male rats and the urine and the faeces of the animals were collected until 48 h after administration: the elimination ratio (renal:faecal) amounted to about 1:1.6; the main elimination product in faeces, anthraquinone amounted to min. 40 % of the totally recovered radioactivity (in the excreta and the carcass 48 h after administration), non conjugated 2-hydroxy-anthraquinone as a minor faecal metabolite was found in approximately 4 %; urine contained as main biotransformation product (approximately 20 % of the totally recovered radioactivity) conjugated 2-hydroxy-anthraquinone, unchanged anthraquinone amounted to about 1 % in the urine

**Source:** Bayer AG Leverkusen

(78)

**Type:**

**Remark:** experiments with mice indicate that anthraquinone has no laxative effect (no further data)

**Source:** Bayer AG Leverkusen

(79)

**Type:**
**Remark:** mice given anthraquinone per os had the substance in feces, but not in urine; no reduction of anthraquinone to anthrone could be demonstrated (no further data)
**Source:** Bayer AG Leverkusen
(79)

**Type:**
**Remark:** anthraquinone does not seem to be toxic to human skin fibroblasts in vitro (no further data)
**Source:** Bayer AG Leverkusen
(80)

**Type:**
**Remark:** adding 0.1 % anthraquinone to food stimulated liver growth in partially hepatectomized rats (no further data)
**Source:** Bayer AG Leverkusen
(81)

**Type:**
**Remark:** a photodynamic effect was observed in guinea pigs after dermal exposure to anthraquinone and exposure to sunlight (no further data)
**Source:** Bayer AG Leverkusen
(82)

**Type:**
**Remark:** rabbits which were treated dermally with anthraquinone and which were subsequently exposed to sunlight, showed a weak photodynamic effect (hyperemia) within a period of 30 minutes to 1 h after the irradiation
**Source:** Bayer AG Leverkusen
(35)

**Type:**
**Remark:** anthraquinone fed to chickens (2 animals used) was shown to have antihaemorrhagic properties
**Source:** Bayer AG Leverkusen
(83)

**Type:**
**Remark:** the secretagogue activity of anthraquinone was investigated by determining its influence on water absorption in the gastrointestinal tract of the rat: 1h after injection of anthraquinone into the tied-off ligated colon segments in vivo, no significant change in water absorption was observable, i.e. there was no significant difference in the water content in the lumen when compared to the control solution
**Source:** Bayer AG Leverkusen
(84)

**Type:**
**Remark:** in vitro assay: anthraquinone at a concentration of 0.00001 m was found to inhibit the ferment activity of desoxyribonuclease from bovine pancreas
**Source:** Bayer AG Leverkusen

(85)

**Type:**
**Remark:** the activity of the sodium plus potassium activated ATPase from the rabbit red cell membrane is inhibited by anthraquinone and the concentration of anthraquinone for maximal inhibition is about 5 mM; the inhibitory action of anthraquinone on the ATPase activity is due to sulfhydryl group or the carboxyl group of the enzyme of NaK ATPase
**Source:** Bayer AG Leverkusen

(86)

**Type:**
**Remark:** in vitro assay: anthraquinone at a concentration of 1 mM was found to inhibit rat heart guanylate cyclase activity (enzyme activity different from the control by 20 %; findings marginally significant)
**Source:** Bayer AG Leverkusen

(87)

**Type:**
**Remark:** in vitro assay: anthraquinone has been shown to inhibit prostaglandin biosynthesis in methylcholanthrene-transformed 3T3 mouse fibroblasts (50 % inhibition by a concentration of 2.4 uM)
**Source:** Bayer AG Leverkusen

(88)

**Type:**
**Remark:** rats were injected i.p. with 3-methyl-4-dimethylaminoazobenzene in arachis oil, other rats received the same injections incorporating anthraquinone: anthraquinone was found to inhibit partially the binding of the aminoazodye to rat liver protein; furthermore, the test substance exerted a definite suppression of the glutathione content of livers of the animals injected with the aminoazodye
**Source:** Bayer AG Leverkusen

(89)

**Type:**
**Remark:** incubation of cell cultures of malignant epithelial ovarian tumors (from women) with anthraquinone at a concentration of 0.1 ug/ml for 1 h at 37 degrees centigrade in a 5 % carbon dioxide 95 % air atmosphere: no clinically useful cytotoxicity in vitro was observed (increase in cell kill by 14.9 or 33.0 %)
**Source:** Bayer AG Leverkusen

(90)

**Type:**
**Remark:** the inhibitory effects of anthraquinone upon the growth of the Twort carcinoma were investigated: tumor-bearing mice were injected i.p., twice daily, with 0.5 c.c. of a 0.1 % solution of the test substance, for 13 d and the inhibitory effect was calculated as the percentage difference between the average increases in tumor size of the treated and the control mice: a percentage inhibition of 46.9 % was determined
**Source:** Bayer AG Leverkusen

(91)

**Type:**
**Remark:** the ability of anthraquinone (concentration: 0.0001 M) to stimulate the formation of superoxide by three flavoprotein enzymes (NADPH-cytochrome P-450 reductase, NADH-cytochrome b5 reductase, NADH:ubiquinone oxidoreductase) was investigated in isolated rat hepatocytes and was found to be quite limited
**Source:** Bayer AG Leverkusen

(92)

**Type:**
**Remark:** the effect of anthraquinone on the survival in vitro of NF mouse sarcoma was investigated by incubation of subcutaneous sarcoma tissue with anthraquinone at the concentration of 0.05, 0.01 or 0.005 %, for 24 h at 4-7 degrees centigrade and by subsequent implantation of the treated sarcoma tissue fragments into the subcutaneous tissue at different sites of one mouse; the growth of tumors resulting from the implantation was observed for two w: no tumoricidal effect (= no anti-cancer action) was observed, i.e., all implants produced tumors (at least three mice were used)
**Source:** Bayer AG Leverkusen

(93)

**Type:**
**Remark:** the metabolism of anthraquinone was studied in rats which received 100 mg of the test substance mixed with the diet: 2-hydroxyanthraquinone (quantity: several per cent of the dosed anthraquinone), traces of 1-hydroxyanthraquinone and several other metabolites (not specified) could be detected in the urine collected within a 24 h period (no further data)
**Source:** Bayer AG Leverkusen

(94)

**Type:**
**Remark:** urine from rats fed anthraquinone and given s.c. injections of S35-sulfate was collected within a 24 h period and examined: a metabolite which decomposed to sulfate and 2-hydroxyanthraquinone was detected, and it was concluded to be a sulfate conjugate of 2-hydroxyanthraquinone (no further data)
**Source:** Bayer AG Leverkusen

(95)

**Type:**
**Remark:** in a study of the metabolism of anthraquinone, rats were maintained for 4 d on a diet containing 5 % of anthraquinone, the urines being collected daily; the following urinary metabolites were detectable: 2-hydroxyanthraquinone and its sulphuric ester, conjugates of 9-hydroxy-, 9,10-dihydroxy- and 2,9,10-trihydroxyanthracene and anthrone
**Source:** Bayer AG Leverkusen

(96)

**Type:**
**Remark:** anthraquinone showed a sedative effect in mammals treated orally with 1 mg/kg bw of anthraquinone (species not clearly specified); the test substance did not show an analgetic effect after s.c. administration of 1 mg/kg bw to mice and revealed no antipyretic activity in rabbits after oral administration of 2 mg/kg bw
**Source:** Bayer AG Leverkusen

(97)

**Type:**
**Remark:** study of the excretion and tissue distribution of radioactivity in male rats following a single oral dose of radiolabelled anthraquinone: 14C-anthraquinone was administered by gavage at 3.5 and 35 mg/kg bw and excretion of the radiolabel in the urine and faeces was monitored over a period of 96 h; the animals were then terminated and tissues were sampled and analyzed for radioactivity: cumulative excretion was similar at both dose levels with approximately 41 % and 55 % of the dosed radioactivity appearing in the urine and faeces respectively; the majority of the radiolabel was excreted within 48 h of dose administration, less than 3 % of the administered radioactivity remained in the tissues; highest tissue concentrations of anthraquinone-derived radioactivity were found in the liver, kidney and blood, preliminary analyses of the urine revealed little unchanged parent compound, but several metabolites (no further data)
**Source:** Bayer AG Leverkusen

(98)

**Type:**
**Remark:** male and female rats were injected i.p. with a suspension of anthraquinone dust at a dose of 50 mg/kg bw, the tissue reaction was examined at 1 month and 3 months after administration (the following tissues were taken for histological examination: omentum, spleen, liver and pancreas): anthraquinone produced no lesions and did not reveal any fibrogenicity
**Source:** Bayer AG Leverkusen

(99)

**Type:**
**Remark:** the cytotoxicity of anthraquinone dust to rat alveolar and peritoneal macrophages was measured as follows: suspension cultures containing 1000000 cells per ml were treated with the stock suspension of anthraquinone dust to give a final concentration of 0.5 mg/1000000 cells, the cultures were incubated for 2 h and samples taken for counting at 0, 1 and 2 h after addition of the dust: anthraquinone showed only low cytotoxicity, i.e., less than 2 per cent of the peritoneal macrophages and less than 5 per cent of the alveolar macrophages were killed following phagocytosis of the dust
**Source:** Bayer AG Leverkusen

(99)

**Type:**
**Remark:** in an in vitro assay human liver carbonyl reductase which was incubated with anthraquinone (test concentration: 15 uM) showed a relative enzyme activity of 22 % (the activity obtained with menadione was arbitrarily set as 100 %, corresponding to 2.3 U/mg protein)
**Source:** Bayer AG Leverkusen

(100)

**Type:**
**Remark:** the excretion and tissue distribution of radiolabelled anthraquinone were examined in male rats following a single oral or intravenous dose: 14C-anthraquinone was administered i.v. at 0.35 mg/kg bw or p.o. at 0.35, 3.5, 35 and 350 mg/kg bw and the excretion of the radiolabel in the urine and faeces was monitored over a period of 96 h; the animals were then killed and tissues were analyzed for radioactivity: cumulative excretion was similar at all dose levels studied with 26-41 % and 52-63 % of the dosed radioactivity appearing in the urine and faeces respectively, the majority of the radiolabel being excreted within 48 h of dose administration; less than 7 % of the administered radioactivity remained in the tissues, the highest tissue concentrations of anthraquinone derived radioactivity being detectable in the liver, kidney and blood; within 6 h of i.v. administration of 14C-anthraquinone at 0.35 mg/kg bw, approximately 35 % of the dosed radioactivity was excreted in the bile; analyses of urine and bile samples revealed little unchanged anthraquinone but several metabolites which are currently being identified (no further data)
**Source:** Bayer AG Leverkusen

(101)

**Type:**
**Remark:** anthraquinone seems to inhibit the function of certain enzymes in the S-9-Mix (rat liver homogenate) by which 3-amino-1-methyl-5H-pyrido(2,3-b)indol, 2-acetylaminofluorene and benzo(a)pyrene are activated: in a mutation assay (according to Ames with some modification) anthraquinone decreased markedly the mutagenicities of the mu-

tagens mentioned above (test strains: S. typhimurium TA 98, TA 100; assay with metabolic activation)
**Source:** Bayer AG Leverkusen

(102)

**Type:**
**Remark:** in a modified tetrazolium-reduction test (screening assay for carcinogenicity) anthraquinone was applied dermally to mice and subsequently tetrazolium reduction in mouse skin was measured in vitro: anthraquinone was considered to be negative in the screen as there was no significant difference between control and test mice, with regard to the amount of formazan deposited in the mouse skin (in this assay, solid test compounds were dissolved or suspended in benzene)
**Source:** Bayer AG Leverkusen

(103)

**Type:**
**Remark:** in the sebaceous-gland-suppression test (a screening assay for carcinogenicity) anthraquinone was applied dermally to mice, twice daily for 3 d (total dose of anthraquinone: 2.4 mg per mouse): a statistically significant reduction in the ratio of sebaceous glands to hair follicles was observed; the degree of suppression was arbitrarily classified as grade 1, i.e. less than 50 % of the glands (in the case of anthraquinone: 28.5 %) were suppressed, compared to the control (generally, the test compounds were dissolved in dimethylsulphoxide containing 10 % v/v benzene)
**Source:** Bayer AG Leverkusen

(104)

**Type:**
**Remark:** the degranulation of rough endoplasmic reticulum and the resultant increase in smooth endoplasmic reticulum was investigated in vitro using an isolated rat liver rough endoplasmic reticulum preparation incubated with anthraquinone (test concentration: 12 ug/ml) for 2 h: a negative test result was obtained, i.e. the percentage degranulation was 4.7 % (screening assay for carcinogenicity)
**Source:** Bayer AG Leverkusen

(105)

**Type:**
**Remark:** phototoxicity testing of anthraquinone in hairless mice: one group of mice was treated dermally (skin of the back) with a saturated solution of anthraquinone 2 times/d and irradiated with U.V. light simultaneously for 72 h, to another group of mice anthraquinone was administered i.p. at a daily dose of 100 mg/kg bw, the animals being exposed to U.V. light for 48 h: anthraquinone did not exhibit phototoxic activity after i.p. or dermal application, neither after completion of the irradiation nor on the next day (in the experiment, one control group remained untreated but was exposed to the U.V. light, a second control group was treated with the test substance but was not irradiated)
**Source:** Bayer AG Leverkusen

(106)

**Type:**
**Remark:** the excretion and tissue distribution of anthraquinone were examined in male rats following a single oral or intravenous dose of 14C-anthraquinone at 0.35 mg/kg bw; excretion of the radiolabel in the urine and faeces was monitored over a period of 96 h; rats were killed at 1, 4, 24 and 96 h after dose administration and tissues were analyzed for radioactivity: cumulative excretion was similar for both routes of administration with 29 % and 54-60 % of the dosed radioactivity appearing in the urine and faeces respectively; anthraquinone was rapidly distributed to all tissues examined with highest initial tissue concentrations of anthraquinone-derived radioactivity detected in adipose tissue, analyses of adipose extracts indicated this to be parent anthraquinone; no accumulation of anthraquinone-derived radioactivity was observed in any tissues examined, less than 5 % of the administered radioactivity remained in the tissues at 96 h; at this time point, highest concentrations of radioactivity were found in the liver, kidney and blood; analysis of urine revealed little unchanged anthraquinone but several metabolites (no further data)
**Source:** Bayer AG Leverkusen

(107)

## 5.11 Experience with Human Exposure

**Remark:** experience with human exposure: anthraquinone (10 % pet.) was photo-patch tested (irradiation with UVA, UVB or visible light) in a 40-year-old patient suffering from subacute dermatitis in face, neck, and dorsum of the hands: the only positive reaction was when anthraquinone was irradiated with UVA; a biopsy was taken of the positive reaction and showed eczema; photopatch tests with anthraquinone were performed in 5 controls and were negative (the test substance used contained 99 % anthraquinone with the following impurities: 0.3 % phenanthrene, 0.05 % anthracene, 0.3 % anthrone, 0.1 % nitrobenzene)
**Source:** Bayer AG Leverkusen

(42)

**Remark:** industrial workers exposed to dust of anthraquinone at concentrations of 0.002-1.65 mg/l complained of headache, general weakness and skin and eye irritations
**Source:** Bayer AG Leverkusen

(35)

**Remark:** the case of a 37-year old man suffering from hyperpigmentation of the face and neck is reported: his skin was tested directly to anthraquinone (among other organic substances to which the patient was exposed); this was carried out by the application of two series of skin tests applied to the skin of the abdomen, one directly, the other followed by ultraviolet irradiation: in the

direct test a solution of anthraquinone in gasoline was painted on the skin over an area one-half inch square, and as soon as the solvent had evaporated, leaving the test material in a thin layer on the skin, a dry gauze bandage was applied with adhesive tape; in the irradiation test, the same procedure was carried out, with the exception that following evaporation of the solvent and immediately preceding bandaging, a suberythema dose of ultraviolet rays, was administered to the test area; in both series the bandages were allowed to remain undisturbed for 18 h: the tests to the anthraquinone showed no visible reactions (no inflammatory changes, no hyperpigmentation) at either site

**Source:** Bayer AG Leverkusen

(108)

**Remark:** Zeneca internal data, based on manufacturing operations, indicates that the 8 hour TWA3 95th percentil exposure are less than 1.0 mg/m3 (range <0.1 to 3.5 mg/m3).
No user data is availlable but it is believed this unlikely to be substantially greater than for manufacture.

**Source:** Zeneca Specialities
Bayer AG Leverkusen

(109)

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Giornale degli Igienisti Industriali;
Massime concentrazioni ammissibili in Unione Sovietica;
Supplemento al n° 1/91

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(3) Banca Dati ACNA C.O.

(4) Banca dati ACNA C.O.

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## 7.1 Risk Assessment

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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September 8, 2003

Mr. Jerry A. Cook
Technical Director
Chemical Products Corporation
Cartersville, Georgia 30120

Re: Request for Reconsideration submitted March 27, 2003

Dear Mr. Cook:

On behalf of the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), I am responding to your March 27, 2002, Request for Reconsideration submitted for the Chemical Products Corporation (CPC) under the NIH's "Guidelines for Ensuring the Quality of Information Disseminated to the Public" (NIH Guidelines). Your Reconsideration Request appealed the NIH's March 19, 2003, decision regarding the CPC's November 15, 2003, Request for Correction contained in the Abstract for Draft National Toxicology Program (NTP) Technical Report # TR-494. A summary of the background information on the study that culminated in draft TR-494, the process I used to consider the appeal, and my conclusions are provided as follows:

*Background:* The NTP conducted a 2-year carcinogenicity study in rodents on a batch of anthraquinone obtained commercially that was shown to be 99.9% pure; results of this study eventually led to a draft report termed TR-494. Once it was peer reviewed, the abstract of draft TR-494 was posted on the NTP website. On July 25, 2000, you sent a letter to Dr. Kenneth Olden, Director of the NTP, stating that the sample of anthraquinone tested contained a 0.1% contamination by 9-nitroanthracene, a mutagenic compound, and noting that the presence of this contaminant called the study interpretations into question. The NTP followed up on your letter, confirming that a contaminant in the anthraquinone sample at about the 0.1% level was indeed 9-nitroanthracene. The NTP then initiated the process, in September 2000, to assess the metabolism of the parent compound, anthraquinone, in rodents, and to assess the relative mutagenicity in an Ames test of anthraquinone, its two major urinary metabolites, the contaminant 9-nitroanthracene, and two isomers of 9-nitroanthracene. You subsequently filed an Information Quality Request for Correction on November 15, 2002, asking that the abstract be immediately removed from the NTP's website in view of errors or misleading statements in the material presented. On March 19, 2003, NIH sent you a response to your Request for Correction stating that additional information would be incorporated into the NTP web site to clarify the material in the abstract of draft TR-494 and informing you about ongoing follow-up studies of

anthraquinone. The NTP amended the abstract of draft TR-494 on April 1, 2003, on its website to include reference to the 9-nitroanthracene contaminant, and the NTP also made mention of ongoing studies to resolve whether or not this contaminant might have affected the 2-year study results. On March 27, 2003, you submitted a Request for Reconsideration to NIH.

*Process:* In the course of my review, I have reviewed the HHS and NIH Guidelines for Ensuring the Quality of Information Disseminated to the Public, read draft TR-494, and read Chemical Products Corporation's letters and the NTP's responses to those letters. I have consulted with NIH and HHS staff familiar with the Information Quality process. I also have reviewed data and ongoing tests with the staff of NIEHS' Environmental Toxicology Program who were responsible for the NTP studies and draft report. I have been assisted in these efforts by staff from the NIEHS Office of Policy, Planning and Evaluation.

*Conclusions:* Following the process outlined above and after careful review of the information that I have described, I have reached the following conclusions:

1. The sample of anthraquinone used in the NTP 2-year study was contaminated with 9-nitroanthracene at a level of about 0.1%.

2. The presence of this contaminant raises doubt as to the effect(s) of anthraquinone itself, or its metabolites, and confounds interpretation of the NTP studies referenced in draft TR-494. In addition, in view of imprecise statements in the text presented on the website, this abstract needs to have greater specificity than it presently has.

3. The abstract of draft TR-494 will immediately be removed from the NTP website.

Further studies are underway on the metabolism of anthraquinone in rodents and on the relative mutagenic potency of this compound, its major metabolites, the contaminant 9-nitroanthracene, and two isomers of 9-nitroanthracene. Additional information from this work will eventually be incorporated into a revised abstract and technical report which will be submitted for peer review and subsequent publication.

I appreciate your comments and hope that the actions that I have taken address your concerns.

Sincerely,

Samuel H. Wilson, M.D.
Deputy Director

cc: Mary Wolfe, Ph.D.
Director, NTP Liaison and Scientific Review Office

**The Contamination Level in the NTP Anthraquinone Bioassay was 0.6% and Not 0.1% as Reported in the Abstract of Technical Report 494**

Submitted to
The National Toxicology Program
National Institutes of Health

by
Orn Adalsteinsson, Ph.D.
Arkion Life Sciences, 3521 Silverside Road
Wilmington, DE 19810

February 2, 2004

**Summary**

The Abstract of TR 494 of the National Toxicology Program (NTP) on the carcinogenicity of anthraquinone (AQ) states that the level of contamination of the test material by GC analysis was 0.1%. HPLC analytical studies in that same report, however, noted a contamination level of 0.5% including unidentified compound(s).

Because of that GC vs. HPLC inconsistency, and because of the discrepancy that the AQ bioassay material has been shown to contain contaminating mutagenic activity that may not all be assigned to the primary contaminant, 9-nitroanthracene (9-NA), Arkion Life Sciences undertook a state-of-the-art analysis of the bioassay material. This new analysis shows that the contamination level of the AQ bioassay material was actually 0.6 %. Contaminants included 9-nitroanthracene, polycyclic aromatic hydrocarbons, and other unidentified organic and nitro-organic compounds. The presence of these chemicals is consistent with the fact that this material was produced by the oxidation of anthracene derived from coal tar.

These data indicate that the level of contamination is actually 6-fold greater than is stated in the Abstract, and suggests that the mutagenic contamination resides with compounds in addition to 9-NA. These observations strengthen the case that it is plausible that all of the tumor induction in the NTP bioassay may be assigned to mutagenic and carcinogenic contaminants.

**Background**

This is a supplemental communication from Arkion Life Sciences (Formerly Environmental Biocontrol, Intl.) regarding the NTP AQ Bioassay (NTP, 2004) (see also the Adalsteinsson Submission to NTP of January 8, 2004). The central issue of concern is that the test material used in the NTP cancer bioassay was mutagenic in the Ames test bacterial strains TA98, TA100, and TA1537 (Butterworth *et al.*, 2001). Removal of the primary contaminant 9-nitroanthracene (9-NA) and other contaminating organics by recrystalization resulted in the complete loss of mutagenic activity (Butterworth *et al.*, 2001). The degree of this contamination was of a magnitude that confounded interpretation of the bioassay (Butterworth *et al.*, 2001).

9-NA was found at 0.12% in the bioassay material (Butterworth *et al.*, 2001). Although other organics were present, the fact that nitroaromatic compounds often exhibit strong

mutagenic and carcinogenic activity suggested that 9-NA was the likely bad actor. Thus, the strength of the argument that the bioassay was flawed was based on the degree of mutagenic activity and no further analytical work was done at that time.

**NTP Approach to the Contamination Problem**

Members of the NTP Technical Reports Review Subcommittee who reviewed the original draft of the AQ bioassays (NTP, 1999) were unaware that there was a contamination problem and approved the report. To address the concerns raised in the Butterworth et al. paper, the NTP withheld release of the original AQ carcinogenesis report, and began their own analytical and mutagenicity evaluations, which are incorporated in the current draft report (NTP, 2004). The question was raised whether the mutagenic activity of 9-NA alone was of sufficient potency to account for the degree of activity seen in the bioassay material. It was possible that the observed contaminating mutagenic and potential carcinogenic activity might reside with more than just the 9-NA. However, this would mean that the degree of contamination would have to be greater than 0.1% as reported in the Abstract of TR 494. Therefore, in the past weeks Arkion Life Sciences has undertaken an extensive, state-of-the-art analytical reevaluation of the AQ bioassay material.

**New Analytical Studies**

GC analysis can often fail to detect substantial contamination with low levels of multiple contaminants because the minimum amount of material is applied to the column to avoid overloading. The GC/FID analysis of the NTP bioassay material indicated a contaminant level of 0.1% (NTP, 2004 - p. J-2) and is so reported in the Abstract of the draft report. However when the same material was evaluated using HPLC/UV analysis, a contaminant level of 0.5% was seen with two impurities of 0.3% and 0.2% relative to the AQ peak (NTP, 2004 - p. J-2). The greater peak was identified as 9-NA. The second peak was not identified. This information is not presented in the Abstract of the study report (NTP, 2004).

Much improved analysis can be conducted if the contaminants can be removed and studied separately from the main material. In the Arkion studies, after the AQ had been removed by recrystalization, the remaining supernatant was quantitatively analyzed for contaminants. The results of this new analysis revealed that the contamination level in the AQ bioassay material was actually 0.6% (Mathre, 2004). Classes of contaminants found are noted below. Identification of individual components is an ongoing longer-term project.

| **Contaminants in the AQ Bioassay Material** | |
|---|---|
| 0.12% | 9-nitroanthracene |
| 0.05% | polycyclic aromatic hydrocarbons |
| 0.45% | unidentified organics and other nitro-organics |
| 0.62% | Total |

**Implications of the New Analytical Studies**

The AQ preparation used in the NTP bioassays was more contaminated than had been previously acknowledged. The report of a 0.1% contamination level in the Abstract of TR 494 is incorrect and misleading. The actual 6-fold higher level of contamination indicates that the contaminating mutagenic activity probably resides with more than just the 9-NA component. It

should be noted that as a class nitroaromatic compounds often exhibit potent mutagenic and carcinogenic activity.

The procedure employed to make the anthraquinone used in the NTP bioassay involves oxidation of anthracene isolated from coal tar. Such preparations are often contaminated with polycyclic aromatic hydrocarbons and nitroaromatic compounds. The Arkion analysis of the NTP AQ bioassay sample is consistent with that history. AQ from that process is neither used nor imported into the United States.

**Conclusion and Recommended Course of Action**

The new analytical data summarized here strengthen the case that it is plausible that the tumor induction in the NTP AQ bioassay was produced by the contaminating material. The weight of evidence indicates that no conclusions as to the carcinogenic activity of AQ can be drawn from the bioassays that were run. This flawed study does not meet the high standards set by the NTP in the performance of cancer bioassays. AQ is an important compound in commerce and it is vital that we have a quantitative understanding of its carcinogenic potential in order to make sound decisions on acceptable exposures. The only avenue to gain this information is to conduct a new bioassay using the uncontaminated, non-anthracene based AQ in common use today. We urge that the current AQ draft report be withdrawn and that the NTP conduct a new bioassay as soon as is practical.

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