12-20-04

Provisional Peer Reviewed Toxicity Values for

Alizarin Red Compounds
(Various CASRNs)

Derivation of Subchronic and Chronic Oral RfDs

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level

MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	**provisional inhalation unit risk**
p-OSF	**provisional oral slope factor**
p-RfC	**provisional inhalation reference concentration**
p-RfD	**provisional oral reference dose**
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	**Provisional Peer Reviewed Toxicity Value**
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

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PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR ALIZARIN RED COMPOUNDS (VARIOUS CASRNS)
Derivation of Subchronic and Chronic Oral RfDs

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

In the chemical literature, several compounds share the common chemical names "alizarin," "alizarin red" or "alizarine red." Seven distinct chemicals were identified in searches of the CAS Registry. Four of the alizarin chemicals are anthraquinone derivatives, two are azo dyes, and one is of an unknown molecular and structural formula. The alizarin red compounds identified include:

Anthraquinone derivatives:

1. alizarin, alizarin red, 1,2-dihydroxy-9,10-anthracenedione, or 1,2-dihydroxy-9,10-anthraquinone (CASRN 72-48-0);

2. alizarin red S, acid red alizarin, [9,10-dihydro-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid, monosodium salt], or sodium 3,4-dihydroxyanthraquinone-2-sulfonate (CASRN 130-22-3);

3. alizarine red 3WS or 9,10-dihydro-3,4,7-trihydro-9,10-dioxo-2-anthracenesulfonic acid, monosodium salt (CASRN 6486-94-8);

4. alizarine red YCAP, 1,2,6-trihydroxy-9,10-anthracenedione, or 1,2,6-trihydroxyanthraquinone (CASRN 82-29-1).

Azo dyes:

5. acid alizarin red B or 2-[(2-hydroxy-3,6-disulfo-1-naphthalenyl)azo]-benzoic acid, trisodium salt (CASRN 1836-22-2);

6. alizarine chrome red G, acid red 97, or 4,4'-bis[(2-hydroxy-1-naphthalenyl)azo]-[1,1'-biphenyl]-2,2'-disulfonic acid, disodium salt (CASRN 10169-02-5).

Unknown structural and molecular formula:

7. alizarine red V2A or V2A alizarine red (molecular and structural formula unspecified) (CASRN 12794-90-0).

Subchronic or chronic RfDs for the alizarin red compounds identified above are not available on IRIS (U.S. EPA, 2002a), the HEAST (U.S. EPA, 1997) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2002b). The CARA list (U.S. EPA, 1991, 1994) includes no documents for these chemicals, and these chemicals have not been reviewed by ATSDR (2002), IARC (2002) or WHO (2002). The NTP (2002) Management Status Report provided no relevant information. In June 1996, literature searches were conducted from 1965 to May 1996 in TOXLINE, CANCERLIT, MEDLINE, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS and TSCATS for relevant studies. In September 2002, update literature searches were conducted in these databases going back to the previous search in 1996. Additional literature search was conducted by NCEA-Cin from October 2003 through May 2004 using TOXLINE, MEDLINE, Chemical and Biological Abstract databases.

REVIEW OF PERTINENT LITERATURE

Human Studies

No reports were located regarding the subchronic or chronic toxicity of any of the alizarin red compounds in humans by oral exposure.

Animal Studies

No reports were located regarding the subchronic or chronic toxicity of any of the alizarin red compounds in animals by oral exposure.

Other Studies

The only toxicity data available for the alizarin red compounds are a few acute studies and case reports. For example, data from acute rabbit studies suggest that alizarin red (CASRN 72-48-0) is a skin sensitizer (Greenburg and Lester, 1954) and a mild eye irritant (500 mg/24 hours) (NIOSH, 2002). In humans, two case reports of contact dermatitis from the perennial herbs *Rubia tinctorum* and *R. peregrina*, both of which contain alizarin red (CASRN 72-48-0) naturally, were published by Castelain and Ducombs (1988). An acute toxicity study in mice is available for alizarin red S (CASRN 130-22-3) wherein an intravenous LD_{50} of 70 mg/kg was reported (NIOSH, 2002). No reports regarding acute, subchronic or chronic toxicity in humans or animals by any route were located for any of the other alizarin red compounds.

FEASIBILITY OF DERIVING A PROVISIONAL SUBCHRONIC OR CHRONIC RfD FOR ALIZARIN RED COMPOUNDS

No pertinent data regarding the oral toxicity of alizarin red compounds in humans or experimental animals were located, precluding derivation of subchronic or chronic p-RfDs for alizarin red compounds.

REFERENCES

ATSDR (Agency for Toxic Substances and Disease Registry). 2002. Toxicological Profile Information Sheet. Online. http://www.atsdr.cdc.gov/toxpro2.html

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12-20-04

Provisional Peer Reviewed Toxicity Values for

Alizarin Red Compounds
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Derivation of Subchronic and Chronic Inhalation RfCs

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
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Acronyms and Abbreviations

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3. alizarine red 3WS or 9,10-dihydro-3,4,7-trihydro-9,10-dioxo-2-anthracenesulfonic acid, monosodium salt (CASRN 6486-94-8);

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REVIEW OF PERTINENT LITERATURE

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No reports were located regarding the subchronic or chronic toxicity of any of the alizarin red compounds in humans by inhalation exposure.

Animal Studies

No reports were located regarding the subchronic or chronic toxicity of any of the alizarin red compounds in animals by inhalation exposure.

Other Studies

The only toxicity data available for the alizarin red compounds are a few acute studies and case reports. For example, data from acute rabbit studies suggest that alizarin red (CASRN 72-48-0) is a skin sensitizer (Greenburg and Lester, 1954) and a mild eye irritant (500 mg/24 hours) (NIOSH, 2002b). In humans, two case reports of contact dermatitis from the perennial herbs *Rubia tinctorum* and *R. peregrina*, both of which contain alizarin red (CASRN 72-48-0) naturally, were published by Castelain and Ducombs (1988). An acute toxicity study in mice is available for alizarin red S (CASRN 130-22-3) wherein an intravenous LD_{50} of 70 mg/kg was reported (NIOSH, 2002b). No reports regarding acute, subchronic or chronic toxicity in humans or animals by any route were located for any of the other alizarin red compounds.

FEASIBILITY OF DERIVING A PROVISIONAL SUBCHRONIC OR CHRONIC RfC FOR ALIZARIN RED COMPOUNDS

No pertinent data regarding the inhalation toxicity of alizarin red compounds in humans or experimental animals were located, precluding derivation of subchronic or chronic p-RfCs for alizarin red compounds.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 2001. 2001 Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinnati, OH.

ATSDR (Agency for Toxic Substances and Disease Registry). 2002. Toxicological Profile Information Sheet. Online. http://www.atsdr.cdc.gov/toxpro2.html

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12-20-04

Provisional Peer Reviewed Toxicity Values for

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Derivation of a Carcinogenicity Assessment

Superfund Health Risk Technical Support Center
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VOC	volatile organic compound

12-20-04

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Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

In the chemical literature, several compounds share the common chemical names "alizarin," "alizarin red" or "alizarine red." Seven distinct chemicals were identified in searches of the CAS Registry. Four of the alizarin chemicals are anthraquinone derivatives, two are azo dyes, and one is of an unknown molecular and structural formula. The alizarin red compounds identified include:

Anthraquinone derivatives:

1. alizarin, alizarin red, 1,2-dihydroxy-9,10-anthracenedione, or 1,2-dihydroxy-9,10-anthraquinone (CASRN 72-48-0);

2. alizarin red S, acid red alizarin, [9,10-dihydro-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid, monosodium salt], or sodium 3,4-dihydroxyanthraquinone-2-sulfonate (CASRN 130-22-3);

3. alizarine red 3WS or 9,10-dihydro-3,4,7-trihydro-9,10-dioxo-2-anthracenesulfonic acid, monosodium salt (CASRN 6486-94-8);

4. alizarine red YCAP, 1,2,6-trihydroxy-9,10-anthracenedione, or 1,2,6-trihydroxyanthraquinone (CASRN 82-29-1);

Azo dyes:

5. acid alizarin red B or 2-[(2-hydroxy-3,6-disulfo-1-naphthalenyl)azo]-benzoic acid, trisodium salt (CASRN 1836-22-2);

6. alizarine chrome red G, acid red 97, or 4,4'-bis[(2-hydroxy-1-naphthalenyl)azo]-[1,1'-biphenyl]-2,2'-disulfonic acid, disodium salt (CASRN 10169-02-5).

Unknown structural and molecular formula:

7. alizarine red V2A or V2A alizarine red (molecular and structural formula unspecified) (CASRN 12794-90-0).

Cancer assessments for the alizarin red compounds identified above are not available on IRIS (U.S. EPA, 2002a), the HEAST (U.S. EPA, 1997) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2002b). The CARA list (U.S. EPA, 1991, 1994) includes no documents for these chemicals. The carcinogenicity of the alizarin red compounds has not been reviewed by IARC (2002), WHO (2002), or ATSDR (2002). The NTP (2002) Management Status Report provided no relevant information. In June 1996, literature searches were conducted from 1965 to May 1996 in TOXLINE, CANCERLIT, MEDLINE, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS and TSCATS for relevant studies. In September 2002, update literature searches were conducted in these databases going back to the previous search in 1996. Additional literature search was conducted by NCEA-Cin from October 2003 through May 2004 using TOXLINE, MEDLINE, Chemical and Biological Abstract databases.

REVIEW OF PERTINENT LITERATURE

Human Studies

No data regarding the possible carcinogenicity of alizarin red compounds in humans by any route of exposure were located.

Animal Studies

No reports of animal studies examining the carcinogenicity of alizarin red compounds by any route of exposure were located.

Other Studies

Alizarin red was negative in bacterial tests for mutagenicity using several strains of *S. typhimurium* (TA98, TA102, TA1535, TA1538) with or without a metabolic activating system (Brown and Brown, 1976; Kawasaki et al., 1992; Matsushima et al., 1986; Tikkanen et al., 1983; Westendorf et al., 1990). In *S. typhimurium* TA100, alizarin red showed both negative (Brown and Brown, 1976; Brown, 1980) and positive (Tikkanen et al., 1983) results. Alizarin red was mutagenic to *S. typhimurium* TA1537 (Brown and Brown, 1976; Brown et al., 1977; Brown, 1980; Westendorf et al., 1990). Alizarin red was mutagenic to *S. typhimurium* TA2637 with or without metabolic activation (Tikkanen et al., 1983). Positive (Brown, 1980) and inconclusive (Fujita et al., 1976) results have been reported for mutagenicity in *B. Subtilis.* In mammalian test systems, alizarin red was not mutagenic in the V79/HGPRT assay (Westendorf et al., 1990), did not cause chromosomal damage in CHO cells *in vitro* (Au and Hsu, 1979), and did not enhance transformation of initiated murine fibroblasts (Westendorf et al., 1990; Wölfle et al., 1990). In tests of unscheduled DNA synthesis using primary rat hepatocytes, alizarin red showed both weakly positive (Westendorf et al., 1990) and negative (Kawai et al., 1986; Wölfle et al., 1990) results. Kawai et al. (1986) reported that alizarin red uncoupled and inhibited oxidative phosphorylation in isolated rat liver mitochondria. Using a ^{32}P-postlabeling technique, Poginsky et al. (1991) found no evidence of *in vitro* DNA adduct formation when alizarin red (740 µmol) was incubated with murine DNA and rat liver S9 fraction. Similarly, no increase in DNA adduct formation was found in hepatic, renal, duodenal or colon DNA isolated from male Parkes mice fed 10 mg/day alizarin red in cheese spread for 4 days (Poginsky et al., 1991).

Alizarin red S did not induce a mutagenic response in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 (tested without exogenous metabolic activation) (Brown et al., 1977). In the *B. subtilis* rec M45 or H17 assay, alizarin red S damaged DNA (Driscoll et al., 1979).

No data regarding the genetic toxicity of alizarine red 3WS were located.

No data regarding the genetic toxicity of alizarine red YCAP were located.

Brown et al. (1978) reported inconclusive results for alizarin red B in *S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 (with and without exogenous metabolic activation).

Alizarine chrome red G was positive in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537, but only when tested with exogenous metabolic activation (Elliott and Gregory, 1980).

No data regarding the genetic toxicity of alizarine red V2A were located.

PROVISIONAL WEIGHT-OF-EVIDENCE CLASSIFICATION

In accordance with the proposed cancer guidelines (U.S. EPA, 1999), the available data are inadequate for an assessment of human carcinogenic potential for each of the seven alizarin red compounds (alizarin red, CASRN 72-48-0; alizarin red S, CASRN 130-22-3; alizarine red 3WS, CASRN 6486-94-8; alizarine red YCAP, CASRN 82-29-1; acid alizarin red B, CASRN 1836-22-2; alizarine chrome red G, CASRN 10169-02-5; alizarine red V2A CASRN 12794-90-0).

QUANTITATIVE ESTIMATES OF CARCINOGENIC RISK

Provisional oral slope factors and inhalation unit risk values for the seven alizarin red compounds cannot be derived because human and animal cancer data are lacking.

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