

SCREENING-LEVEL HAZARD CHARACTERIZATION SPONSORED CHEMICAL

Acetoacet-*o*-anisidide (CASRN 92-15-9)

SUPPORTING CHEMICALS

Acetoacetanilide (CASRN 102-01-2)

Acetoacet-*o*-toluidide (CASRN 93-68-5)

The High Production Volume (HPV) Challenge Program¹ was conceived as a voluntary initiative aimed at developing and making publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the United States in quantities greater than one million pounds per year. In the Challenge Program, producers and importers of HPV chemicals voluntarily sponsored chemicals; sponsorship entailed the identification and initial assessment of the adequacy of existing toxicity data/information, conducting new testing if adequate data did not exist, and making both new and existing data and information available to the public. Each complete data submission contains data on 18 internationally agreed to “SIDS” (Screening Information Data Set^{1,2}) endpoints that are screening-level indicators of potential hazards (toxicity) for humans or the environment.

The Environmental Protection Agency’s Office of Pollution Prevention and Toxics (OPPT) is evaluating the data submitted in the HPV Challenge Program on approximately 1400 sponsored chemicals by developing hazard characterizations (HCs). These HCs consist of an evaluation of the quality and completeness of the data set provided in the Challenge Program submissions. They are not intended to be definitive statements regarding the possibility of unreasonable risk of injury to health or the environment.

The evaluation is performed according to established EPA guidance^{2,3} and is based primarily on hazard data provided by sponsors; however, in preparing the hazard characterization, EPA considered its own comments and public comments on the original submission as well as the sponsor’s responses to comments and revisions made to the submission. In order to determine whether any new hazard information was developed since the time of the HPV submission, a search of the following databases was made from one year prior to the date of the HPV Challenge submission to the present: (ChemID to locate available data sources including Medline/PubMed, Toxline, HSDB, IRIS, NTP, ATSDR, IARC, EXTOXNET, EPA SRS, etc.), STN/CAS online databases (Registry file for locators, ChemAbs for toxicology data, RTECS, Merck, etc.) and Science Direct. OPPT’s focus on these specific sources is based on their being of high quality, highly relevant to hazard characterization, and publicly available.

OPPT does not develop HCs for those HPV chemicals which have already been assessed internationally through the HPV program of the Organization for Economic Cooperation and Development (OECD) and for which Screening Initial Data Set (SIDS) Initial Assessment

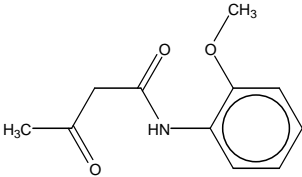
¹ U.S. EPA. High Production Volume (HPV) Challenge Program; <http://www.epa.gov/chemrtk/index.htm>.

² U.S. EPA. HPV Challenge Program – Information Sources; <http://www.epa.gov/chemrtk/pubs/general/guidocs.htm>.

³ U.S. EPA. Risk Assessment Guidelines; <http://cfpub.epa.gov/ncea/raf/rafguid.cfm>.

Reports (SIAR) and SIDS Initial Assessment Profiles (SIAP) are available. These documents are presented in an international forum that involves review and endorsement by governmental authorities around the world. OPPT is an active participant in these meetings and accepts these documents as reliable screening-level hazard assessments.

These hazard characterizations are technical documents intended to inform subsequent decisions and actions by OPPT. Accordingly, the documents are not written with the goal of informing the general public. However, they do provide a vehicle for public access to a concise assessment of the raw technical data on HPV chemicals and provide information previously not readily available to the public.

<p>Chemical Abstract Service Registry Number (CASRN)</p>	<p>92-15-9</p>
<p>Chemical Abstract Index Name</p>	<p>Butanamide, N-(2-methoxyphenyl)-3-oxo-</p>
<p>Structural Formula</p>	
<p style="text-align: center;">Summary</p> <p>This chemical is a solid with high water solubility and moderate vapor pressure. It is expected to have high mobility in soil. Volatilization is expected to be low based on the estimated Henry's Law constant. The rate of atmospheric photooxidation is considered moderate. Hydrolysis is expected to be negligible under environmental conditions. This chemical is expected to have low persistence (P1) and low bioaccumulation potential (B1).</p> <p>The acute oral toxicity of this chemical in rats is low. It is irritating to rabbit eye and guinea pig skin and is not a skin sensitizer in guinea pigs. Repeated oral exposures to rats with CASRNs 92-15-9, 93-68-5 and 102-01-2 showed effects in the spleen and changes in hematology parameters at 80, 75 and 100 mg/kg-bw/day, respectively; the NOAEL for systemic toxicity was not established for CASRN 92-15-9 and was 25 and 12 mg/kg-bw/day for the supporting chemicals, CASRN 93-68-5 and CASRN 102-01-2, respectively. In oral combined repeated-dose/reproductive/developmental toxicity screening tests in rats, both of the supporting chemicals, CASRN 93-68-5 and CASRN 102-01-2, showed effects on adults at 80 mg/kg-bw/day and 30 mg/kg-bw/day, respectively, but no reproductive or prenatal and postnatal developmental toxicity; the NOAEL for maternal toxicity was 25 mg/kg-bw/day for CASRN 93-68-5 and 3 mg/kg-bw/day for CASRN 102-01-2. The NOAELs for reproductive/developmental toxicity were 250 mg/kg-bw/day (highest dose tested) for CASRN 93-68-5 and 100 mg/kg-bw/day (highest dose tested) for CASRN 102-01-2. CASRN 92-15-9 did not induce gene mutations in bacteria and mammalian cells. The supporting chemical CASRN 102-01-2 did not induce chromosomal aberrations <i>in vitro</i> but the supporting chemical CASRN 93-68-5 gave a positive response.</p> <p>The 96-hour LC₅₀ of CASRN 92-15-9 to fish is 332 mg/L. The 96-hour EC₅₀ and the 48-hour EC₅₀ to aquatic invertebrates is > 100 mg/L and 931 mg/L, respectively, for the two supporting chemicals, CASRN 102-01-2 and CASRN 93-68-5. The 96-hour EC₅₀ to aquatic plants is 318 mg/L (growth rate) from the supporting chemical, CASRN 102-01-2.</p> <p>There were no data gaps identified under the HPV Challenge Program.</p>	

The sponsor, The Color Pigments Manufacturers Association, Inc. - Diarylide Intermediates Task Force, submitted a Test Plan and Robust Summary to EPA for Acetoacet-*o*-anisidide (CASRN 92-15-9; CA Index name: butanamide, N-(2-methoxyphenyl)-3-oxo-), on June 28, 2001. EPA posted the submissions on the ChemRTK HPV Challenge Website on August 6, 2001 (<http://www.epa.gov/chemrtk/pubs/summaries/cpma/c13103tc.htm>). EPA comments on the original submission were posted to the website on February 11, 2002. Public comments were also received and posted to the website. The sponsor has not submitted a response to EPA comments or revised/updated documents.

Justification for Supporting Chemicals

The sponsor indicated in the test plan that CASRN 92-15-9 was manufactured and transported in closed systems; however, no formal claims for reduced testing for closed system intermediates were made under the HPV Challenge Program. The sponsor used data for acetoacetanilide (CASRN 102-01-2; CA Index name: butanamide, 3-oxo-N-phenyl-) and acetoacet-*o*-toluidide (CASRN 93-68-5; CA Index name: butanamide, N-(2-methylphenyl)-3-oxo-) to represent the toxicity of CASRN 92-15-9 based on their similar structures and target organ toxicity. The chemical structures of CASRNs 102-01-2, 92-15-9 and 93-68-5 only differ by the presence of a methyl or a methoxy group in the ortho position. This single substitution does not significantly alter the basic physicochemical properties or their basic biological effects. All three compounds have a similar acute toxicity value and repeated exposure studies showed similar toxicity targeting the red-blood-cells inducing a methemoglobinemia and subsequent anemia. EPA agrees that the use of data for CASRNs 102-01-2 and CASRN 93-68-5 are appropriate.

The data for CASRN 102-01-2 and CASRN 93-68-5 were used to address the chromosomal aberrations, reproductive and developmental toxicity endpoints and supplement data for the repeated-dose toxicity endpoint for CASRN 92-15-9. In addition, data for CASRN 102-01-2 were used to supplement computer model estimations for toxicity to aquatic plants. In its comments to the Sponsor (dated January 29, 2002) EPA stated that the submitted data for the acute toxicity to aquatic invertebrates endpoint were invalid. However, data for CASRN 102-01-2 and CASRN 93-68-5 can be used to address this endpoint and were used by EPA in the hazard characterization. CASRN 102-01-2 and CASRN 93-68-5 have been reviewed in the OECD HPV program where the data are available for review at the following website: <http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html> (these documents were reviewed for this characterization and provided some details not found in the US HPV Challenge submission).

1 Chemical Identity

1.1 Identification and Purity

The following description is taken from the Test Plan (2001):

CASRN 92-15-9 is a chemically stable material of very high purity. Its sole use is as a chemical intermediate in the production of pigments.

1.2 Physical-Chemical Properties

The physical-chemical properties of CASRN 92-15-9 and its supporting chemicals are summarized in Table 1. CASRN 92-15-9 is a solid with high water solubility and moderate vapor pressure.

Property	Sponsored Chemical	Supporting Chemicals	
	Acetoacet- <i>o</i> -anisidide	Acetoacetanilide	Acetoacet- <i>o</i> -toluidide
CASRN	92-15-9	102-01-2	93-68-5
Molecular Weight	207.23	177.20	191.09
Physical State	Solid	Solid	Solid
Melting Point	86.6°C (measured)	83–85°C (measured)	106°C (measured)
Boiling Point	375.47°C (estimated)	351.81°C (estimated) ²	363.41°C (estimated) ²
Vapor Pressure	1.5×10 ⁻⁴ mm Hg at 25°C (estimated)	5.2×10 ⁻⁵ mm Hg at 25°C (extrapolated) ³	1.1×10 ⁻⁵ mm Hg at 25°C (estimated) ²
Water Solubility	3,340 mg/L (measured)	7,657 mg/L (estimated) ²	6,896 mg/L (estimated) ²
Dissociation Constant (pK _a)	Not applicable under environmental conditions	10.68 (measured)³	Not applicable under environmental conditions
Henry's Law Constant	2.46×10 ⁻¹³ atm-m ³ /mole (estimated)	4.15×10 ⁻¹² atm-m ³ /mole (estimated) ²	4.58×10 ⁻¹² atm-m ³ /mole (estimated) ²
Log K _{ow}	0.53 (estimated)	1.01 (estimated) ²	0.99 (estimated) ²

Color Pigments Manufacturers Association, Inc. Diarylide Intermediates Task Force. June 28, 2001. Robust Summary and Test Plan for Acetoacet-*o*-Anisidide. <http://www.epa.gov/chemrtk/pubs/summaries/cpma/c13103tc.htm>.

²U.S. EPA. 2008. Estimation Programs Interface Suite™ for Microsoft® Windows, v3.20. United States Environmental Protection Agency, Washington, DC, USA. <http://www.epa.gov/opptintr/exposure/pubs/episuite.htm>.

³SRC. The Physical Properties Database (PHYSPROP). Syracuse, NY: Syracuse Research Corporation. Available from <http://www.srcinc.com/what-we-do/free-demos.aspx> as of January 30, 2009.

2 General Information on Exposure

2.1 Production Volume and Use Pattern

This chemical had an aggregated manufactured and/or imported production volume in the United States of 1 million to 10 million pounds in 2005.

The IUR submissions did not contain any information on industrial processing and uses, or commercial and consumer uses. The HPV submission for the chemical claims that the chemical is manufactured and transported in closed-systems and sold to a limited number of customers to be used as a chemical intermediate in the production of pigments. However, this chemical does not meet the Agency's closed-system intermediate criteria.

2.2 Environmental Exposure and Fate

No quantitative information is available on releases of this chemical to the environment.

The environmental fate properties are provided in Table 2. CASRN 92-15-9 is expected to have high mobility in soil. CASRN 92-15-9 was not readily biodegradable using a modified MITI (OECD 301C); however, the substance degraded rapidly and was characterized as inherently biodegradable using a Zahn-Wellens (OECD 302B) test. Moreover, the supporting chemical CASRN 102-01-2- was readily biodegradable using the modified MITI test, suggesting that CASRN 92-15-9 and its supporting chemicals will not be persistent in the environment.

Hydrolysis is expected to be negligible under environmental conditions. CASRN 92-15-9 is expected to have low persistence (P1) and low bioaccumulation potential (B1).

Table 2. Environmental Fate Characteristics of Acetoacet-<i>o</i>-anisidide and its Supporting Chemicals¹			
Property	Sponsored Chemical	Supporting Chemicals	
	Acetoacet-<i>o</i>-anisidide	Acetoacetanilide	Acetoacet-<i>o</i>-toluidide
CASRN	92-15-9	102-01-2	93-68-5
Photodegradation Half-life	10.1 hours (estimated)	9.8 hours (estimated) ²	8.0 hours (estimated) ²
Hydrolysis Half-life	Hydrolytically stable	131.4 days at pH 1.5 and 37°C	No data
Biodegradation	18% after 28 days (not readily biodegradable); 25–28% after 28 days (not readily biodegradable)³; 97% after 10 days (inherently biodegradable)	79% after 28 days (readily biodegradable)³	No data
Bioconcentration	BCF = 3.2 (estimated) ²	BCF = 1.19 (estimated) ²	BCF = 3.2 (estimated) ²
Log K _{oc}	1 (estimated) ²	1 (estimated) ²	1.3 (estimated) ²
Fugacity (Level III Model)	Air = 1.05% Water = 50.7% Soil = 48.2% Sediment = 0.103%	Air = <0.1% Water = 34.5% Soil = 65.4% Sediment = <0.1%	Air = <0.1% Water = 40.8% Soil = 59.2% Sediment = <0.1%
Persistence ⁴	P1 (low)	P1 (low)	P1 (low)
Bioaccumulation ⁴	B1 (low)	B1 (low)	B1 (low)

¹Color Pigments Manufacturers Association, Inc. Diarylide Intermediates Task Force. June 28, 2001. Robust Summary and Test Plan for Acetoacet-*o*-Anisidide.

<http://www.epa.gov/chemrtk/pubs/summaries/cpma/c13103tc.htm>.

²U.S. EPA. 2008. Estimation Programs Interface Suite™ for Microsoft® Windows, v3.20. United States Environmental Protection Agency, Washington, DC, USA.

<http://www.epa.gov/opptintr/exposure/pubs/episuite.htm>.

³National Institute of Technology and Evaluation. 2002. Biodegradation and Bioaccumulation of the Existing Chemical Substances under the Chemical Substances Control Law.

http://www.safe.nite.go.jp/english/kizon/KIZON_start_hazkizon.html.

⁴Federal Register. 1999. Category for Persistent, Bioaccumulative, and Toxic New Chemical Substances. *Federal Register* 64, Number 213 (November 4, 1999) pp. 60194–60204.

3 Human Health Hazard

Acute Oral Toxicity

Acetoacet-*o*-anisidide (CASRN 92-15-9)

CrI:CD (SD) rats (5/sex/dose) were administered CASRN 92-15-9 via gavage at 1260, 1600, or 2000 mg/kg-bw. (Observation period not stated.) Deaths occurred within three days of dosing: one male at 1260 mg/kg-bw; three males and two females at 1600 mg/kg-bw; three males and five females at 2000 mg/kg-bw. Terminal necropsies of survivors did not reveal any abnormalities.

LD₅₀ = 1637 mg/kg-bw

Repeated-Dose Toxicity

Acetoacetanilide (CASRN 102-01-2, Supporting Chemical)

In a 28-day study, Sprague-Dawley rats (10/sex/dose) were administered CASRN 102-01-2 via oral gavage at 0, 12, 100 or 850 mg/kg-bw/day. An additional group of high-dose recovery animals was observed for 14 days following cessation of treatment. The control group received 1% methylcellulose. Statistically significant dose-dependent changes in hematology and serum chemistry that were indicative of hemolytic anemia and methemoglobinemia occurred in the mid- and high-dose groups. Compensatory erythropoiesis was confirmed in the evaluation of bone marrow smears. By the end of the 14-day recovery period, hematologic parameters were near normal with no evidence of anemia or methemoglobinemia. Spleen weights after 4 weeks of treatment were significantly greater in the mid- and high-dose animals compared to controls. Higher spleen weights were also evident after recovery. Microscopic evaluation of tissues indicated extramedullary hematopoiesis in the liver with siderosis in the spleen. Evidence of renal excretion of heme was also present.

LOAEL = 100 mg/kg-bw/day (based on effects on spleen weights with associated histopathology and changes in hematology and serum chemistry)

NOAEL = 12 mg/kg-bw/day

Acetoacet-o-toluidide (CASRN 93-68-5, Supporting Chemical)

In a combined repeated-dose/reproductive/developmental toxicity screening test, Crl:CD (SD) rats (10/sex/dose) were administered CASRN 93-68-5 via oral gavage at 0, 8, 25, 80 or 250 mg/kg-bw/day for 44 days for males and for females, from 14 days before mating, gestation to Day 3 of lactation. The control group received 1% methylcellulose. At 80 and 250 mg/kg-bw/day, a decrease in erythrocyte counts and an increase in mean corpuscular volume (MCV) and serum bilirubin were observed. Histological examination indicated hemosiderin deposits in spleen and liver. In addition, at 250 mg/kg-bw/day, decreases in hemoglobin concentration and hematocrit values, increases in mean corpuscular hemoglobin (MCH) and reticulocyte counts and the appearance of Heinz-bodies in erythrocytes were also observed. The absolute and relative weights of spleen were increased and histopathology of the spleen showed extramedullary hematopoiesis and congestion. An increased incidence of eosinophilic bodies was noted in renal proximal tubular epithelial cells.

LOAEL = 80 mg/kg-bw/day (based on effects on hematological parameters and organ weights)

NOAEL = 25 mg/kg-bw/day

Acetoacet-o-anisidide (CASRN 92-15-9)

In a 14-day study, rats (5; sex and strain not specified) were administered CASRN 92-15-9 in the diet *ad libitum* at concentrations of 0, 0.1 and 1% (approximately 0, 75 or 709 mg/kg-bw/day, respectively). For the control group, 1% corn oil was mixed into the diet. No mortality or treatment-related clinical signs were observed at either dose level. Histopathological evaluation revealed a minor splenic congestion in 3/5 animals at the low dose. Hematological changes consisted of decreases in RBC count, hemoglobin and hematocrit. Changes in RBC appearance consisted of anisocytosis (macrocytes and spherocytes), poikilocytosis, polychromasia, target cells and Howell-Jolly bodies. Enlarged and/or dark spleens were noted in 4 of 5 animals.

LOAEL = 75 mg/kg-bw/day (based on effects on spleen)

NOAEL = Not established

Reproductive/Developmental Toxicity

Acetoacet-o-toluidide (CASRN 93-68-5, Supporting Chemical)

In a combined repeated-dose/reproductive/developmental toxicity screening test described previously, systemic toxicity was evident at 80 and 250 mg/kg-bw/day with effects on hematology parameters, spleen and liver. There were no effects on any mating or fertility parameters at any dose. The test substance did not induce reproductive or developmental toxicity under the conditions of this assay.

LOAEL (parental/maternal toxicity) = 80 mg/kg-bw/day (based on effects on hematology parameters and organ weights)

NOAEL (parental/maternal toxicity) = 25 mg/kg-bw/day

NOAEL (reproductive/developmental toxicity) = 250 mg/kg-bw/day

Acetoacetanilide (CASRN 102-01-2, Supporting Chemical)

In a combined reproductive/developmental toxicity screening test, Sprague-Dawley rats (10/sex/dose) were administered CASRN 102-01-2 via gavage at 0, 3, 30 or 100 mg/kg-bw/day. Males were treated for 6 weeks beginning 14 days before breeding; females were treated from 2 weeks before breeding through gestation and Day 4 of lactation. At 3 mg/kg-bw/day, no adverse effects were noted. At 30 mg/kg-bw/day, methemoglobinemia was noted in both sexes. Methemoglobinemia and other effects (excessive salivation [males only] and other hematological effects and increased spleen weights) were seen at 100 mg/kg-bw/day. Mating and fertility were unaffected by treatment and there were no microscopic lesions in the sex organs. There were no effects on gestation, implantation or viability and no effects were observed in the pups.

LOAEL (parental/maternal toxicity) = 30 mg/kg-bw/day (based on observed effects of methemoglobinemia)

NOAEL (parental/maternal toxicity) = 3 mg/kg-bw/day

NOAEL (reproductive/developmental toxicity) = 100 mg/kg-bw/day

Genetic Toxicity – Gene Mutation

In vitro

Acetoacet-o-anisidide (CASRN 92-15-9)

(1) *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to CASRN 92-15-9 at concentrations of 25–10,000 µg/plate with and without metabolic activation. Positive and negative controls were used which showed appropriate responses. The cytotoxic concentration was greater than 10,000 µg/plate. The test material did not induce an increase in mutation frequency in any tester strains in the presence or absence of metabolic activation.

CASRN 92-15-9 was not mutagenic in this assay.

(2) Chinese hamster ovary (CHO) cells were exposed to CASRN 92-15-9 at 1.0 – 3.0 mg/mL with and without metabolic activation. The test included both positive and negative controls but their responses were not provided in the robust summary. Cytotoxic concentration was 3 mg/mL. CASRN 92-15-9 did not induce an increase in mutant colonies both in the presence and absence of metabolic activation.

CASRN 92-15-9 was not mutagenic in this assay.

Genetic Toxicity – Chromosomal Aberrations

In vitro

Acetoacetanilide (CASRN 102-01-2, Supporting Chemical)

Human peripheral blood lymphocytes were exposed to CASRN 102-01-2 at 232, 929 or 1860 µg/mL without metabolic activation and 464, 1860 or 3710 µg/mL with metabolic activation. Positive and negative controls were used which showed appropriate responses. The cytotoxic concentration was 3710 µg/mL with and without metabolic activation. No treatment-related increases in cells with chromosomal aberrations were observed.

CASRN 102-01-2 did not induce chromosomal aberrations in this assay.

Acetoacet-o-toluidide (CASRN 93-68-5, Supporting Chemical)

Chinese Hamster lung cells were exposed to CASRN 93-68-5 at concentrations up to 5 mg/mL with and without metabolic activation. Positive and negative controls were used which showed appropriate responses. The cytotoxic concentration was 3.5 mg/mL. Structural chromosomal aberrations (including gaps) were induced without metabolic activation under the following conditions: 24-hour continuous treatment (2.5 mg/mL, 10.0%); 48-hour continuous treatment (1.8 mg/mL, 5.0 %); short-term treatment without S9 (5mg/mL, 9%); short-term treatment with S9 (5 mg/mL, 5%). The increase in structural chromosomal aberrations was dose dependent. The test substance was positive without metabolic activation and showed equivocal response with metabolic activation.

CASRN 93-68-5 induced chromosomal aberrations in this assay.

Genetic Toxicity – Other

In vitro

Acetoacet-o-anisidide (CASRN 92-15-9)

In an unscheduled DNA synthesis (UDS) assay, CD-1 rat liver cells were exposed to CASRN 92-15-9 at concentrations up to 3.2 mg/mL. Positive and negative controls were used which showed appropriate responses. The cytotoxic concentration was 3.2 mg/mL. The test substance did not induce an increase in UDS.

CASRN 92-15-9 was not mutagenic in this assay.

Additional Information

Skin Irritation

Acetoacet-o-anisidide (CASRN 92-15-9)

Application of CASRN 92-15-9 to the depilated abdomen of guinea pigs (5/sex not stated) for 24 hours under an impervious cuff produced a slight irritation with no evidence of percutaneous absorption. Repeated application to the clipped backs of guinea pigs for 10 days did not exacerbate the irritative response. <http://www.srcinc.com/what-we-do/databaseforms.aspx?id=384>

CASRN 92-15-9 was a skin irritant to guinea pigs in this assay.

Eye Irritation

Acetoacet-o-anisidide (CASRN 92-15-9)

Rabbits (6/sex not stated) were instilled with the test substance in the conjunctival sacs. Three rabbits' eyes were washed. Slight eye irritation was present. Retention of fluorescein dye was observed in the nictitating membrane of 1/3 unwashed eyes. No staining was observed in washed eyes. <http://www.srcinc.com/what-we-do/databaseforms.aspx?id=384>

CASRN 92-15-9 was irritating to rabbit eye in this assay.

Skin Sensitization

Acetoacet-o-anisidide (CASRN 92-15-9)

Guinea pigs (10/sex not stated) treated with CASRN 92-15-9 to the skin were not sensitized.

<http://www.srcinc.com/what-we-do/databaseforms.aspx?id=384>

CASRN 92-15-9 was not a skin sensitizer in this assay.

Conclusion: The acute oral toxicity of this chemical in rats is low. It is irritating to rabbit eye and guinea pig skin and is not a skin sensitizer in guinea pigs. Repeated oral exposures to rats with CASRNs 92-15-9, 93-68-5 and 102-01-2 showed effects on spleen and changes in hematology parameters at 80, 75 and 100 mg/kg-bw/day, respectively; the NOAEL for systemic toxicity was not established for CASRN 92-15-9 and was 25 and 12 mg/kg-bw/day for the supporting chemicals, CASRN 93-68-5 and CASRN 102-01-2, respectively. In oral combined repeated-dose/reproductive/developmental toxicity screening tests in rats, both of the supporting chemicals, CASRN 93-68-5 and CASRN 102-01-2, showed effects on adults at 80 mg/kg-bw/day and 30 mg/kg-bw/day, respectively, but no reproductive or prenatal and postnatal developmental toxicity; the NOAEL for maternal toxicity was 25 mg/kg-bw/day for CASRN 93-68-5 and 3 mg/kg-bw/day for CASRN 102-01-2. The NOAELs for reproductive/developmental toxicity were 250 mg/kg-bw/day (highest dose tested) for CASRN 93-68-5 and 100 mg/kg-bw/day (highest dose tested) for CASRN 102-01-2. CASRN 92-15-9 did not induce gene mutations in bacteria and mammalian cells. The supporting chemical CASRN 102-01-2 did not induce chromosomal aberrations *in vitro* but the supporting chemical CASRN 93-68-5 gave a positive response.

4 Hazards to the Environment

Acute Toxicity to Fish

Acetoacet-o-anisidide (CASRN 92-15-9)

Zebrafish (*Brachydanio rerio*) were exposed to CASRN 92-15-9 at nominal concentrations of 0, 100, 220 and 500 mg/L under static conditions for 96 hours. Mortality showed a clear dose-response with 100% death at 500 mg/L.

96-h LC₅₀ = 332 mg/L

Acute Toxicity to Aquatic Invertebrates

Acetoacetanilide (CASRN 102-01-2, Supporting Chemical)

Water fleas (*Daphnia magna*) were exposed to CASRN 102-01-2 at nominal concentrations for 96 hours. The daphnia behavior was comparable to the controls at all test concentrations at all observation time periods.

96-h EC₅₀ > 100 mg/L

Acetoacet-o-toluidide (CASRN 93-68-5, Supporting Chemical)

Water fleas (*Daphnia magna*) were exposed to CASRN 93-68-5 at nominal concentrations of 0, 198, 296, 444, 667 or 1000 mg/L under static conditions for 48 hours. The measured concentrations were 96.4 to 103% of the nominal concentrations.

48-h EC₅₀ = 931 mg/L

Toxicity to Aquatic Plants

Acetoacetanilide (CASRN 102-01-2, Supporting Chemical)

Green algae (*Pseudokirchneriella subcapitata*) were exposed to CASRN 102-01-2 at nominal concentrations of 11.3, 22.5, 45, 90, 180, 360, and 720 mg/L for 72 hours. No deformed cells were noted.

72-h EC₅₀ (growth rate) = 318 mg/L

Acetoacet-o-anisidide (CASRN 92-15-9)

The toxicity of CASRN 92-15-9 to green algae was estimated by ECOSAR.

96-h EC₅₀ (growth rate) = 2058 mg/L (estimated)

Conclusion: The 96-hour LC₅₀ of CASRN 92-15-9 to fish is 332 mg/L. The 96-hour EC₅₀ and the 48-hour EC₅₀ to aquatic invertebrates is > 100 mg/L and 931 mg/L, respectively, for the two supporting chemicals, CASRN 102-01-2 and CASRN 93-68-5. The 96-hour EC₅₀ to aquatic plants is 318 mg/L (growth rate) from the supporting chemical, CASRN 102-01-2.

**Table 3. Summary Table of the Screening Information Data Set
as submitted under the U.S. HPV Challenge Program**

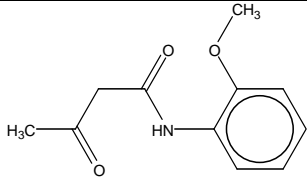
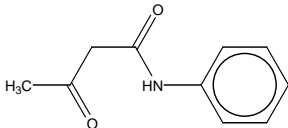
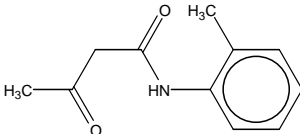
Endpoints	SPONSORED CHEMICAL Acetoacet-<i>o</i>-anisidide (CASRN 92-15-9)	SUPPORTING CHEMICAL Acetoacetanilide (CASRN 102-01-2)	SUPPORTING CHEMICAL Acetoacet-<i>o</i>-toluidide (CASRN 93-68-5)
Structure			
Summary of Human Health Data			
Acute Oral Toxicity LD₅₀ (mg/kg-bw)	1637	—**	—**
Repeated-Dose Toxicity (Oral) NOAEL/LOAEL (mg/kg-bw/day)	No data NOAEL = 12 LOAEL=100 (RA)	(28-day) NOAEL = 12 LOAEL=100	NOAEL = 25 LOAEL= 80
Reproductive/Develo pmental Toxicity NOAEL/LOAEL (mg/kg-bw/day) Systemic/Maternal Toxicity	No data NOAEL = 3 LOAEL = 30	NOAEL = 3 LOAEL = 30	NOAEL = 25 LOAEL = 80
Reproductive/ Developmental Toxicity	NOAEL = 100 (RA)	NOAEL = 100	NOAEL = 250
Genetic Toxicity— Gene Mutation <i>In vitro</i>	Negative	—**	—**
Genetic Toxicity – Chromosomal Aberrations <i>In vitro</i>	No data Negative (RA)	Negative	Positive
Genetic Toxicity – UDS <i>In vitro</i>	Negative	—**	—**
Additional Information Skin Irritation Eye Irritation Skin Sensitization	Slight Slight Negative	—**	—**

Table 3. Summary Table of the Screening Information Data Set as submitted under the U.S. HPV Challenge Program			
Endpoints	SPONSORED CHEMICAL Acetoacet-<i>o</i>-anisidide (CASRN 92-15-9)	SUPPORTING CHEMICAL Acetoacetanilide (CASRN 102-01-2)	SUPPORTING CHEMICAL Acetoacet-<i>o</i>-toluidide (CASRN 93-68-5)
Summary of Environmental Effects – Aquatic Toxicity Data			
Fish 96-h LC₅₀ (mg/L)	332	–**	–**
Aquatic Invertebrates 48-h EC₅₀ (mg/L)	No Data 931 (RA)	>100 (96-h)	931
Aquatic Plants 96-h EC₅₀ (mg/L)	No Data 318 (RA)	318	–**

Measured data in bold text; (RA) = read across; –** indicates endpoint was not addressed for this chemical