

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Anthraquinone

EC Number: 201-549-0

CAS Number: 84-65-1

Index Number: -

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	anthraquinone
EC number:	201-549-0
CAS number:	84-65-1
Annex VI Index number:	-
Degree of purity:	≥ 98.5%
Impurities:	See confidential Annex

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	-
Current proposal for consideration by RAC	Carc. 1B, H350
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc. 1B, H350

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification ¹⁾	Reason for no classification ²⁾
2.1.	Explosives				Not assessed in this dossier.
2.2.	Flammable gases				Not assessed in this dossier.
2.3.	Flammable aerosols				Not assessed in this dossier.
2.4.	Oxidising gases				Not assessed in this dossier.
2.5.	Gases under pressure				Not assessed in this dossier.
2.6.	Flammable liquids				Not assessed in this dossier.
2.7.	Flammable solids				Not assessed in this dossier.
2.8.	Self-reactive substances and mixtures				Not assessed in this dossier.
2.9.	Pyrophoric liquids				Not assessed in this dossier.
2.10.	Pyrophoric solids				Not assessed in this dossier.
2.11.	Self-heating substances and mixtures				Not assessed in this dossier.
2.12.	Substances and mixtures which in contact with water emit flammable gases				Not assessed in this dossier.
2.13.	Oxidising liquids				Not assessed in this dossier.
2.14.	Oxidising solids				Not assessed in this dossier.
2.15.	Organic peroxides				Not assessed in this dossier.
2.16.	Substance and mixtures corrosive to metals				Not assessed in this dossier.
3.1.	Acute toxicity - oral				Not assessed in this dossier.
	Acute toxicity - dermal				Not assessed in this dossier.
	Acute toxicity - inhalation				Not assessed in this dossier.
3.2.	Skin corrosion / irritation				Not assessed in this dossier.
3.3.	Serious eye damage / eye irritation				Not assessed in this dossier.
3.4.	Respiratory sensitisation	None		None	Not assessed in this dossier.
3.4.	Skin sensitisation				Not assessed in this dossier.
3.5.	Germ cell mutagenicity	None		None	Conclusive but not sufficient for classification.
3.6.	Carcinogenicity	Carc. 1B		None	

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3.7.	Reproductive toxicity	None		None	Not assessed in this dossier.
3.8.	Specific target organ toxicity –single exposure				Not assessed in this dossier.
3.9.	Specific target organ toxicity – repeated exposure				Not assessed in this dossier.
3.10.	Aspiration hazard				Not assessed in this dossier.
4.1.	Hazardous to the aquatic environment				Not assessed in this dossier.
5.1.	Hazardous to the ozone layer				Not assessed in this dossier.

¹⁾Including specific concentration limits (SCLs) and M-factors

²⁾Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Pictogram:



_____ GHS08

Signal word: Danger

Hazard statements: H350 (May cause cancer.)

Proposed notes assigned to an entry:

2 BACKGROUND TO THE CLH PROPOSAL

This CLH proposal aims to classify and label anthraquinone (AQ) for carcinogenicity. For the purpose of this dossier the available REACH registration data has been taken into account (January 2015).

AQ can be applied in the production of cellulose fibres. In case of use of such fibres for manufacturing of paper and board for food contact any traces of the substance that remain in the cellulose can transfer to foodstuffs.

So far, AQ was not listed in any priority list of Existing Substance Regulation (Regulation 793/93/EC).

AQ was evaluated in the framework of Directive 91/414/EEC (concerning the placing of plant protection products on the market). As result of that review AQ has not been included in the Annex I to Directive 91/414/EEC (list of active substances that are authorised for the use in plant protection products). An EFSA opinion (2012) stating, that a carcinogenic potential for AQ cannot be excluded.

There is also a decision not to include AQ in the Annex I, IA and IB to Directive 98/8/EC concerning the placing of biocidal products on the market (Commission Directive 2007/565/EC).

2.1 History of the previous classification and labelling

AQ is not listed in Annex VI to Regulation (EC) 1272/2008 on Classification, Labelling and Packing of Dangerous Substances.

Based on the results of two carcinogenicity studies (oral administration of AQ to mice and rats; NTP 2005) it was concluded by IARC in 2012 that there is sufficient evidence in the experimental animals for carcinogenicity. Therefore, AQ was evaluated as possible carcinogenic to human (group 2B). Since this evaluation no further animal data has become available. The same experimental studies are re-evaluated for justification of classification of AQ as carcinogenic according to CLP Regulation.

2.2 Short summary of the scientific justification for the CLH proposal

The available carcinogenicity studies on AQ as tested by the NTP (2005) showed that there was clear evidence of carcinogenic potential of AQ in female F344/N rats as well as clear evidence of carcinogenic activity in male and female B6C3F₁ mice. The tumour findings are consistently observed in target organs showing toxic effects and precursor lesions in repeated dose studies and in organs with highest tissue concentrations (see toxicokinetic data). It is not possible to determine to what extent, if any, the impurity 9-nitroanthracene (9-NA) has influenced the outcomes of the NTP carcinogenicity study on AQ. The low exposure level, the bioavailability, and the weak mutagenicity (positive effects in bacterial gene mutation tests but equivocal effect (L5178Y cells) or marginal positive effect (h1A1v2 cells) in gene mutation tests) make it unlikely and implausible that 9-NA was solely and totally responsible for the carcinogenic response. Due to biotransformation processes mutagenic metabolites of AQ appear, which are at least five times more potent and present at systemically higher concentration than 9-NA. Therefore, the results are attributable to AQ (and its active metabolites) and AQ is considered to be carcinogenic. AQ was not clearly demonstrated as mutagenic in the available tests; however, a contribution of mutagenicity (by mutagenic metabolites) cannot be excluded.

Overall, the NTP studies are valid and there is sufficient evidence for carcinogenicity. A classification of AQ as carcinogenic Category 1B with the hazard phrase ‘H350: May cause cancer.’ is proposed according to CLP Regulation.

2.3 Current harmonised classification and labelling

AQ is not classified according Annex VI to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of Dangerous Substances.

2.4 Current self-classification and labelling

Table 4: Notified classification and labelling according to CLP criteria (December 2014)

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)			
Skin Sens. 1	H317	H317		GHS07 Wng			96
Not Classified							33
Acute Tox. 4	H312	H312		GHS07			24
Skin Sens. 1	H317	H317		Wng			
Acute Tox. 4	H332	H332					
Acute Tox. 4	H312	H312		GHS07			4
Acute Tox. 4	H332	H332		GHS09			
Aquatic Acute 1	H400	H400		Wng			
Skin Sens. 1B	H317	H317		GHS07 Wng			3
		H351		GHS07 GHS08			2
		H317		Wng			
Acute Tox. 4	H302	H302		GHS07			1
Acute Tox. 4	H312	H312		Wng			
Acute Tox. 4	H332	H332					
Acute Tox. 4	H332	H332		GHS07 Wng			1

Number of Aggregated Notifications: 8

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Harmonised classification and labelling of AQ as Carc. 1B, H350 (May cause cancer) is proposed, which is considered a community wide action according to Article 36 regulation (EC) No 1272/2008. It is recommended that the classification proposal is considered for inclusion on Annex VI to Regulation (EC) No 1272/2008, table 3.1, with regard to Article 42.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

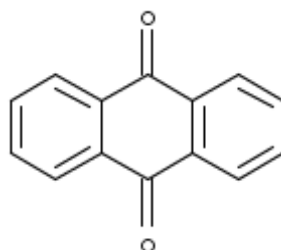
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	201-549-0
EC name:	anthraquinone
CAS number (EC inventory):	84-65-1
CAS number:	84-65-1
CAS name:	9,10-Anthracenedione
IUPAC name:	9,10-anthraquinone
CLP Annex VI Index number:	-
Molecular formula:	C ₁₄ H ₈ O ₂
Molecular weight range:	208.2 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks

Current Annex VI entry:

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks

Current Annex VI entry:

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry:

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Light yellow to almost colorless crystals	O'Neil 2001	Handbook data (Merck Index)
	Yellow odourless powder	DEZA a.s. 2010	EPA OPPTS 830.6302 (Colour) EPA OPPTS 830.6303 (Physical State) EPA OPPTS 830.6304 (Odour)
Melting/freezing point	ca. 284.6 °C, ca. 100 kPa	DEZA a.s. 2008	OECD Guideline 102 (capillary method)
Boiling point	377 °C, 760 mm Hg	O'Neil 2001	Handbook data (Merck Index)
Relative density	1.42 — 1.44, 20 °C	O'Neil 2001	Handbook data (Merck Index)
	1.261, 20 °C	DEZA a.s. 2008	OECD Guideline 109 (buoyancy method)
Vapour pressure	0.000000116 mm Hg 25 °C	Shimizu et al. 1987	Experimental result
Surface tension			water solubility is below 1 mg/l at 20 °C
Water solubility	74.6 µg/L at 20 °C, pH 7	Cook 2010	EPA Pesticide Assessment Guidelines Subdivision N, Section 161-1 (column elution method)
	190 µg/L at 20 °C, pH: ≥5.5 - ≤ 6.5	DEZA a.s. 2009	OECD Guideline 105, (column elution method)
	0.17 mg/L at 20 °C, pH 5.6	Lewandowska 2012	OECD Guideline 105, EU Method A.6 (flask method) Value might not be valid as for soluble amount of 0.17mg/L the wrong method was used, as the column elution method should be used for water solubility's < 10 mg/L
Partition coefficient n-octanol/water	Log Pow = 3.4 at pH=7, 30 °C	Cook 2010	ASTM E 1147-92 (HPLC Method)
Flash point			
Flammability			
Explosive properties			
Self-ignition temperature			
Oxidising properties			
Granulometry	Mass median diameter: 70.98 — 71.62 µm D10: 15.52 µm D50: 53.74 µm D90: 141.97 µm	Al Amin 2012	volumetric distribution, OECD Guideline 110 (Coulter LS 230)
	Mass median diameter:	VUOS a.s. 2010	counted distribution, OECD

	15.49 μm D10: 8.05 μm D90: 32.46 μm		Guideline 110 (LA-950 Laser Scattering Particle Size Distribution Analyzer - WET was used)
Stability in organic solvents and identity of relevant degradation products	-		
Dissociation constant	-		
Viscosity	-		

2 MANUFACTURE AND USES

AQ can be applied in the production of cellulose fibres.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this report.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

An overview on toxicokinetics of AQ is given for information.

4.1.1 Non-human information

Table 10: Summary table of toxicokinetics studies

Method	Results	Remarks	Reference
<p>Fischer 344 rat/ B6C3F₁ mice</p> <p>1. Imbedded toxicokinetic study (in NTP carcinogenicity assay): 18M rats dosed 469, 938, 1,875 or 3,750 ppm and 36M mice dosed 833, 2,500, or 7,500 ppm for 8 d. Also 10M/10F rats dosed 469, 938, 1,875 or 3,750 ppm and 10M/10F mice dosed 833, 2,500, or 7,500 ppm.</p> <p>Blood collection and analysis after 8 d, 3, 6, 12, and 18 months (rats) / 8 d, 12 months (mice).</p> <p>2. Single dose toxicokinetics in 18 months old animals: 100 mg/kg in rats (14F/14M) and 200 mg/kg in mice (13F/14M) per gavage</p> <p>Blood collection and analysis after 2, 6, 12, 24, and 36 h (rats) / 1, 2, 4, 8, and 12 h (mice).</p>	<p>AQ absorbed from gastrointestinal tract and distributed to tissues. No bioaccumulation. Majority of AQ eliminated in the faeces and urine within 24 h. 96 h after dosing, less than 5% of the dose remained in the tissues.</p> <p>Metabolites identified: yes Details on metabolites: 1-hydroxyanthraquinone (1-OH-AQ) 2-hydroxyanthraquinone (2-OH-AQ)</p>	<p>Test material (EC name): 9,10-¹⁴C-anthraquinone</p>	<p>NTP 2005</p>
<p>Chester-Beatty rat (4 rats) metabolism study</p> <p>diet containing 5% (w/w) for 4 d</p> <p>Urine collection and analysis.</p>	<p>Sulfate and glucuronide conjugates of 2-hydroxyanthraquinone, 9,10-dihydroxyanthracene and 2,9,10-trihydroxyanthracene were found in pooled urine samples.</p>	<p>Test material (EC name): 9,10-anthraquinone</p>	<p>Sims 1964</p>

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<p>rat</p> <p>metabolism study</p> <p>100 mg in diet</p> <p>Urine collection and analysis.</p>	<p>Detection of 1-hydroxy and 2-hydroxy-anthraquinone in the urine</p>	<p>Test material (EC name): 9,10-anthraquinone</p>	<p>Sato et al. 1956</p>
<p>Male Mura SPRA (SPF 68 Han) rat (M/F)</p> <p>Dose levels: 0.1, 1.0 and 3.0 mg/kg bw</p> <p>Route of exposure: intravenous and oral</p> <p>Measurement of toxicokinetic parameters. Deviation from official protocol: Too limited description.; no high dose; no repeated doses; no observation of toxicity.</p> <p>GLP: no</p>	<p>Almost complete absorption and distribution throughout the body (highest relative concentrations in liver and kidney). 90-95 % AQ excreted via urine and faeces within 48 h.</p> <p>Metabolites identified: no</p>	<p>Test material (EC name): 9,10-¹⁴C-anthraquinone</p>	<p>Bayer AG 1983</p>
<p>Sprague-Dawley rats (5M)</p> <p>Single dose: 5.0 mg/kg bw</p> <p>Urine and faeces were collected until 48 h post administration. Metabolites were studied and identified in naïve urine and in methanolic extracts of faeces.</p> <p>Deviation from official protocol: Too limited description. No proposed biotransformation pathway.</p> <p>GLP: no</p>	<p>Metabolites identified: yes</p> <p>Details on metabolites: 1-OH-AQ 2-OH-AQ</p>	<p>Test material (EC name): 9,10-¹⁴C-anthraquinone</p>	<p>Bayer AG 1985</p>

Absorption

The National Toxicology Program (NTP 2005) found in their toxicokinetic experiments that doses of 0.35 to 350 mg/kg bw [¹⁴C] AQ were well absorbed (> 99% of the administered dose from the gastrointestinal tract in male F334 rats). Studies conducted by Bayer AG (1983 and 1985) also suggested that AQ is almost completely absorbed after oral administration and that a short lag period of approximately 2-3 minutes occurs before absorption commences).

Distribution

After absorption AQ was distributed to various tissues, with the highest concentration observed in the adipose tissue, but no bioaccumulation was shown in any particular tissue (NTP 2005). The plasma concentration of AQ in female rats was approximately twice the concentration in male rats (NTP 2005). In addition to the NTP studies, Bayer AG (1983) identified that the liver and the kidneys are the organs with the highest relative concentration. The concentrations of rat organs at 48 h following a single oral administration of 1 mg/kg bw were up to 0.47 % and 0.55 % in the liver or kidney, respectively, in female rats and up to 0.44 % and 0.29 % in the liver or kidney, respectively, in male rats. The maximum plasma level (C_{max}) was reached after 2.5 h after 0.1 mg/kg bw, or after 5 h or 12 h for male or female rats treated with 1 mg/kg bw, respectively (Draft Assessment Report – Anthraquinone 2006). Also, results of repeated oral administration studies showed distribution of AQ throughout the whole body (incidences of non-neoplastic lesions of the kidney, liver, spleen, and bone marrow in male and female rats, the liver, urinary bladder, and spleen in male and female mice, and the thyroid gland and kidney in male mice; NTP 2005).

Metabolism

The major metabolite detected by the NTP (2005) in the urine samples of rats after oral or intravenous exposure was 2-hydroxyanthraquinone (2-OH-AQ). This is in accordance with earlier studies, where 1-hydroxyanthraquinone (1-OH-AQ) and 2-OH-AQ were identified in the urine of rats after daily oral administration of AQ (Sato et al. 1956). Bayer AG (1985) found that approximately 25% of the administered dose was metabolised and 2-OH-AQ was the main metabolite (20% of the total recovered radioactivity in urine, 4% in faeces) followed by 1-OH-AQ. In this study 41% of the recovered radioactivity (urine + faeces + body) consisted of not metabolised AQ, with only 1% unchanged AQ detected in urine. Furthermore, sulphate and glucuronide conjugates (of 2-OH-AQ, 8,10-dihydroxyanthracene and 2,9,10-trihydroxyanthracene) were found in the pooled urine samples of 4 male Chester-Beatty rats, which had received a diet containing 5% AQ for 4 days (Sims 1964). Last, anthrone was reported as metabolite by the NTP (2005). However, no further robust evidence for its occurrence as metabolite was given.

Excretion

In the NTP studies the majority of the radiolabeled substance was eliminated in the faeces and the urine by 48 h after administration. Within 96 h more than 95% of the administered dose was metabolised and eliminated in the bile, faeces and urine at all dose levels (NTP 2005). Bayer AG (1983) reported an excretion of 90-95% of the administered dose (oral and intravenous) within 48 h, whereby 55-59% were represented in the faeces and 33-37% in the urine. A single dose intraduodenal administration demonstrated that the biliary route is an important pathway for faecal excretion of AQ (Bayer AG 1983). Bayer AG (1985) found 40% excretion of the radioactivity via urine and 60% via faeces within 48 h after oral administration of a single oral dose of 5 mg/kg bw AQ.

4.1.2 Human information

No information is available.

4.1.3 Summary and discussion on toxicokinetics

AQ is readily, rapidly and completely absorbed in the gastrointestinal tract of rats and distributed throughout the whole body. The highest concentrations after distribution with no indication of bioaccumulation have been found in adipose tissue as well as in liver and kidney. Identified metabolites comprise 1-OH-AQ and 2-OH-AQ as major ones as well as probably their sulphate and glucuronide conjugates. Sulphate and glucuronide conjugates of 2-OH-AQ, 8,10-dihydroxyanthracene and 2,9,10-trihydroxyanthracene were identified. Besides urine bile fluid represented an important route of excretion. The majority (95%) of the radiolabeled substance is eliminated within 48-96 h via faeces and urine, whereby over 50% of the eliminated radioactivity was found in the faeces.

4.2 Acute toxicity

Not evaluated for this dossier.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated for this dossier.

4.4 Irritation

Not evaluated for this dossier.

4.5 Corrosivity

Not evaluated for this dossier.

4.6 Sensitisation

Skin sensitisation as well as respiratory sensitisation was not evaluated for this dossier.

4.7 Repeated dose toxicity

The studies on repeated dose toxicity are reported for purpose of background information only. The findings are of relevance to identify toxic effects and possible precursor lesions with regard to hazard class toxicity.

4.7.1 Non-human information

Table 11: Summary table of repeated dose toxicity studies

Method	Results	Remarks	Reference
<p>rat (Wistar) (5M/5F/per dose group)</p> <p>subacute (oral: gavage)</p> <p>0, 2, 10, 20, 50 and 250 mg/kg (actually ingested)</p> <p>Exposure: 28 days</p> <p>GLP: no</p> <p>Not compliant with 67/548/EEC Annex V methods or OECD 407 (no guidelines existed at that time)*</p>	<p>NOAEL: 2 mg/kg bw/day LOAEL: 10 mg/kg bw/day</p> <p><u>M/F ≥ 10 mg/kg bw/day:</u></p> <p>- Rel. liver weight ↑ (8%/10% M/F) associated with minimal hepatocyte enlargement centrilobular/ midzonal/perportal</p> <p>- Rel. kidney weight ↑ (10% F)</p> <p>- Rel. spleen weight ↑ (13%/22% M/F) associated with minimal spleen congestion</p>	<p>experimental result</p> <p>Test material (EC name): Anthraquinone</p>	Bayer AG 1976
<p>rat (Wistar) (20M/20F per dose group)</p> <p>subchronic (oral: feed)</p> <p>0, 15, 150, 1,500 ppm (0, 1.36, 12.58, 126.25 mg/kg bw/d for M, 0, 1.79, 16.79 and 175.21 mg/kg bw/d for F) (nominal in diet)</p> <p>Exposure: 3 months (daily)</p> <p>GLP: no</p> <p>Not compliant with 67/548/EEC Annex V methods or OECD 408 (no guidelines existed at that time)**</p>	<p>NOAEL: 15 ppm (1.36/1.79 mg/kg bw M/F)</p> <p>Not taking into account the slightly reduced body weight (5% in M/F), increased reticulocyte count (108%/92% M/F week 6, 11%/54% M/F week 13) and minimal decreases in RBC counts (6%/5% M/F week 6, 2%/3% M/F week 13)</p> <p>LOAEL: 150 ppm (12.58/16.79 mg/kg bw M/F)</p> <p><u>M/F ≥ 150 ppm:</u> reduced body weight (↓6%/↓7% M/F) and food consumption (↓10 % M/F)</p> <p>Increases in reticulocyte counts ↑149%/↑92% M/F week 6, ↑99%/↑136% M/F week 13</p> <p>Increased RBC ↑6%/↑6% M/F week 6, ↑5%/↑6% M/F week 13</p> <p>Cholesterol ↑ F</p> <p>Albumin ↑ M/F</p> <p>Abs. liver weight (↑22%)</p> <p>Same effects at <u>M/F ≥ 1,500 ppm</u> with increasing effect strength and at this concentration also changes in abs. thyroid weight (↑42%) appeared.</p>	<p>experimental result</p> <p>Test material (EC name): Anthraquinone</p>	Bayer AG 1979

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<p>F344/N rats (10M/10F)</p> <p>subchronic (oral: diet)</p> <p>0, 1,875, 3,750, 7,500, 15,000, and 30,000 ppm (0, 135, 275, 555, 1,130 and 2,350 mg/kg bw)</p> <p>Exposure: 14 weeks (daily)</p>	<p>LOAEL: 1,875 ppm</p> <p><u>M/F ≥1,875 ppm:</u> Slight anaemia (Hct, Hb and RBC↓, levels ≤10%), MCV↑, MCHC↓, reticulocytes↑, platelet counts ↑ ALP↓, creatinine ↑, total protein↑, albumin ↑</p> <p><u>M≥7,500 ppm</u> urea nitrogen↑</p> <p><u>M≥1,875 ppm, F ≥15000 ppm:</u> Normalized urine AST↑, NAD↑, urine protein↑</p> <p><u>M/F ≥1,875 ppm:</u> Abs/rel. liver weight ↑ Abs/rel. kidney weight↑ Abs. testis weight ↑ (rel from 7500 ppm) F ≥15,000 ppm: Estrous cycle length↑</p> <p><u>Liver:</u> M/F ≥1,875 ppm: Centrolobular liver cell hypertrophy: minimal to moderate</p> <p><u>Kidney:</u> M/F ≥1,875 ppm: Renal tubular hyaline droplet accumulation: M mild-moderate (PAS negative), F Minimal-mild (PAS positive) M ≥1,875 ppm; F ≥ 15,000 ppm: Nephropathy in all males including controls, lesion more severe in all exposed groups (positive immune response to alpha2 u-globulin) of M and F ≥ 15,000 ppm</p> <p><u>Spleen:</u> M/F ≥1,875 ppm extramedullar haematopoiesis ↑,(iron-positive) pigmentation↑</p> <p><u>Bone marrow:</u> M ≥3,750 ppm,F ≥1,875 ppm Haematopoiesis↑ minimal-slight</p> <p><u>Thyroid :</u> M/F ≥3,750 ppm Follicular cell hypertrophy, minimal</p> <p>Urinary bladder: F 30,000 ppm Inflammation & transitional epithelial hyperplasia</p>	<p>experimental result</p> <p>Test material (EC name): Anthraquinone</p>	<p>NTP 2005</p>
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<p>B6C3F₁ mice (10M/10F) subchronic (oral: diet)</p> <p>0, 1,875, 3,750, 7,500, 15,000, or 30,000 ppm (250, 500, 1,050, 2,150, or 4,300 mg/kg in males and 300, 640, 1,260, 2,600, or 5,300 mg/kg in females)</p> <p>Exposure: 14 weeks (daily)</p>	<p>LOAEL: 1875 ppm.</p> <p>M ≥7,500 ppm F ≥1,875 ppm Slight anaemia reticulocytes↑, (Hct↓ (high dose M 12%, high dose F 8%), Hb ↓ (6%, 5%) and RBC↓ (13%, 12%)), M/F ≥15,000 ppm MCV↑, MCH↑, MCHC↑, M ≥15,000 ppm F ≥1875 ppm platelet counts ↑ (no data on clinical biochemistry and urine)</p> <p><u>M/F ≥1,875 ppm</u> Abs/rel liver weight ↑ <u>M/F 30,000 ppm</u> Abs/rel kidney weight ↑</p> <p><u>Liver</u> M/F ≥3,750 ppm Centrilobular hypertrophy, mild-moderate Urinary bladder cytoplasmic alteration (intracellular eosinophilic granules) minimal - severe <u>Spleen</u> M/F ≥1,875 ppm Haematopoiesis (minimal/mild), pigmentation (minimal-mild)</p>	<p>experimental result</p> <p>Test material (EC name): Anthraquinone</p>	<p>NTP 2005</p>
<p>rat</p> <p>chronic (inhalation: dust) (whole body)</p> <p>12.2 ± 0.7 mg/m³ (analytical conc.) 5.2 ± 0.8 mg/m³ (analytical conc.)</p> <p>Vehicle: air</p> <p>Exposure: 5-6 h (daily, 4 months)</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 413 is possible***</p>	<p>NOAEC: 5.2 mg/m³ air (analytical)</p> <p>LOAEC: 12.2 mg/m³ air (analytical)</p> <p>↓body weight Slight anaemia (↓Hb, ↓RBC, reticulopenia)</p> <p><u>Lungs</u>: emphysema, atelectasis, cellular proliferation, in particular perivascular hyperaemia of the capillaries and exsudation in the alveolar lumen without any information on affected species, number of animals, sexes and treatment.</p>	<p>Test material (EC name): Anthraquinone</p> <p><u>Clear limitations</u>: Number and species of exposed animals is unclear, only two dose groups were tested, it is not specified which organs were examined (in histological investigations).</p>	<p>Volodchenko et al. 1970</p>

* Deviation from to date standard protocol: No weight of epididymes and thymus, no histology of nerve, thymus and trachea, no reactivity test, only one observation a day, no measurement of electrolytes and total proteins in blood and no measurement of food consumption (Draft Assessment Report – Anthraquinone 2006).

** Deviation from to date standard protocol: No reactivity test and weekly checking out of cage, no platelets count, no weighing of the brain, epididymes and uterus, no histology of a nerve and the uterus, blood tests on 5 rats/sex/dose only, no measurement of electrolytes and no ophthalmological tests (Draft Assessment Report – Anthraquinone 2006).

*** Deviation from to date standard protocol: number and species of exposed animals is not clear, only two dose groups were tested, no statement concerning clinical and cage-side observation, food consumption not reported, it is not sound enough specified which parameters were measured (clinical chemistry haematology) and which organs were examined (in the histological investigations); the same is true for a in-depth analysis of the respiratory tract.

Repeated dose toxicity: oral

1. Subacute study (Bayer AG 1976)

The study is summarised in the Draft Assessment Report – Anthraquinone (2006), which was the base for the following paragraph.

In a 28 day subchronic oral study male and female Wistar rats were administered 0, 2.0, 10, 20, 50 and 250 mg/kg bw AQ per gavage. Clinical observations reported that the animals showed lowered general conditions (effects not specified) at doses of 10 mg/kg bw/d. Body weight was decreased at 50 mg/kg bw/d onwards during week 1 up to week 4 in males and at 20 mg/kg bw/d onwards during week 2 up to week 4 in females. In haematology and clinical chemistry no adverse effects or abnormalities were reported. Sometimes absolute organ weights were altered, but no dose relationship was observed. Therefore, these effects were considered of no toxicological relevance. Relative liver weights as well as relative spleen weights were increased beginning at 10 mg/kg bw/d. Relative kidney weights were increased at 50 mg/kg bw/d and at higher doses. Histological investigations showed enlarged hepatocytes (which frequently involve periportal as well as centrilobular and midzonal hepatocytes) beginning at doses of 10 mg/kg bw/d and becoming more pronounced at 250 mg/kg bw/d. Males were more severely affected than females at all dose levels. This effect was viewed as adaptive, because no clinical chemistry alterations or adverse histological lesions (such as hyperplasia, necrosis or degeneration) were found. Congestion of the spleen was observed in some rats at 10 and 20 mg/kg bw and in all rats dosed at 50 mg/kg and 250 mg/kg bw. This effect was considered to be compound-related. Table 12 summarises the results:

Table 12: Effects of the subacute study

Endpoints/dose	0		2.0		10		20		50		250 mg/kg bw/d	
sex	M	F	M	F	M	F	M	F	M	F	M	F
Body weight								↓5-6%	↓%4-11%	↓4-12%	↓%11-16%	↓5-10%
Relative organ weight:												
Liver					↑8%	↑10%	↑10%	↑15%	↑24%	↑24%	↑60%	↑64%
Spleen					↑13%	↑22%	↑51%	↑33%	↑22%	↑28%	↑23%	↑20%
Thyroid									↑25%		↑75%	
Kidney						↑10%			↑9%	↑9%	↑13%	↑12%
Testes									↑13%		↑15%	↓25%
Adrenals								↓15%	↑15%	↓16%		↓16%
Brain									↑7%		↑5%	
Histopathology:												
Liver: hepatocyte enlargement centrilobular/midzonal/periportal												
minimal					4/5	2/5	0/5	0/5	1/5	4/5	0/5	4/5
moderate									4/5	1/5	5/5	1/5
Spleen:												
congestion												
minimal					3/5	3/5	5/5	5/5	4/5	1/5	2/5	2/5
moderate					0/5	2/5	0/5	0/5	1/5	4/5	3/5	3/5

A NOAEL of 2 mg/kg bw/d is proposed, based on increased relative spleen weight associated with minimal splenic congestion at 10 mg/kg bw/d.

2. Subchronic study (Bayer AG 1979)

The study is summarised in the Draft Assessment Report – Anthraquinone (2006), which was the base for the following paragraph.

In a 90-day dietary study with Wistar rats, males and females were exposed to 0, 15, 150, 1,500 ppm AQ. Clinical observations showed no difference in appearance, behaviour or mortality. Food consumption was reduced for males and females at 150 ppm and 1,500 ppm. Body weight was decreased in female rats at all doses and in male rats at 1,500 ppm. Reticulocyte counts raised and RBC counts were slightly reduced at all concentrations, suggesting haemolytic anaemia. However, these effects were seen at week 6 of exposure, but decreased in males at week 13, while still being increased in females. At doses of 150 ppm and 1,500 ppm blood cholesterol and albumin were increased in males and females. Albumin was also increased in urine at these doses, whereas reduced urea was measured at all doses levels. Liver weight was increased in male rats at 150 ppm and 1,500 ppm. Relative thyroid and liver weight was increased in males at 1,500 ppm. No evidence of pathological organ changes was revealed in the histological investigations. A summary of the results is given in table 13.

Table 13: Effects of the subchronic study

Endpoints/dose	0		15		150		1,500 ppm	
sex	M	F	M	F	M	F	M	F
mortality								
Body weight			↓5%	↓5%*	↓6%	↓7%*	↓16%*	↓15%*
Food consumption					↓10%	↓10%	↓14 %	↓11 %
Hematology								
Week 6								
RBC count			↓6%	↓5%	↓6 %	↓6 %*	↓6%	↓4%
Reticulocyte count			↑108%*	↑92%*	↑149%*	↑92%*	↑191%*	↑128%*
Week 13								
RBC count			↓2%	↓3%	↓5 %*	↓6 %	↓5%	↓4%
Reticulocyte count			↑11%	↑54%*	↑99%*	↑136%*	↑16%	↑218%*
Clinical chemistry wk 13:								
Albumin						↑7%*		↑15%*
Cholesterol							↑41%*	↑88%*
Urinalysis wk 13								
Albumin					↑29%*	↑39%*	↑64%*	↑86%*
Organ weight								
Rel. liver weight								
Abs. Thyroid weight							↑42%	
Abs. Liver weight					↑22%		↑33%	

* Statistically significantly different from control.

A NOAEL of 15 ppm (1.36 mg/kg bw/d) was proposed, not taking into account the slightly reduced body weight, the increased reticulocyte counts and slightly, non-significantly decreased RBC counts, which could be considered as not adverse at that dose level due to the slight decreases in RBC counts (less than 10% level).

3. 14 week study in rats (NTP 2005)

This abstract has been extracted from the NTP Technical Report 494 - Anthraquinone (NTP 2005).

Groups of 10 male and 10 female F344/N rats were fed diets containing 0, 1,875, 3,750, 7,500, 15,000 or 30,000 ppm AQ (equivalent to average daily doses of approximately 135, 275, 555, 1,130 or 2,350 mg AQ/kg bw) for 14 weeks. All rats survived until the end of the study. Mean body weights of females were significantly lower in the exposed groups than in the control group. Feed consumption by the exposed and control groups was similar at the end of the study. Liver and kidney weights of exposed groups were greater than those of the controls, as were testis weights of males exposed to 7,500 ppm or greater. A minimal, responsive anaemia was apparent in groups of male and female rats exposed to 3,750 ppm or greater by day 26 of the study. The (regenerative haemolytic) anaemia persisted and involved all exposed groups of rats at the end of the study. Renal function was also affected by AQ exposure as demonstrated by increases in urine protein and glucose concentrations and aspartate aminotransferase and N-acetyl- β -D-glucosaminidase activities. Estrous cycles were longer in 15,000 and 30,000 ppm females than in the controls. Groups of exposed rats showed liver hypertrophy (M/F \geq 1875 ppm); eosinophilic hyaline droplets in the kidney (M/F \geq 1875 ppm); congestion, hematopoietic cell proliferation, iron-positive pigmentation of the spleen (indicative of hemosiderin deposition; /F \geq 1875 ppm); and bone marrow hyperplasia (M \geq 3,750 ppm, F \geq 1,875 ppm). The incidences of nephropathy in 15,000 and 30,000 ppm females were significantly greater than those in the controls, and the severities of nephropathy were increased in exposed groups of males and in 30,000 ppm females. The concentrations of α 2u-globulin in the kidneys were significantly greater in all exposed groups of males. Thyroid gland follicular cell hypertrophy was present in all males and females exposed to 3,750 ppm or greater. Incidences of inflammation and transitional cell hyperplasia in the urinary bladder of 30,000 ppm females were greater than those in the controls. No NOAEL could be derived.

4. 14 week study in mice (NTP 2005)

This abstract has been extracted from the NTP Technical Report 494- Anthraquinone (NTP 2005).

Groups of 10 male and 10 female B6C3F1 mice were fed diets containing 0, 1,875, 3,750, 7,500, 15,000 or 30,000 ppm AQ (equivalent to average daily doses of approximately 0, 250, 500, 1,050, 2,150 or 4,300 mg/kg in males and 0, 300, 640, 1,260, 2,600 or 5,300 mg/kg in females) for 14 weeks. All mice survived until the end of the study. Mean body weights and feed consumption were similar among exposed and control groups. A responsive anaemia occurred in exposed mice at week 14 (M \geq 7,500 ppm, F \geq 1,875 ppm; in both sexes without unequivocal dose-response relationship). Liver weights of exposed groups of mice were significantly greater than those of the control groups. The incidences of centrilobular hypertrophy in the liver of mice exposed to 3,750 ppm or greater were significantly greater than those in the controls, and the severities increased with increasing exposure concentration. Cytoplasmic alteration of the urinary bladder was observed in all exposed mice, and the severities increased with increasing exposure concentration. The incidences of hematopoietic cell proliferation were increased in all exposed groups of males and females, and pigmentation was observed in the spleen of all exposed mice (except one male and one female in the 30,000 ppm groups). No NOAEL could be set.

Repeated dose toxicity: inhalation

Different experiments (chronic inhalation toxicity, acute and subacute oral toxicity, eye irritation, tests for photodynamic effects) were conducted in a study by Volodchenko et al. (1970). Test animals were 96 rats, 10 rabbits and 50 mice. A not specified number of animals (probably rats) was exposed to doses of 5.2 ± 0.8 and 12.2 ± 0.7 mg/m³ (analytical) dust concentrations of AQ for 6-8 hours daily in the 4 month repeated dose inhalation toxicity study. At 12.2 mg/m³ the following symptoms occurred: Body weight loss and changes in blood parameters, i.e. lowered levels of haemoglobin (76.2% after two months, 74.4% after 4 months), erythrocytopenia (7.5 million vs 9.08 million in the control animals), relative reticulopenia. The blood parameters normalized during the experimental period. Histopathological investigations showed several effects in the lungs: emphysema, atelectasis, cellular proliferation, in particular perivascular hyperaemia of the capillaries and exsudation in the alveolar lumen. The microstructural changes of the lung regenerated within the first month after termination of the experiment. No severe effects were observed in the 5.2 mg/m³ dose groups. This study is considered as not valid, because of the scarce information available.

Repeated dose toxicity: dermal

No information are available.

Repeated dose toxicity: other routes

No information are available.

Human information

No information is available.

Other relevant information

No information is available.

Summary and discussion of repeated dose toxicity

Four oral toxicity studies with repeated doses were performed. In two of them, both conducted by NTP (2005) no NOAEL can be derived. In these studies the doses were much higher than in the two older studies, performed by the Bayer AG (1976 and 1979). Target organs were the liver, kidneys, urinary bladder and the haematopoietic system.

The 28 day study with rats performed by Bayer AG (1976) included AQ doses from 2 up to 250 mg/kg bw/d given by gavage. A lowered general health condition of the laboratory animals was reported at 10 mg/kg bw/d onwards. They showed body weight decreases for doses beginning with 20/50 mg/kg bw/d (F/M). In addition, altered organ weights were seen, but these were considered to be related to the former effect (decreased body weight). Minimal congestion and increased weight was reported for the spleen. Hepatocyte enlargement (liver cell hypertrophy) and increased liver weights were reported. This effect was more severe in male rats and was not found to be associated with alterations in clinical chemistry. Probably this effect indicates an adaptive response.

A 90-day dietary rat study (Bayer AG 1979) was conducted at doses of 15, 150 and 1,500 ppm (ca. 1.4, 13 and 175 mg/kg bw/d). Reduction in body weight and food consumption was observed beginning at 15 ppm. Increased reticulocytes and slightly decreased RBC count were measured from doses of 15 ppm onwards, indicative of haemolytic anaemia. However, after weighing and histological examination of the spleen (and other organs) no haemosiderin depositions or other related effects were seen in this study, the red blood cell reduction was quite low and no firm explanation could be given for the highly altered reticulocyte count. A NOAEL of 1.36 mg/kg bw/d is proposed due to the fact that the observed effects on body weight and blood count at this doses level were considered as minimal and non-adverse.

In the two 14-week NTP studies in rats (NTP 2005) and mice (NTP 2005) a mild, responsive anaemia was seen beginning with doses of 1,870 ppm (rat) and 3,750 ppm (mouse) and starting at day 26 of the study. Also, altered renal function and thyroid gland follicular cell hypertrophy were apparent in rats. Additionally, centrilobular hypertrophy of liver cells was observed in mice and rats at doses of 1,875 ppm and above. All exposed mice showed cytoplasmic alteration of the urinary bladder. Inflammation & transitional epithelial hyperplasia of the urinary bladder was observed in female rats at 30000 ppm. Hematopoietic cell proliferation and pigmentation in the spleen of mice and rats are effects that corresponded to the haemolytic and regenerative nature of the anaemia.

Induction of regenerative anaemia is consistent in studies conducted by NTP (2005) and the Bayer AG (1976 and 1979). Mild regenerative anaemia has been induced at doses of 1,875 ppm and above in rats and mice in the NTP studies (2005). Reductions in Hct, Hb and RBC did not exceed the 10% level in rats at 30,000 ppm and reached maximum -13% in mice at this dose. Anaemia observed in rats and mice is consistent with the findings in the 90 day study in rats conducted by the Bayer AG (1979). The lowest dose where anaemic effects have been observed was 150 ppm in the 3 month study of the Bayer AG (1979). However, no special staining on haemosiderin depositions were conducted in any of these studies and overall anaemic effects were weak. The NTP studies (2005) reported organ effects secondary to the anaemia such as spleen congestion, iron-positive pigmentation (hemosiderin desposition) as well as increased (extramedullar) hematopoietic cell proliferation, and bone marrow hyperplasia, whereby the overall effect was considered to be a regenerative haemolytic anaemia.

One study on chronic inhalation toxicity in rats is available (Volodchenko et al. 1970). The study has various limitations as it is only available in summary, only two dose groups were tested, number and species of exposed animals is not clear and it is not specified, which organs were examined in histological investigations. Hence, it consists of less valid information and is just reported for the sake of completeness. Effects on body weight, blood parameters and the lungs were observed, however the study documentation is insufficient to allow any conclusion from these data.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

The presented studies on repeated dose were reported for purpose of background information only. The findings in repeated dose studies are of relevance to identify toxic effects and possible precursor lesions with regard to the hazard class carcinogenicity.

4.9 Germ cell mutagenicity (Mutagenicity)

Mutagenicity of AQ is discussed controversially in literature, mainly due to conflicting data from bacterial testing systems, where contamination of AQ samples with 9-NA play a major role. With regard to the carcinogenicity study of the National Toxicology Program (NTP 2005), especially the contaminant 9-NA and also metabolites of AQ have to be examined concerning their mutagenicity.

AQ is produced by different production methods, which generate varying grades of purity. The most common methods for AQ production are Oxidation of anthracene (AQ-OX), Friedel-Crafts technology (AQ-FC) and Diels-Alder chemistry (AQ-DA) (Butterworth et al. 2001).

Table 14: Summary table of relevant in vitro and in vivo mutagenicity studies with AQ

Method	Results	Remarks	Reference
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p>S. typhimurium TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p>E. coli WP2 uvr A (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 60, 125, 250, 500, 1,000 and 2,000 µg/plate</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 471</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>precipitations: with and without S9-mix at the highest tested dose of 2,000 µg/plate</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation: 'Purified NTP AQ-OX' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p>	<p>Butterworth et al. 2001</p>
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p>S. typhimurium TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p>E. coli WP2 uvr A (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 60, 125, 250, 500, 1,000 and 2,000 µg/plate</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 471</p>	<p>Evaluation of results: positive without and with S9-mix</p> <p>without S9-mix:</p> <ul style="list-style-type: none"> - TA 98 (≥ 125 µg/plate); dose-dependent effect - TA 1537 (≥ 250 µg/plate); dose-dependent effect - TA 100 (2,000 µg/plate) <p>with S9-mix:</p> <ul style="list-style-type: none"> - TA 98 ($\geq 1,000$ µg/plate); dose-dependent effect - TA 1537 ($\geq 1,000$ µg/plate); dose-dependent effect <p>cytotoxicity: no</p> <p>precipitations: with and without S9-mix at the highest tested dose of 2,000 µg/plate</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation: 'NTP AQ-OX' with about 0.1 % 9-nitroanthracene as impurity</p> <p>purity: 99 %</p>	<p>Butterworth et al. 2001</p>

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<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p>S. typhimurium TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p>E. coli WP2 uvr A (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 60, 125, 250, 500, 1,000 and 2,000 µg/plate</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 471</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>precipitations: with and without S9-mix at the highest tested dose of 2,000 µg/plate</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation: 'AQ-FC' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p>	<p>Butterworth et al. 2001</p>
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p>S. typhimurium TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p>E. coli WP2 uvr A (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 60, 125, 250, 500, 1,000 and 2,000 µg/plate</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 471</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>precipitations: with and without S9-mix at the highest dose tested of 2,000 µg/plate</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation: 'AQ-DA' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p>	<p>Butterworth et al. 2001</p>
<p>Mouse lymphoma assay (L5178Y cells; TK locus)</p> <p>Test concentrations (with and without S9-mix): 0, 1.57, 3.13, 6.25, 12.5, 25, 37.5 and 50 µg/mL</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 476</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation: 'AQ-DA' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p> <p>The highest tested concentration is about twice the solubility limit in medium.</p>	<p>Butterworth et al. 2001</p>

<p>Chromosome aberration test (CHO cells)</p> <p>Test concentrations (with and without S9-mix.): 0, 12.5, 25, 37.5 and 50 µg/mL</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 473</p>	<p>Evaluation of results: negative cytotoxicity: no</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation: 'AQ-DA' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p> <p>Solubility considerations determined the highest dose to be 50 µg/mL.</p>	<p>Butterworth et al. 2001</p>
<p>Micronucleus test (bone marrow)</p> <p>Mice: CrI:CD-1 (ICR) BR mice (5 males and 5 females for each dose and each harvest time)</p> <p>Test concentrations: gavage of 250, 2,500 and 5,000 mg/kg bw</p> <p>Sampling times: 24, 48 and 72 h after administration</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 474</p>	<p>Evaluation of results: negative toxicity: 'lack of significant toxicity' (only information)</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation 'AQ-DA' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p>	<p>Butterworth et al. 2001</p>

4.9.1 Non-human information

By means of a weight of evidence approach the existing information for the toxicological endpoint mutagenicity is sufficient for the conclusion that AQ is neither mutagenic in vitro nor in vivo.

In tests with positive results AQ is generally not highly purified or definitively burdened with mutagenic 9-NA as contamination. On the other hand, the metabolites 1-OH-AQ and 2-OH-AQ show mutagenic properties in vitro.

For justification of classification/non-classification of AQ only those mutagenicity studies are of major relevance, which were carried out in accordance with the corresponding OECD test guideline. Mutagenicity tests whose test performance was carried out similar to the corresponding OECD test guideline should be also considered for the description of mutagenic effects.

In vitro data

Bacterial gene mutation tests with AQ

Table 15: Summary table of bacterial gene mutation tests with AQ

Method	Results	Remarks	Reference
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p>S. typhimurium TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p>E. coli WP2 uvrA (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 60, 125, 250, 500, 1,000 and 2000 µg/plate</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 471</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>precipitations: with and without S9-mix at the highest tested dose of 2,000 µg/plate</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation: 'Purified NTP AQ-OX' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p>	<p>Butterworth et al. 2001</p>
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p>S. typhimurium TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p>E. coli WP2 uvrA (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 60, 125, 250, 500, 1,000 and 2,000 µg/plate</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 471</p>	<p>Evaluation of results: positive without and with S9-mix</p> <p>without S9-mix:</p> <ul style="list-style-type: none"> - TA 98 (≥ 125 µg/plate); dose-dependent effect - TA 1537 (≥ 250 µg/plate); dose-dependent effect - TA 100 (2,000 µg/plate) <p>with S9-mix:</p> <ul style="list-style-type: none"> - TA 98 ($\geq 1,000$ µg/plate); dose-dependent effect - TA 1537 ($\geq 1,000$ µg/plate); dose dependent effect <p>cytotoxicity: no</p> <p>precipitations: with and without S9-mix at the highest tested dose of 2,000 µg/plate</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation: 'NTP AQ-OX' with about 0.1 % 9-nitroanthracene as impurity</p> <p>purity: 99 %</p>	<p>Butterworth et al. 2001</p>

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<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p>S. typhimurium TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p>E. coli WP2 uvr A (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 60, 125, 250, 500, 1,000 and 2,000 µg/plate</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 471</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>precipitations: with and without S9-mix at the highest tested dose of 2,000 µg/plate</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation: 'AQ-FC' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p>	<p>Butterworth et al. 2001</p>
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p>S. typhimurium TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p>E. coli WP2 uvrA (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 60, 125, 250, 500, 1,000 and 2,000 µg/plate</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 471</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>precipitations: with and without S9-mix at the highest dose tested of 2,000 µg/plate</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation: 'AQ-DA' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p>	<p>Butterworth et al. 2001</p>
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p>S. typhimurium TA 98, TA 100 and TA 102 (with and without S9-mix)</p> <p>Test concentrations: 0, 100, 333, 1,000, 3,333 and 10,000 µg/plate</p> <p>GLP: no information</p> <p>Similar to OECD Guideline 471</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>precipitations: with and without S9-mix ≥ 1,000 µg/plate</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: 100 %</p>	<p>NTP 2005 (Table E2)</p>

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<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98, TA 100 and TA 1537 (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 100, 300, 1,000, 3,000 and 10,000 µg/plate</p> <p>GLP: no information</p> <p>Similar to OECD Guideline 471</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>precipitations: without S9-mix \geq 30 µg/plate; with S9-mix \geq 100 µg/plate</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: 99.8 %</p>	<p>NTP 2005 (Table E3)</p>
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98 and TA 100 (S9-mix: with and without)</p> <p>Test concentrations: 0, 33, 100, 333, 1,000 and 2,500 µg/plate</p> <p>GLP: no information</p> <p>Similar to OECD Guideline 471</p>	<p>Evaluation of results: positive without and with S9-mix</p> <p>without S9-mix:</p> <ul style="list-style-type: none"> - TA 98 (\geq 33 µg/plate); dose-dependent effect - TA 100 (\geq 1,000 µg/plate); dose-dependent effect <p>with S9-mix:</p> <ul style="list-style-type: none"> - TA 98 (\geq 333 µg/plate); dose-dependent effect - TA 100 (\geq 1,000 µg/plate); dose-dependent effect <p>precipitations: without and without S9-mix at 2,500 µg/plate</p> <p>cytotoxicity: no</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: 97 %</p>	<p>NTP 2005 (Table E1) (primary literature: Zeiger et al., 1988)</p>
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98 and TA 100 (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 100, 300, 1,000, 3,000 and 10,000 µg/plate</p> <p>GLP: no information</p> <p>Similar to OECD Guideline 471</p>	<p>Evaluation of results: negative</p> <p>precipitations: with and without S9-mix \geq 1,000 µg/plate</p> <p>cytotoxicity: no</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>preparation: A 65343</p> <p>purity: not known</p>	<p>NTP 2005 (Table E4)</p>

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<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98 and TA 100 (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 100, 300, 1,000, 3,000 and 10,000 µg/plate</p> <p>GLP: no information</p> <p>Similar to OECD Guideline 471</p>	<p>Evaluation of results: negative</p> <p>precipitations: with and without S9-mix \geq 1,000 µg/plate</p> <p>cytotoxicity: no</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>preparation: A 54984</p> <p>purity: not known</p>	<p>NTP 2005 (Table E5)</p>
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98 and TA 100 (with and without S9-mix)</p> <p>Test concentrations: 0, 30, 100, 300, 1,000, 3,000 and 10,000 µg/plate</p> <p>GLP: no information</p> <p>Similar to OECD Guideline 471</p>	<p>Evaluation of results: weakly positive in TA98 without S9-mix (all other positive results with and without S9-mix have been induced by concentrations, which also induced precipitations)</p> <p>without S9-mix:</p> <ul style="list-style-type: none"> - TA 98 \geq 100 µg/plate; dose-dependent effect - TA 100 = 10,000 µg/plate <p>with S9-mix:</p> <ul style="list-style-type: none"> - TA 98 \geq 1,000 µg/plate ; dose-dependent effect - TA 100 = 10,000 µg/plate <p>precipitations: with and without S9-mix \geq 300 µg/plate</p> <p>cytotoxicity: no</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: 99.4 %</p>	<p>NTP 2005 (Table E6)</p>
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98, TA 100, TA 1535, TA 1537, TA 1538 and TA1538 (with and without S9-mix)</p> <p>Test concentrations: 0, 0.2, 2.0, 10 and 20 µg/plate</p> <p>GLP: not reported</p> <p>Similar to OECD Guideline 471</p>	<p>Evaluation of results: weakly positive</p> <p>without S9-mix:</p> <ul style="list-style-type: none"> - TA 98 \geq 10 µg/plate - TA 1537 \geq 10 µg/plate - TA 1538 \geq 10 µg/plate <p>with S9-mix:</p> <ul style="list-style-type: none"> - TA 100 \geq 20 µg/plate - TA 1537 = 20 µg/plate <p>cytotoxicity: no</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: not known</p>	<p>Lieberman et al. 1982</p>

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<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p><i>E. coli</i> WP2 uvrA (with and without S9-mix)</p> <p>Test concentrations: 0, 1.5, 5.0, 15, 50, 150 and 1,000 µg/plate</p> <p>GLP: yes</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 471 is possible.</p>	<p>Evaluation of results: equivocal with S9 mix</p> <p>cytotoxicity: not determined</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>4 (not assignable)</p> <p>purity: not known</p> <p>(no detailed description of experimental results)</p>	<p>Täublová 2009</p>
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 97, TA 98, and TA 100 (with and without S9-mix)</p> <p>Test concentrations: 0, 5.0, 10, 50 and 200 µg/plate</p> <p>GLP: not reported</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 471 is possible.</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: yes</p> <p>- without S9-mix: TA 98 at 200 µg/plate</p> <p>- with S9-mix: TA 97 at 200 µg/plate</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: not reported</p>	<p>4 (not assignable)</p> <p>purity: not known</p>	<p>Sakai et al. 1985</p>
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98, TA 100 and TA 2637 (with and without S9-mix)</p> <p>Test concentrations: “0.1 to 1,000” µg/plate (only range reported)</p> <p>GLP: not reported</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 471 is possible.</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: not reported</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>4 (not assignable)</p> <p>purity: not known</p>	<p>Tikkanen et al. 1983</p>

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<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98, TA 100, TA 1535, TA 1537 and TA 1538 (with and without S9-mix)</p> <p>Test concentrations: “0.1 to 1,000” µg/plate (only range reported)</p> <p>GLP: not reported</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 471 is possible.</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: not reported</p> <p>vehicle controls valid: not reported</p> <p>positive controls valid: not reported</p>	<p>4 (not assignable)</p> <p>purity: not known</p>	<p>Salamone et al. 1979</p>
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98, TA 1535, TA 1537 and TA 1538 (with and without S9-mix)</p> <p>Test concentrations: “104 to 520” µg/plate (only range reported)</p> <p>GLP: not reported</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 471 is possible.</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>vehicle controls valid: not reported</p> <p>positive controls valid: not reported</p>	<p>4 (not assignable)</p> <p>purity: not known</p>	<p>Gibson et al. 1978</p>
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p>Test concentrations: not reported</p> <p>GLP: not reported</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 471 is possible.</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: not reported</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>4 (not assignable)</p> <p>purity: not known</p>	<p>Anderson and Styles 1978</p>

<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p>S. typhimurium TA 98, TA 100, TA 1535, TA 1537 and TA1538 (with and without S9-mix)</p> <p>Test concentrations: not reported</p> <p>GLP: not reported</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 471 is possible.</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: not reported</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>4 (not assignable)</p> <p>purity: not known</p>	<p>Brown and Brown 1976</p>
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Overall, AQ induced negative responses in bacterial tests.

Four bacterial gene mutation tests are available whose test performance is in accordance with OECD guideline 471 (Butterworth 2001). AQ samples with high purity (samples AQ-FC and AQ-DA; purity: 99 %; without 9-NA as impurity) were clearly negative. One sample of AQ (AQ-OX; purity 99 %) with about 0.1 % 9-NA as impurity induced positive effects with and without S9-mix. After the removal of this impurity AQ (sample AQ-OX purified; purity: 99 %) induced no mutagenic effects in bacteria. This suggests the conclusion that 9-NA affects the outcome of the bacterial experiments. For more information on 9-NA see section 4.9.3.

Tests whose test performance is closely related to OECD Test Guideline 471 support the negative results of the guideline-conform bacterial gene mutation tests (NTP 2005, tables E2, E3, E4 and E5). The results of two positive bacterial gene mutation tests (NTP 2005, table E1; Liberman et al. 1982) must be subjected a critical evaluation, because the tested AQ sample has only a purity of 97 % or is unknown and it is not known if e.g. mutagenic 9-NA was present as impurity. For another positive result (NTP 2005, table E6) the biological relevance is questionable, because the weak effect was induced only in one tester strain without S9-mix at a concentration near the solubility limit. Apart from that, further effects with and without S9-mix were induced only at concentrations, which also induced precipitations.

Mammalian cell tests with AQ**Table 16: Summary table of in vitro soma cell tests with AQ**

Method	Results	Remarks	Reference
<p>Mammalian cell gene mutation test (V79 cells)</p> <p>Test concentrations: 0, 1.25, 2.5, 5, 10, 20 µg/mL (with and without S9-mix)</p> <p>GLP: yes</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 476 is possible.</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>4 (not assignable)</p> <p>purity: not known</p>	<p>Bednáriková 2010</p>
<p>Mouse lymphoma assay (L5178Y cells; TK locus)</p> <p>Test concentrations (with and without S9-mix): 0, 1.57, 3.13, 6.25, 12.5, 25, 37.5 and 50 µg/mL</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 476</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation: 'AQ-DA' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p> <p>The testing limit is about twice the solubility limit in medium.</p>	<p>Butterworth et al. 2001</p>
<p>Gene mutation test (h1A1v2 cells; TK locus)</p> <p>Test concentrations: 0, 1, 10, 100, 250 and 5,000 ng/mL</p> <p>GLP: no information</p> <p>Not in accordance with OECD Guideline 476</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: 97 %</p> <p>The test was only done with metabolic activation. Cells of the h1A1v2 cell line constitutively express cytochrome P4501A1, which is known to be necessary for the metabolism of many promutagens.</p> <p>Insufficient data presentation (only pooled data of two independent experiments were given).</p>	<p>Durant et al. 1996</p>
<p>Chromosome aberration test</p>	<p>Evaluation of results: negative</p>	<p>1 (reliable without</p>	<p>Butterworth et</p>

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<p>(CHO cells)</p> <p>Test concentrations (with and without S9-mix.): 0, 12.5, 25, 37.5 and 50 µg/mL</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 473</p>	<p>cytotoxicity: no</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>restrictions)</p> <p>key study</p> <p>preparation: 'AQ-DA' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p> <p>Solubility considerations determined the highest dose to be 50 µg/mL.</p>	<p>al. 2001</p>
<p>Chromosome aberration test (V79 cells)</p> <p>Test concentrations: 0, 1.25, 2.5, 5.0, 10 and 20 µg/mL (with and without S9-mix)</p> <p>GLP: yes</p> <p>Not in accordance with OECD 473</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>4 (not assignable)</p> <p>purity: not known</p>	<p>Lazová 2010</p>
<p>Micronucleus test (SHE cells)</p> <p>Test concentrations: 3.13, 6.25, 12.5 and 25 µg/mL</p> <p>GLP: no information</p> <p>Not in accordance with OECD Guideline 487 (SHE cells are not included in the guideline)</p>	<p>Evaluation of results: weakly positive</p> <p>metabolically active SHE cells: - weakly positive effect at the highest tested concentration of 25 µg/mL (doubling of the micronucleus frequency compared to the negative control)</p> <p>cytotoxicity: no</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: not known</p>	<p>Gibson et al. 1997</p>

AQ does not induce mutagenic effects in mammalian cell cultures.

AQ (without 9-NA as impurity) induces neither a mutagenic effect in a mouse lymphoma assay (Butterworth et al. 2001) nor in a chromosome aberration test (Butterworth et al. 2001). Both test performances are equivalent to OECD Test Guideline 476, respectively OECD Test Guideline 473.

A gene mutation test with h1A1v2 cells is also negative (Durant et al. 1996). In a micronucleus assay in SHE cells (Gibson et al. 1997) AQ induced a weak positive effect (doubling of the micronucleus frequency compared to the negative control) at the highest tested concentration of 25 µg/mL. At both tests there exist methodological limitations. However, the biological meaning of the micronucleus test in SHE cells remains questionable, because the weak positive effect is induced by a charge of AQ with unknown purity, and it is not known whether e. g. mutagenic 9-NA was contained as an impurity in the tested sample.

In vivo data**Table 17: Summary table of in vivo mutagenicity studies with AQ**

Method	Results	Remarks	Reference
<p>Micronucleus test (bone marrow)</p> <p>Mice: CrI:CD-1 (ICR) BR mice (5 males and 5 females for each dose and each harvest time)</p> <p>Test concentrations: gavage of 250, 2,500 and 5,000 mg/kg bw</p> <p>Sampling times: 24, 48 and 72 h after administration</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 474</p>	<p>Evaluation of results: negative</p> <p>toxicity: 'lack of significant toxicity' (only information)</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>preparation 'AQ-DA' without 9-nitroanthracene as impurity</p> <p>purity: 99 %</p>	Butterworth et al. 2001
<p>Micronucleus assay (bone marrow)</p> <p>Mice: B6C3F₁ mice (5 males only for each harvest time)</p> <p>Test concentrations: i.p. of 500, 1,000 and 2,000 mg/kg bw; each dose was injected three times at 24-hour intervals</p> <p>Sampling time: 24 h after third injection</p> <p>GLP: no information</p> <p>Similar to OECD Guideline 474</p>	<p>Evaluation of results: negative</p> <p>toxicity: no information</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: not known</p>	NTP 2005 (Table 12)
<p>Micronucleus assay (peripheral blood erythrocytes)</p> <p>Mice: B6C3F₁ mice (5 males and 5 females for each concentration)</p> <p>Test concentrations: 1,875, 3,750, 7,500, 15,000 and 30,000 ppm for 14 weeks in the feed</p> <p>GLP: no information</p> <p>Not in accordance with OECD Guideline 474</p>	<p>Evaluation of results: positive</p> <p>Weak positive effect in males at the highest dose tested (doubling of the micronucleus frequency compared to the negative control) and positive effects in females at all doses tested without a clear dose-dependency.</p> <p>negative controls valid: yes</p> <p>positive controls valid: a positive control is lacking</p>	<p>4 (not assignable because of the lack of a positive control)</p> <p>purity: 99.8 %</p>	NTP 2005 (Table 13)

<p>DNA damage (alkaline elution technique coupled with a microfluorimetric method)</p> <p>Mice: Swiss CD1</p> <p>Test concentration: i.p. of 250 mg/kg bw</p> <p>Sampling time: 4 h after administration</p> <p>GLP: no information</p> <p>Not in accordance with a OECD guideline; lack of a positive control; screening test without detailed information.</p>	<p>Evaluation of results: positive in cells of liver and kidney</p> <p>A three-fold increase of single-strand DNA-breaks was observed in liver and kidney cells compared to the solvent control.</p> <p>negative control: valid</p> <p>positive control: a positive control is lacking</p>	<p>4 (not assignable)</p> <p>purity: not known</p>	<p>Cesarone et al, 1982</p>
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No induction of mutagenic effects was observed in soma cells.

In a bone marrow micronucleus assays whose test performance is equivalent to OECD Test Guideline 474 a negative result was observed in male and female rats after oral administration of AQ (Butterworth et al. 2001).

A negative bone marrow micronucleus assay after i.p. injection of AQ (NTP 2005, table 12) has only limited evidence, because the test performance is not equivalent to OECD Test Guideline 474. There is no justification why only males were tested and not also females.

4.9.2 Human information

No data available.

4.9.3 Other relevant information

The description of mutagenicity of metabolites and impurities of the AQ sample used for the carcinogenicity testing of the NTP (2005) should be taken into account with regard to the discussion on induction of carcinogenic effects.

Of the impurities only the mutagenicity data of the 9-NA were considered. For other impurities such as anthrone, phenanthrene, and anthracene it is stated that they are not or only very weakly genotoxic (NTP 2005).

The mutagenicity of the main metabolite 2-OH-AQ as well as of the metabolite 1-OH-AQ were considered with regard to the carcinogenicity. Anthrone is also reported to be a minor metabolite, but its role in carcinogenicity is expected to be low and its mutagenicity is considered to be equivocal (NTP 2005).

With 9-NA, 2-OH-AQ and 1-OH-AQ only a few mutagenic studies were performed.

Impurity (9-NA)

Table 18: Summary table of bacterial gene mutation tests with 9-NA

Method	Results	Remarks	Reference
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p>TA 98 and TA 100, (with and without S9-mix)</p> <p>Test concentrations: 0, 2.9, 11.4, 45.6 and 182.5 nmol/plate (equivalent to: 0, 0.6, 2.3, 9.4 and 37 µg/plate)</p> <p>GLP: no information</p> <p>Not in accordance with OECD Guideline 471</p>	<p>Evaluation of results: weakly positive without and with S9-mix</p> <p>without S9-mix: - TA 98 (≥ 45.6 nmol/plate)</p> <p>with S9-mix: - TA 98 (at 182.5 nmol/plate)</p> <p>cytotoxicity: no</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: no</p>	<p>4 (not assignable)</p> <p>purity: > 99%</p>	<p>Fu et al. 1985</p>
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p>S. typhimurium TA 98 and TA 100 (with and without S9-mix)</p> <p>Test concentrations: 0, 0.1, 0.3, 1.0, 3.0 and 10 µg/plate</p> <p>GLP: yes</p> <p>Similar to OECD Guideline 471</p>	<p>Evaluation of results: positive without S9-mix; negative with S9-mix</p> <p>without S9-mix: - TA 98 (≥ 0.3 µg/plate); dose-dependent effect ($F_{max} = 3.5$) - TA 100 (10 µg/plate; $F_{max} = 2.0$)</p> <p>cytotoxicity: no</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: 97 %</p>	<p>Butterworth et al. 2004</p>
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p>TA 98 and TA 100 (with and without S9-mix)</p> <p>Test concentrations: 0, 100, 333, 1,000, 3,333 and 6,667 µg/plate</p> <p>GLP: no information</p> <p>Similar to OECD Guideline 471</p>	<p>Evaluation of results: positive without and with S9-mix</p> <p>without S9-mix: - TA 98 (≥ 100 µg/plate); dose-dependent effect ($F_{max} = 5.2$) - TA 100: negative</p> <p>with S9-mix: - TA 98 (≥ 100 µg/plate); dose-dependent effect ($F_{max} = 5.2$) - TA 100 (≥ 100 µg/plate); dose-dependent effect ($F_{max} = 8.2$)</p> <p>precipitations: with and without S-9 mix $\geq 1,000$ µg/plate</p> <p>cytotoxicity: no</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: not known</p>	<p>NTP 2005 (Table 11)</p>
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p>	<p>Evaluation of results: weakly positive without and with S9-mix</p> <p>without S9-mix:</p>	<p>4 (not assignable)</p> <p>purity: 'highly purified'</p>	<p>Pitts et al. 1982</p>

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<p>TA 98 and TA 98R (with and without S9-mix)</p> <p>Test concentrations: not specified</p> <p>GLP: no information</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 471 is possible.</p>	<p>- TA 98: 0.30 rev/μg - TA 98NR: 0.14 rev/μg</p> <p>with S9-mix: - TA 98: 0.32 rev/μg - TA 98NR: 0.13 rev/μg</p> <p>cytotoxicity: not reported</p> <p>vehicle controls valid: not reported</p> <p>positive controls valid: yes</p>	<p>TA 98R is a nitro-reductase deficient strain</p>	
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p>S. typhimurium TA 98 and TA 100 (with and without S9-mix)</p> <p>Test concentrations: not clearly specified</p> <p>GLP: no information</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 471 is possible.</p>	<p>Evaluation of results: positive</p> <p>cytotoxicity: no</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>4 (not assignable)</p> <p>purity: not known</p>	<p>Zeiger et al. 1988</p>

Table 19: Summary table of in vitro soma cell tests with 9-NA

Method	Results	Remarks	Reference
<p>Mouse lymphoma assay (L5178Y cells)</p> <p>Test concentrations without S9-mix: 0, 0.1, 1.0, 10, 20 and 30 µg/mL</p> <p>Test concentrations with S9-mix: 0, 0.1, 1.0, 5.0, 10 and 50 µg/mL</p> <p>GLP: yes</p> <p>Similar to OECD Guideline 476</p>	<p>Evaluation of results: equivocal with S9-mix</p> <p>- with S9-mix: questionable effect ≥ 5.0 µg/mL; although the weak dose-dependent increase in small colonies suggesting predominance of a clastogenic mechanism this effect is associated with increasing strong toxicity</p> <p>cytotoxicity:</p> <p>- without S9-mix: strongly toxic ≥ 20 µg/mL</p> <p>- with S9-mix: strongly toxic ≥ 5.0 µg/mL</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: not known</p>	Butterworth et al. 2004
<p>Gene mutation test (h1A1v2 cells; TK locus)</p> <p>Test concentrations: 0, 1, 10, 100, 1,000 and 6,000 ng/mL</p> <p>GLP: no information</p> <p>Not in accordance with OECD Guideline 476</p>	<p>Evaluation of results: weakly positive (doubling of the mutant frequency at the highest test concentration of 6,000 ng/mL)</p> <p>cytotoxicity: no</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>purity: > 99 %</p> <p>Cells of the h1A1v2 cell line constitutively express cytochrome P4501A1, which is known to be necessary for the metabolism of many promutagens.</p>	Durant et al. 1996

9-NA induced positive effects in bacterial gene mutation tests and was weakly positive in mammalian cell cultures.

Two positive bacterial gene mutation tests were carried out similar by OECD Test Guideline 471 (Butterworth et al. 2004; NTP 2005).

Two mammalian cell gene mutation tests support the conclusion on mutagenicity, but indicate only a weak potency. Durant et al. (1996), found a weak positive result at the TK-locus in h1A1v2 cells. The result of a mouse lymphoma assay in L5178Y cells, carried out similar by OECD Test Guideline 476, should be considered as equivocal, because with S9 mix positive effects were only observed at concentrations that induced strong to extreme toxic effects (Butterworth et al. 2004).

Metabolites (1-OH-AQ; 2-OH-AQ)

Table 20: Summary table of bacterial gene mutation tests with two in vivo metabolites of AQ (1-OH-AQ / 2-OH-AQ)

Method	Results	Remarks	Reference
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p><i>E. coli</i> WP2 uvr A (with and without S9-mix)</p> <p>Test concentrations: 0, 3.3, 10, 33.3, 100, 333 and 1,000 µg/plate</p> <p>GLP: yes</p> <p>Equivalent to OECD Guideline 471</p>	<p>Evaluation of results: positive without and with S9-mix</p> <p>without S9-mix: - TA 1537 (≥ 3.3 µg/plate); without dose-dependency</p> <p>with S9-mix: - TA 1537 (≥ 3.3 µg/plate); without dose-dependency</p> <p>cytotoxicity: no</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>1-hydroxyanthraquinone</p> <p>purity: not known</p>	<p>Butterworth et al. 2004</p>
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98, TA 100 and TA 102 (with and without S9-mix)</p> <p>Test concentrations: 0, 100, 333, 1,000, 3,333 and 10,000 µg/plate</p> <p>GLP: no information</p> <p>Similar to OECD Guideline 471</p>	<p>Evaluation of results: negative</p> <p>cytotoxicity: no</p> <p>precipitations: with and without S9-mix $\geq 1,000$ µg/plate</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>1-hydroxyanthraquinone</p> <p>purity: not known</p>	<p>NTP 2005 (Table E7)</p>
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix)</p> <p><i>E. coli</i> WP2 uvr A (with and without S9-mix)</p> <p>Test concentrations: without S9-mix: 0, 1.0, 3.3, 10, 33.3, 100, 333 and 1,000 µg/plate with S9-mix: 0.3, 1.0, 3.3, 10, 33.3, 100 and 1,000 µg/plate</p>	<p>Evaluation of results: positive with S9-mix</p> <p>with S9-mix: - TA 1537 (≥ 0.3 µg/plate); dose-dependent effect</p> <p>cytotoxicity: - with S9-mix: increased toxicity from 33 µg/plate upwards</p> <p>negative controls valid: yes</p> <p>positive controls valid: yes</p>	<p>1 (reliable without restrictions)</p> <p>key study</p> <p>2-hydroxyanthraquinone</p> <p>purity: not known</p>	<p>Butterworth et al. 2004</p>

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<p>GLP: yes</p> <p>Equivalent to OECD Guideline 471</p>			
<p>Bacterial reverse mutation test (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98 and TA 100 (with and without S9-mix)</p> <p>Test concentrations: 0, 3.3, 10, 33, 100, 200, 333 and 450 µg/plate</p> <p>GLP: no information</p> <p>Similar to OECD Guideline 471</p>	<p>Evaluation of results: positive without and with S9-mix</p> <p>without S9-mix: - TA 98 (≥ 10 µg/plate); dose-dependent effect</p> <p>with S9-mix: - TA 98 (≥ 33 µg/plate); dose-dependent effect</p> <p>precipitations: with and without S9-mix ≥ 333 µg/plate</p> <p>cytotoxicity: no</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>2 (reliable with restrictions)</p> <p>support study</p> <p>2-hydroxy-anthraquinone</p> <p>purity: not known</p>	<p>NTP 2005 (Table 8)</p>
<p>Bacterial reverse mutation assay (Ames test; gene mutation)</p> <p><i>S. typhimurium</i> TA 98, TA 100, TA and TA 2637 (with and without S9-mix)</p> <p>Test concentrations: 0.1 to 1,000 µg/plate (only range reported)</p> <p>GLP: not reported</p> <p>Due to the lack of information, no statement of compliance with OECD Guideline 471 is possible.</p>	<p>Evaluation of results: positive</p> <p>with S9-mix: - TA 100 (3.4 rev/nmol) - TA 2637 (9.6 rev/nmol)</p> <p>cytotoxicity: not reported</p> <p>vehicle controls valid: yes</p> <p>positive controls valid: yes</p>	<p>4 (not assignable)</p> <p>2-hydroxy-anthraquinone</p> <p>purity: not known</p>	<p>Tikkanen et al. 1983</p>

2-OH-AQ is positive in bacterial gene mutation tests.

2-OH-AQ was positive with S9-mix in a bacterial gene mutation test that was carried out equivalent to OECD Test Guideline 471 (Butterworth et al. 2004). In a further study, whose test performance was similar to OECD Test Guideline 471 (NTP 2005, table 8) positive results were induced with and without S9 mix.

1OH-AQ is equivocal in bacterial gene mutation tests.

With respect to the induction of gene mutations in bacteria, results from two available studies give no firm conclusion for 1-OH-AQ. In a bacterial gene mutation test that meets the requirement of OECD guideline 471 (Butterworth et al. 2004), mutagenic effects were induced with and without

S9-mix. In contrast, another bacterial gene mutation test, whose test performance was similar to OECD guideline 471 (NTP 2005, table E7) exhibited negative results with and without S9 mix.

4.9.4 Summary and discussion of mutagenicity

For the justification of classification only data of studies are to be considered whose test performance is in accordance with the corresponding OECD test guideline. All studies with AQ that meet this requirement showed negative findings in vitro (bacterial gene mutation test, mouse lymphoma assay, chromosome aberration test) and in vivo (micronucleus test).

4.9.5 Comparison with criteria

According to the ECHA guidance to CLP (point 3.5.2.2) substances shall be classified as germ cell mutagens, when:

Category 1A: there is positive evidence from human epidemiological studies,

Category 1B: there are positive results from in vivo heritable germ cell mutagenicity tests in mammals or in vivo somatic germ cell mutagenicity tests in mammals (in combination with evidence that the substance has potential to cause mutations to germ cells) or tests showing mutagenic effects in germ cells of humans, without demonstration of transmission to progeny,

Category 2: there is positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, e.g.: somatic cell mutagenicity tests in vivo, in mammals or other in vivo somatic cell genotoxicity tests, which are supported by positive results from in vitro mutagenicity assays.

Relevant mutagenicity studies with AQ were negative in vitro (bacterial gene mutation test, mouse lymphoma assay, chromosome aberration test) and in vivo (micronucleus test). Accordingly no mutagenicity was induced in somatic cells (criterion for Category 2). Data for induction of mutagenic effects in germ cells (criterion for Category 1B) or epidemiological studies (criterion for Category 1A) are not available.

4.9.6 Conclusions on classification and labelling

With regard to mutagenicity no classification is proposed for AQ.

4.10 Carcinogenicity

Table 21: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
B6C3F ₁ Mice 50M/50F 2-year study Test concentrations in diet: 0, 833, 2,500, or 7,500 ppm (eq. to average daily doses of app. 90, 265 or 825 mg/kg bw to M and 80, 235 or 745 mg/kg bw to F) GLP conform	AQ caused liver cancer in M/F; and thyroid gland tumours in mice may have been related to AQ. No NOAEL can be proposed.	1 (reliable without restriction) key study Test material (EC name): Anthraquinone purity: 99.8 %	NTP 2005
F344/N Rats 50M/50F 2-year study Test concentrations in diet: 469, 938, 1,875 or 3,750 ppm (eq to average daily doses of approximately 135, 275, 555, 1,130 or 2,350 mg /kg bw) GLP conform	AQ caused cancer of the kidney and urinary bladder in M/F rats and of the liver in F rats. The occurrence of some liver tumours in M rats may have been related to AQ exposure. No NOAEL can be proposed.	1 (reliable without restriction) key study Test material (EC name): Anthraquinone purity: 99.8 %	NTP 2005

4.10.1 Non-human information

Carcinogenicity: oral

Table 22: Summary table of carcinogenicity studies

Method	Results	Remarks	Reference
B6C3F ₁ Mice 50M/50F 2-year study Test concentrations in diet: 0, 833, 2,500, or 7,500 ppm (eq. to average daily doses of app. 90, 265 or 825 mg/kg bw to M and 80, 235 or 745 mg/kg bw to F) GLP conform	AQ caused liver cancer in M/F; and thyroid gland tumours in mice may have been related to AQ. No NOAEL can be proposed.	1 (reliable without restriction) key study Test material (EC name): Anthraquinone purity: 99.8 %	NTP 2005
F344/N Rats 50M/50F 2-year study Test concentrations in diet: 469, 938, 1,875 or 3,750 ppm (eq to average daily doses of approximately 135, 275, 555, 1,130 or 2,350 mg /kg bw) GLP conform	AQ caused cancer of the kidney and urinary bladder in M/F rats and of the liver in F rats. The occurrence of some liver tumours in M rats may have been related to AQ exposure. No NOAEL can be proposed.	1 (reliable without restriction) key study Test material (EC name): Anthraquinone purity: 99.8 %	NTP 2005

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<p>B6C3F1 mice, 18 M/18F</p> <p>B6AKF1 mice, 18 M/18F</p> <p>464 mg/kg bw/d AQ in 0.5% gelatin on days 7-28 followed by 1206 ppm in diet (ad libitum) after day 28</p> <p>Oral (gavage/diet) study, 18 mo, started at the age of 7 days</p> <p>AQ and 119 other test compounds tested</p> <p>Histologic examination of major organs (except cranium)</p> <p>(no data on organs included)</p>	<p>No significant increase in tumour incidence (concluded in the IUCLID dataset and Innes, 1969)</p> <p>Original data from NTP report PB-223 159, 1968: pulmonary adenoma in 2 F B6C3F1 and in 2F B6aKF1 mice, 1 reticulum cell sarcoma in F B6AKF1, 2 hepatoma in M B6C3F1)</p> <p>4 M + 4F B6C3F1 mice and 2 F B6AKF1 mice died during experiment.</p>	<p>4 (not assignable) (available information is not sufficient for a relevant evaluation)</p> <p>Test material (EC name): Anthraquinone</p> <p>purity: not known</p>	<p>Innes et al, 1969</p>
<p>ACI/N Rats, 60M</p> <p>480 d</p> <p>Test concentrations: basal diet as control and basal diet containing 1-Hydroxyanthraquinone at a concentration of 1 %</p> <p>no standardised test protocol</p>	<p>Evaluation of results: positive</p> <p>Test results:</p> <p>Positive: intestinal and liver neoplasms (adenoma, adenocarcinoma, carcinoma) as well as benign stomach neoplasm</p>	<p>4 (not assignable) (no standardised test protocol)</p> <p>Test material (EC name): 1-Hydroxy-anthraquinone</p>	<p>Mori et al. 1990</p>

The paragraphs below are in accordance with the summaries of the two carcinogenicity assays of NTP (2005) and were adapted, where necessary.

F344/N rats (groups of 50 male and 50 female) were fed diets containing 469, 938, 1,875, or 3,750 ppm AQ (equivalent to average daily doses of approximately 135, 275, 555, 1,130, or 2,350 mg/kg bw) for 105 weeks (2 years). Survival of the exposed females was greater than that of the control group, whereas the survival of all males was similar. Mean body weights were lower in exposed females throughout the major part of the study (89%, 84%, 83% and 81% of controls at the end of the study for 469, 938, 1,875 and 3,750 ppm, respectively; NTP 2005, table 6) and were lower in exposed males during the later part of the study (97%, 95%, 96% and 94% of control for 469, 938, 1,875 and 3,750 ppm, respectively; NTP 2005, table 5). The feed consumption of controls and exposed animals was similar in males and females.

Pathological investigations revealed an increased occurrence of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) in all exposed females. Also, the incidences of renal tubule adenoma in all exposed males exceeded the historical control range and the incidence was significantly increased in the male 938 ppm group. AQ exposure was associated with an increased incidence of non-neoplastic lesions of the kidney including hyaline droplet accumulation, pigmentation, and mineralization in the renal medulla and transitional epithelial hyperplasia in males and females, as well as renal tubule hyperplasia in females. Exposed females exhibited an increased incidence of nephropathy and exposed males showed an increased severity of nephropathy. Thereby the concentration of α 2u-globulin of males exposed to 3,750 ppm exceeded the value of the control group at 3 months, but the overall evaluation did not point to male rat-specific α 2u nephropathy (see 4.10.4.).

Compared to the controls, a significantly increased incidence of urinary bladder transitional epithelial papilloma was observed in the 1,875 ppm male group. Furthermore, the incidences in males exposed 938 ppm or greater to exceeded the historical control range. A positive trend was demonstrated in the incidences of transitional epithelial hyperplasia and papilloma or carcinoma (combined) of the urinary bladder in females.

The incidences of hepatocellular adenoma or carcinoma (combined) were slightly increased in exposed males and females and at the upper end of the historical control range. Thereby hepatocellular adenomas occurred in all groups of exposed female rats, and the incidence in the 938 ppm group was significantly greater compared to the control incidence. The incidences of hepatocellular adenoma in 938, 1,875, and 3,750 ppm female rats exceeded the historical control range. Hepatocellular adenomas were also present in all exposed groups of male rats, and hepatocellular carcinoma was present in two males (one in the 1,875 ppm dose group and the other in the 3,750 ppm dose group). However, (as noted in NTP 2005) the occurrence of hepatocellular neoplasms was considered an uncertain finding (Dossier Submitter, DS: and not a negative one due to the low incidences), because of the increased incidences of several non-neoplastic liver lesions, the presence of hepatocellular carcinomas in the two highest dose groups and the increased incidences of hepatocellular adenomas in females.

Congestion, pigmentation, and hematopoietic cell proliferation of the spleen occurred at an elevated incidence in exposed males and females. In most groups of exposed rats the incidences of bone marrow hyperplasia (DS: assumed to be erythropoietic) were increased, and exposed females exhibited also an increased incidence of bone marrow atrophy (DS: assumed to be a lymphopoietic atrophy, which might occur in parallel).

In comparison to the control group the incidences of mononuclear cell leukaemia were significantly reduced in all exposed rats, and the incidences were less than the historical control ranges.

B6C3F₁ mice (groups of 50 males and 50 females) were fed diets containing 0, 833, 2,500, or 7,500 ppm (equivalent to average daily doses of approximately 90, 265, or 825 mg/kg to males and 80, 235, or 745 mg/kg to females) for 105 weeks (2 years). In the 7,500 ppm male group less animals survived than in controls. Mean body weights were lower compared to the control group in 7,500 ppm females at the end of the study (96% of the controls, NTP 2005, table 16) and in 7,500 ppm males during the last 6 study months (95% of the controls, NTP 2005, table 15). The feed consumption of controls and exposed animals was similar in males and females.

Pathological investigations showed an increase with a positive trend in incidences of hepatocellular neoplasms (including multiple neoplasms) in males and females, whereby the incidences were increased in all exposed groups. Significantly increased incidences of hepatoblastoma were observed in males exposed to 2,500 or 7,500 ppm. In exposed mice there were increased incidences of non-neoplastic lesions of the liver. Incidences of neoplasms of thyroid gland follicular cells in males and females were marginally increased. There were higher incidences of intracytoplasmic inclusion bodies of the urinary bladder in nearly all of the exposed males and females in all dose groups, hematopoietic cell proliferation of the spleen in males and females in all groups, as well as higher incidences of thyroid gland follicular cell hyperplasia and kidney pigmentation compared to controls in exposed males.

The following table summarizes the findings of NTP (copied from the report):

Table 23: Summary of the 2-Year Carcinogenesis Studies

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Concentrations in feed	0, 469, 938, 1,875 or 3,750 ppm	0, 469, 938, 1,875 or 3,750 ppm	0, 833, 2,500 or 7,500 ppm	0, 833, 2,500 or 7,500 ppm
Body weights	Exposed groups less than control group	Exposed groups less than control group	7,500 ppm group less than control group	7,500 ppm group slightly less than control group
Survival rates	22/50, 23/50, 22/50, 26/50, 22/50	23/50, 40/50, 35/50, 37/50, 40/50	45/50, 41/50, 43/50, 23/50	35/50, 42/50, 35/50, 42/49
Nonneoplastic effects	<p><u>Kidney</u>: hyaline droplet accumulation (3/50, 14/50, 10/50, 16/50, 16/50); severity of nephropathy (2.2, 3.1, 3.1, 3.0, 3.0); pigmentation (25/50, 31/50, 36/50, 38/50, 33/50); medulla, mineralization (30/50, 42/50, 46/50, 47/50, 49/50); transitional epithelium, hyperplasia (28/50, 45/50, 44/50, 48/50, 48/50)</p> <p><u>Liver</u>: centrilobular hypertrophy (0/50, 4/50, 21/50, 13/50, 29/50); cystic degeneration (9/50, 31/50, 36/50, 28/50, 29/50); inflammation (13/50, 30/50, 28/50, 30/50, 27/50); eosinophilic focus (9/50, 22/50, 30/50, 29/50, 20/50); mixed cell focus (4/50, 12/50, 15/50, 13/50, 10/50); cytoplasmic vacuolization (5/50, 18/50, 23/50, 17/50, 23/50)</p> <p><u>Spleen</u>: congestion (6/50, 35/50, 37/50, 30/50, 31/50); pigmentation (12/50, 36/50, 38/50, 33/50, 28/50); hematopoietic cell proliferation (37/50, 45/50, 44/50, 43/50, 39/50)</p>	<p><u>Kidney</u>: hyaline droplet accumulation (33/50, 48/50, 45/50, 44/50, 44/49); nephropathy (39/50, 49/50, 47/50, 49/50, 49/49); pigmentation (27/50, 50/50, 48/50, 50/50, 47/49); medulla, mineralization (17/50, 25/50, 27/50, 28/50, 20/49); renal tubule, hyperplasia (0/50, 12/50, 13/50, 15/50, 11/49); transitional epithelium, hyperplasia (0/50, 5/50, 12/50, 3/50, 10/49)</p> <p><u>Liver</u>: centrilobular hypertrophy (0/50, 18/50, 23/50, 19/50, 26/49); cystic degeneration (0/50, 5/50, 10/50, 10/50, 6/49); inflammation (25/50, 46/50, 44/50, 38/50, 46/49); eosinophilic focus (8/50, 32/50, 34/50, 39/50, 34/49); mixed cell focus (3/50, 30/50, 20/50, 23/50, 13/49); angiectasis (3/50, 15/50, 18/50, 15/50, 21/49)</p> <p><u>Spleen</u>: congestion (1/50, 46/50, 42/50, 44/50, 45/49); pigmentation (33/50, 45/50, 48/50, 48/50, 47/49); hematopoietic cell proliferation (39/50, 50/50, 47/50, 47/50, 46/49)</p>	<p><u>Liver</u>: centrilobular hypertrophy (24/50, 34/50, 41/50, 33/49); degeneration, fatty, focal (0/50, 7/50, 6/50, 0/49); hepatocyte, erythrophagocytosis (1/50, 9/50, 13/50, 6/49); eosinophilic focus (14/50, 17/50, 24/50, 20/49); focal necrosis (2/50, 3/50, 3/50, 8/49)</p> <p><u>Urinary Bladder</u>: intracytoplasmic inclusion body (0/50, 46/49, 46/49, 42/45)</p> <p><u>Thyroid Gland</u>: follicular cell hyperplasia (7/50, 10/50, 15/49, 21/46)</p> <p><u>Spleen</u>: hematopoietic cell proliferation (12/50, 14/50, 12/49, 30/42)</p> <p><u>Kidney</u>: pigmentation (0/50, 2/50, 2/50, 18/47)</p>	<p><u>Liver</u>: centrilobular hypertrophy (1/49, 27/50, 22/50, 39/49); degeneration, fatty, focal (2/49, 3/50, 1/50, 9/49); eosinophilic focus (6/49, 15/50, 11/50, 22/49)</p> <p><u>Urinary Bladder</u>: intracytoplasmic inclusion body (0/44, 40/48, 43/46, 46/48)</p> <p><u>Spleen</u>: hematopoietic cell proliferation (9/45, 17/49, 17/48, 26/48)</p>

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Nonneoplastic effects (continued)	<u>Bone Marrow:</u> hyperplasia (25/50, 28/50, 37/50, 36/50, 33/50)	<u>Bone Marrow:</u> hyperplasia (19/50, 31/50, 28/50, 19/50, 23/50); atrophy (4/50, 13/50, 13/50, 11/50, 13/50)		
Neoplastic effects	<u>Kidney:</u> renal tubule adenoma (1/50, 3/50, 9/50, 5/50, 3/50); transitional epithelial papilloma (0/50, 0/50, 2/50, 0/50, 1/50) <u>Urinary Bladder:</u> transitional epithelial papilloma (0/50, 1/50, 3/50, 7/50, 3/49)	<u>Kidney:</u> renal tubule adenoma (0/50, 4/50, 9/50, 7/50, 12/49); renal tubule adenoma or carcinoma (0/50, 6/50, 9/50, 8/50, 14/49) <u>Urinary Bladder:</u> transitional epithelial papilloma or carcinoma (0/49, 0/49, 0/49, 1/50, 2/49) <u>Liver:</u> hepatocellular adenoma (0/50, 2/50, 6/50, 4/50, 3/50)	<u>Liver:</u> hepatocellular adenoma (21/50, 32/50, 38/50, 41/49); hepatocellular carcinoma (8/50, 13/50, 17/50, 21/49); hepatoblastoma (1/50, 6/50, 11/50, 37/49); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (26/50, 35/50, 43/50, 48/49)	<u>Liver:</u> hepatocellular adenoma (6/49, 28/50, 27/50, 40/49); hepatocellular carcinoma (2/49, 3/50, 8/50, 8/49); hepatocellular adenoma or carcinoma (6/49, 30/50, 30/50, 41/49)
Equivocal findings	<u>Liver:</u> hepatocellular adenoma or carcinoma (1/50, 3/50, 4/50, 5/50, 3/50) <u>Liver:</u> hepatocellular carcinoma (0/50, 0/50, 0/50, 1/50, 1/50)	None	<u>Thyroid Gland:</u> follicular cell adenoma (0/50, 0/50, 2/49, 2/46)	<u>Thyroid Gland:</u> follicular cell adenoma (1/45, 1/48, 2/48, 2/48); follicular cell carcinoma (0/45, 0/48, 0/48, 2/48); follicular cell adenoma or carcinoma (1/45, 1/48, 2/48, 4/48)
Decreased incidences	<u>Mononuclear Cell Leukemia:</u> (25/50, 2/50, 1/50, 5/50, 7/50)	<u>Mononuclear Cell Leukemia:</u> (18/50, 1/50, 1/50, 2/50, 0/50)	None	None
Level of evidence of carcinogenic activity	Some evidence	Clear evidence	Clear evidence	Clear evidence

Carcinogenicity: inhalation

No data available.

Carcinogenicity: dermal

No data from guideline compliant studies available. No robust information can be taken from other non-guideline studies using non-standard routes and test design:

In a skin painting study 15 male and 15 female mice with 0.1% or 0.25% solutions of AQ (ICI 1991). No information was given on test substance purity, vehicle, strain, duration/frequency of treatment, ('every day or every other day' reported), parameters examined at study end or numbers of survivors/sex/group at day 200. The results of 38 mice surviving over 200 d were reported as 1/38 papilloma, 2/38 lung cancer without any test group in which the findings were observed and 1/46 papilloma in the control group (benzene). The information is only given in the IUCLID dataset

(IUCLID 2000) and is too limited to identify the study type (the benzene only control group could indicate that this was a initiation-promotion study).

A single subcutaneous injection of 1000 mg/kg bw AQ was administered to 18 male and female mice. After 18 months observation period no significant increase in tumours were reported (Bionetics Research Laboratories 1968; cited from IUCLID 2000). A study on subcutaneous implantation of discs of 13 mm diameter with pore size of 0.22 µm containing AQ and assessment of the implantation site after 3 months did not reveal tumours at the implantation site in 10 male and 10 female Swiss mice (Longstaff 1978; cited from IUCLID 2000).

4.10.2 Human information

There is no sound information available on the exposure of humans with AQ and its effects on cancer *per se*. But, IARC (2012) summarised several studies from the dye and resin industry in the USA, where workers can potentially be exposed to AQ during the production process. The results are often equivocal, because in these industrial branches a 5 year period of co-exposure with epichlorohydrin, a chemical which is associated with an increased risk of lung cancer could confound the findings and no data on exposure levels were available.

An initial retrospective cohort study conducted by Delzell et al. (1989) found excess of lung cancer and central nervous system tumours in certain subgroups of dye and resin workers (all 2642 male employees were included in the study) employed at the investigated plant. In the two follow-up nested case-control studies (Barbone et al. 1992 and 1994) a more detailed evaluation of both cancer types was performed (IARC 2012). The lung cancer investigation (Barbone et al. 1992) showed significantly elevated risks for lung cancer among workers in the AQ and epichlorohydrin production area (Odds Ratio (OR): 2.4; 95% confidence interval (CI) 1.1-5.2; 21 exposed cases, 24 exposed controls) and, within this area, for AQ production (OR: 12; 95% CI: 1.4-99; six exposed cases, one exposed controls) and AQ dye standardisation (OR: 3.3; 95% CI: 1.0-11; eight exposed cases, six exposed controls). In the AQ intermediate dye production the odds ratio among workers was 1.8 (95% CI: 0.6-5.1; eight exposed cases, 10 exposed controls). The second study (Barbone et al. 1994) further investigated associative of tumours of the central nervous system (11 cases, eight decedents, three living) and epichlorohydrin exposure. Here the authors found statistically significantly increased risks for central nervous system tumours among workers in the AQ dye production, but there were no controls (OR: ∞, 95% CI: 1.7-∞; three exposed cases). Also a statistically significantly increased odds ratio was reported for acute and routine epichlorohydrin exposure (OR: ∞, 95% CI: 1.5-∞; three exposed cases; and OR: 4.2; 95% CI: 0.7-26; four exposed cases). Therein rested a positive, statistically significant association with 'cumulative potential exposure' ($P_{\text{trend}} = 0.11$) and 'duration of routine potential exposure' ($P_{\text{trend}} = 0.11$). IARC (2012) stated that three of the four epichlorohydrin-exposed cases also worked in the AQ intermediated dye or azo dye areas. In later years a follow up from the original cohort study (Delzell et al. 1989) conducted by Sathiakumar and Delzell (2000) included an expanded cohort and all workers of the evaluated plant (3266, men and women; average follow up 27 years). An increased but not statistically significant mortality for several cancers (including lymphosarcoma, cancer of the colon, lung, liver, genital tissue, bladder and central nervous system) was reported. A statistically significant elevated risk for mortality of lung cancer was in association with working in the AQ production area (standardised mortality ratio (SMR): 1.68; 95% CI: 1.15-2.37; relative risk for ever versus never exposure, 1.7; 95% CI: 1.1-2.6; 32 exposed cases for both analyses). The risk was higher in long term than in short term workers (up to 20 years, thereafter the risk did no rise further).

The studies above summarised showed an excess risk of mortality from lung cancer in workers in the dye and resin processing in a single facility in the USA who were potentially exposed to AQ, AQ dye intermediates, anthracene, vanadium pentoxide, and epichlorohydrin. There was a 12-fold increased risk for lung cancer for workers involved in the production of AQ, but this was based on only few exposed cases. Even fewer cases were the basis for an excess incidence of central nervous system tumours. There are three major limitations of these studies: 1. risks were estimated with the employment in AQ and AQ dye production, but no exposure to AQ *per se* was analysed; 2. due to the low number of cases only a limited statistical power (to detect effects for specific cancers) emerges; 3. there is a limited ability to evaluate the potential confounding factors from other occupational exposures. Therefore IARC concluded there is inadequate evidence in humans for the carcinogenicity of AQ.

4.10.3 Other relevant information

Data from toxicokinetic as well as repeated dose studies and genotoxicity experiments should be taken into account. In brief, AQ was found with the highest concentrations in toxicokinetic studies in the adipositas (fat) tissue (NTP 2005) and the liver and the kidney (Bayer AG 1983). Main metabolites were 1-OH-AQ and 2-OH-AQ. In repeated dose experiments hypertrophic effects in the liver were seen in rats and mice in the 14 day studies of the NTP (2005) as well as in the 28 and 90 day studies conducted by Bayer AG (1976 and 1979). Additionally, the subchronic NTP studies showed histological lesions in kidney, liver, spleen, bone marrow and thyroid glands of male and female rats and the urinary bladder of female rats. AQ showed some positive evidence for genotoxicity, but there is no sufficient evidence to classify AQ as genotoxic taken into account all information available. More information on these issues is described in detail in the sections above.

A mammalian cell transformation test in Chang (human liver) cells and BHK-21 C13 (baby Syrian hamster kidney) cells were incubated separately with solutions of 120 chemicals including AQ in DMSO or water (no detailed data for AQ), with and without metabolic activation by rat liver postmitochondrial supernatant (concentrations of AQ (0.08, 0.4, 2, 10, 50, 250 µg/ml of the cell suspension) was negative (Styles 1978).

4.10.4 Summary and discussion of carcinogenicity

In the two 2-year carcinogenicity studies conducted by NTP (2005), AQ was carcinogenic in rats and mice. Increased tumour rates were seen in the kidney, urinary bladder, thyroid gland and liver. The only organ with consistent increased tumour rates in both species was the liver; however, the increase in liver tumours was rather weak in rats.

In male F344/N rats there was some evidence of carcinogenic activity of AQ based on elevated incidences of renal tubule adenoma and of transitional epithelial papillomas of the kidney and urinary bladder. In addition, hepatocellular neoplasms were observed, which may have been associated with AQ exposure. In female F344/N rats there was clear evidence of carcinogenic activity of AQ based on increased incidences of renal tubule neoplasms. Also, elevated incidences of urinary bladder transitional epithelial papilloma or carcinoma (combined) and of hepatocellular adenoma in female rats were related to exposure to AQ. In male and female B6C3F₁ mice there was clear evidence of carcinogenic activity based on elevated incidences of liver neoplasms. Weaker, but some evidence was seen for an association between thyroid gland follicular cell neoplasms in male and female mice and AQ exposure.

Renal tubule adenomas are rare in male rats and even more uncommon in female rats. In feeding studies by NTP they occurred with a historical control incidence of 7/902 in males and had not been

observed in any control female rats before (NTP 2005). Papillomas of the transitional epithelium of the kidney in male rats and the transitional epithelium of the urinary bladder in male and female rats are also uncommon neoplasms (NTP 2005). The increased incidences of renal tubule hyperplasia and hyperplasia of the transitional epithelium of the kidney and urinary bladder, as well as the increased incidences of rare neoplasms in these tissues, are clearly associated with chemical exposure. Concerning the presence of protein droplets in the kidney of male and female rats it is not sure, if they were involved in the induction of renal neoplasms. Despite the increased α 2u-globulin concentration in male rat kidneys, no renal tubule cell hyperplasia has been observed at the 3- or 12-month interim evaluations of the 2 years study. Also, no elevated (proliferation) labelling indices were found in the kidneys of males or females after BrdU incorporation during a 32-day study (NTP 2005). Hence, the expanded evaluation did not point to male rat-specific α 2u nephropathy. These findings and the fact, that kidney tumours and tubular lesions were also observed in female rats, are in agreement with the NTP conclusion that α 2u nephropathy cannot be considered as the mode of carcinogenic action for the kidney tumours. Overall, the dossier submitter agrees on the interpretation of the NTP authors.

The NTP report further stated that the incidences of renal tubule hyperplasia in males and females, renal tubule adenomas in males, most non-neoplastic lesions in the liver of males and females and hepatocellular adenomas in females were all nonuniform dose response relationships (DS: in the meaning that incidences were elevated in all dose groups compared to controls, but no clear monotonic dose-related increase in incidences was seen). Overall, female rats were more responsive to AQ exposure than male rats. This can be explained by the different internal doses of AQ measured in the NTP toxicokinetic experiments, which was in females approximately twice the concentration as in male rats (at 3, 6, 12, and 18 months and at each exposure concentration). In both species, rats and mice, exposure to AQ produced similar responses in the liver, which included significant increases in liver weights and incidences of centrilobular hypertrophy characterized by increased amounts of eosinophilic cytoplasm.

The reported target organs of the NTP carcinogenicity study are in accordance with the results from repeated dose studies, where (minimal to moderate centrilobular/midzonal/periportal) hypertrophy of liver cells was observed in a 28-day oral rat study (Bayer AG 1976). In a 14 week study in mice histological investigations of NTP found minimal to moderate centrilobular hypertrophy of liver cells with enlarged nuclei. Nevertheless, information on subcellular structural changes with regard to the pronounced centrilobular enlargement of hepatocytes (hypertrophy) was lacking in the 2 year study. The liver weight of all treated male and female mice was increased in the 14 week NTP study as well as the liver weight of male and female rats in the 28 day and 90 day study performed by the Bayer AG (1976 and 1979). The subchronic NTP studies showed histological lesions in kidney, liver, spleen, bone marrow and thyroid glands of male and female rats and the urinary bladder of female rats. Thereby, eosinophilic hyaline droplets of variable size occurred within the renal tubules in exposed males and females, with α 2u-globulin increases of the same degree in all treated male rats. In female rats a similar phenotype is reported, albeit weaker in severity. Furthermore, toxicokinetic studies demonstrated that the liver and the kidneys were the organs with the highest relative concentrations (Bayer AG 1983). Last, in a 32-day study (NTP 2005) cell proliferation was increased in the urinary bladder but not in the kidney of male and female rats.

According to the Draft Assessment Report - Anthraquinone (2006) Doi et al. (2005) stated that despite the mechanism for AQ carcinogenicity in the liver, kidney and urinary bladder remains unclear, different modes of action can be proposed, which do not include mutagenicity of AQ itself. These comprise i. a. the intercalative binding to DNA or the electron reduction to semiquinone radicals. The first involves sliding of the planar AQ ring between adjacent stacked base pairs (Neidle and Abraham 1984). The latter functions via catalysation processes (many enzymes are able

to do this) and ultimately results in peroxidative DNA damage due to reoxidation to quinones in the presence of oxygen species, while these transform to superoxides and other reactive oxygen species (Hartman and Goldstein 1989; Fisher et al. 1992; Barasch et al. 1999). Another alternative is that AQ undergoes ring hydroxylation or is reduced to hydroquinones, and is subsequently conjugated. Thereby, DNA affinity and redox activity of AQ derivatives depend on the pattern and type of substitution. AQ induces CYP2B1 as well as CYP1A2 activity (in the liver, but not in intestine mucosa (Longo et al. 2000)) in Fisher rats. The NTP (2005) conducted a 32 day study in F344/N rats, where treatment- and dose-related elevations in the liver levels of CYP1A1 (two to threefold increase above control) and CYP2B1 (80-fold above controls in males, 40-fold above control in females) were observed. Hence, cytochrom P-450 enzymes possibly play a role in formation of active metabolites. In the NTP 32-day study, 8-hydroxy-2'-deoxyguanosine and 2'-deoxyguanosine concentrations in the kidney were decreased (at the same time point as the increased cytochrome levels), whereas in the liver variable results were seen.

The cancer results of NTP are controversially discussed in literature, especially due to contamination of the NTP samples with 0.1% 9-NA, which is a mutagen, while AQ showed only equivocal mutagenic activity. Butterworth, 2004, claimed that 9-NA was solely responsible for the positive carcinogenicity findings, based on the mutagenicity of 9-NA and non-mutagenicity of AQ, and it was stated that neither AQ nor its metabolites contribute to the positive cancer results. The concentration of 9-NA in the test substance administered to rats and mice in the NTP studies was below 0.1%. If a concentration of 0.1% 9-NA was solely responsible for the positive mutagenic findings in the NTP sample tested by Butterworth et al 2001 (for which the NTP was unable to confirm the results), it would be more potent than benzo[a]pyrene (B[a]P) and about equally as potent as the positive control 2-nitrofluorene (based on the revertants per microgram) (Butterworth et al. 2001). This is not reflected by the following experimental data. Butterworth et al. 2004 tested the mutagenicity of 9-NA and found that it was mutagenic in TA98 and TA100 (in TA100 only at the highest tested dose) in the absence of S9 and not mutagenic in the presence of S9. In the experiment 9-NA induced 53 revertants/ μg in TA98 without S9 while the positive control, 2-nitrofluorene, which was stated to have an equally potency, induced 370 revertants/ μg . Pitts et al. (1982) demonstrated with purified compounds that 9-NA is a weak mutagen in TA98 (0.3 revertants/ μg , without S9) in comparison to 2-nitrofluorene (417 revertants/ μg , without S9) and significantly weaker than benzo[a]pyrene (700 revertants/ μg , with S9). These findings are in concordance with Fu et al. (1985), who found 9-NA to be a weak mutagen in TA98 and TA100. NTP (2005) reported results with a potency of 0.315 revertants/ μg for 9-NA in TA98 without S9 - similar to that found by Pitts et al. (1982). Furthermore, the potency of 9-NA in an in vitro forward mutation assay in h1A1v2 human lymphoblastoid cells (these cells constitutively express CYP1A1) was only 0.0032 times that of the positive control, B[a]P (Durant et al. 1996). In conclusion the experimental mutagenicity data suggest that 9-NA is a substantially weaker mutagen than it is proposed by Butterworth et al. (2001).

In addition, the lack of activity in mutagenicity assays does not give proof of non-carcinogenicity of AQ, because carcinogenicity of AQ could also be mediated by other mechanisms. Also metabolites appear in situ (regardless of the method of manufacture) can evoke carcinogenicity. At least one of those, namely 2-OH-AQ, is a bacterial mutagen. Experimental data indicated that 2-OH-AQ exhibits a clearly greater mutagenic potency than 9-NA: Butterworth et al. (2004) estimated that 2-OH-AQ is a bacterial mutagen twice as potent as the impurity 9-NA. The data of NTP (2005) provide further confirmatory evidence that the mutagenic potency of 2-OH-AQ in TA98 is higher than that of 9-NA. Moreover, measurements in the male rat urine showed, that 2-OH-AQ is systemically present at several-fold higher amounts than it is theoretically possible for the 0.1% 9-NA contamination, even if the latter was 100% bioavailable (NTP 2005, p. 92 table 23). The NTP concluded that 2-OH-AQ is an in situ metabolite of AQ that, based on experimental mutagenicity

data, is as likely to be of comparable carcinogenicity or (with higher probability) more carcinogenic than 9-NA. Hence, AQ has the potential to act through a mechanism involving mutagenicity, and 9-NA is not a necessary component of this action. Last, it is neither demonstrated that 9-NA is an *in vivo* carcinogen nor that it has a potential to be carcinogenic at the proposed contamination concentration. In fact, it should be taken into account further that carcinogenicity has not been examined for 100% pure AQ or AQ-DA or AQ-FC. Thus, it is to conclude that AQ as tested in the NTP studies (containing 0.1% 9-NA) was carcinogenic. A possible contribution of the impurity cannot be assessed.

Addressing structure-activity relationships, as stated in the NTP report, both AQ and substituted AQs are able to interact directly with DNA via intercalation, because of the size and planarity of their ring system (Islam et al. 1985; Tanious et al. 1992). Looking at NTP data for similar structures it becomes apparent that several substituted AQs are carcinogenic in long-term animal studies (see NTP 2005, table 21). While the planar ring system endows AQs with the capability of intercalating with the DNA, its substituents determined the metabolism, the ultimate mutagenicity, the carcinogenic response and the target organs involved. Most of the AQ compounds of the NTP data base carried halogen, amino, or nitro substitutions. The bladder, kidney, and liver of rats and liver of mice were the major sites of tumourigenesis (similar to the pattern observed for AQ) but also tumours in the gastrointestinal tract in rats and mice and the kidney and urinary bladder in rats were seen. 1-amino-2,4-dibromoanthraquinone was the only compound administered in feed other than AQ that produced renal neoplasms in both male and female rats. Looking at hydroxylated compounds 1,8-dihydroxyanthraquinone and 1-OH-AQ administered in feed targeted clearly the large intestine, while investigations with emodin (1,3,8-trihydroxy-6-methylantraquinone) in female rats and male mice only exhibited equivocal results (NTP 2005). Other literature (Mori et al. 1990) reported induction of tumours of the liver, stomach and large intestine in male ACI/N rats for 1-OH-AQ after dietary administration.

In conclusion, the available carcinogenicity studies on AQ as tested by the NTP (2005) showed that there was clear evidence of carcinogenic activity of AQ in female F344/N rats as well as clear evidence of carcinogenic activity in male and female B6C3F₁ mice. The tumour findings are consistently observed in target organs showing toxic effects and precursor lesions in repeated dose studies and in organs with highest tissue concentrations (see toxicokinetic data). Additionally, the target organs of carcinogenic responses are in accordance with those of other AQs. It is not possible to determine to what extent, if any, 9-NA has influenced the outcomes of the NTP carcinogenicity study on AQ. The low exposure level, the bioavailability, and the weak mutagenicity make it unlikely and implausible that 9-NA was solely and totally responsible for the carcinogenic response. Due to biotransformation processes mutagenic metabolites of AQ appear, which are at least five times more potent and present at systemically higher concentration than 9-NA. Therefore, the results are attributable to AQ (and its active metabolites) and AQ is considered to be carcinogenic. AQ was not clearly demonstrated as mutagenic in the available tests, however, a contribution of mutagenicity (by mutagenic metabolites) cannot be excluded.

4.10.5 Comparison with criteria

According to the CLP directive a substance shall be classified as carcinogenic if:

Category 1A

‘It is known to have carcinogenic potential for humans; classification is largely based on human evidence.’

Based on the results given above, classification as Category 1A is not appropriate, because there is insufficient evidence from the few indirect epidemiological studies with confounding effects from co-exposure to other (carcinogenic) substances.

Category 1B

‘It is presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.’

This category depends on the strength of evidence, which consists of animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity. This means a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in

- (a) two or more species of animals or in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols;
- (b) in both sexes of a single species;
- (c) occurrence of malignant neoplasm to an unusual degree with regard to the incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

Concerning animal evidence the NTP studies showed some evidence for carcinogenicity of AQ in male F344/N rats, clear evidence for carcinogenicity of AQ in female F344/N rats as well as clear evidence of carcinogenic activity in male and female B6C3F₁ mice. In comparison to the given criteria for the CLP regulation AQ fulfils the criteria for category 1B with regard to

- (a) two positive species,
- (b) both sexes, and
- (c) (rare) tumours types above the background incidence as well as several target organs of tumour response. In additional considerations there are structurally related substances (from the NTP data base) with shown carcinogenic potential as well as a possible contribution of mutagenicity (in particular of metabolites) as a mode of action. Furthermore, other conclusive modes of tumourigenic action like DNA intercalation, occurrence of semiquinone radicals and reactive oxygen species cannot be excluded.

Category 2

‘It is a suspected human carcinogen, but the evidence is not sufficient for Category 1A or 1B.’

Following consideration would lead to classification as category 2:

- (a) the evidence is limited to a single experiment;
- (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies;
- (c) the agent increases the incidence only of benign neoplasm or lesions of uncertain neoplastic potential; or
- (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

Category 2 is not appropriate, because the criteria are not fulfilled. The evidence is

- (a) neither limited to a single experiment (two species were tested in the NTP study)
- (b) nor limited with regard to benign neoplasms (there was clear evidence for renal tubule carcinoma as well as transitional epithelial urinary bladder carcinoma in female rats, hepatocellular carcinoma in male and female mice, and, in addition, there was some evidence for hepatocellular carcinoma in male rats and follicular cell carcinoma in female mice)
- (c) nor limited to only promoting activity.

With regard to (b) the impurity 9-NA is discussed extensively in the literature. Concerns about misleading results due to contamination issues with low levels of 0.1% 9-NA measured in the NTP material are rebutted by considerations regarding the mutagenic potential of the impurity as well as the potency of the metabolites of AQ and calculations concerning the systemically present in vivo concentrations of the compounds. In addition, it is not conclusively demonstrated that 9-NA is a carcinogen or that the occurring concentrations are sufficiently high to cause the noted tumourigenic effects. 9-NA is judged to be neither strong enough mutagenic nor abundant enough to solely and totally cause the observed carcinogenicity.

Therefore, the NTP results are valid; there is sufficient evidence for carcinogenicity and classification of AQ as carcinogenic Category 1B with the hazard phrase H350: *May cause cancer* is proposed.

This classification is in accordance with the Draft Assessment Report - Anthraquinone (2006), which classified AQ according to the DSD as carcinogenic category 2 with the assigned symbol T and the risk phrase R45. It is also in accordance with the classification of IARC, who ranked AQ in group 2B (possibly carcinogenic to humans).

4.10.6 Conclusions on classification and labelling

Due to clear evidence of carcinogenic activity in animals the following classification and labelling is proposed according to the Classification, Labelling and Packaging Regulation (CLP):

Category 1B carcinogen, H 350 (May cause cancer).

4.11 Toxicity for reproduction

This toxicological endpoint was not evaluated for this dossier.

4.12 Other effects

Not evaluated for this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated for this report.

6 REFERENCES

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