# SCREENING-LEVEL HAZARD CHARACTERIZATION

## Alkyldimethylbenzylammonium Chloride (ADBAC) Category

**Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16) CASRN 68424-85-1**
**Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18) CASRN 68391-01-5**
**Benzyldimethyloctadecyl ammonium chloride CASRN 122-19-0**

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| **Chemical Abstract Service Registry Number (CASRN)** | **68424-85-1<br>68391-01-5<br>122-19-0** |
| **Chemical Abstract Index Name** | **Quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides<br><br>Quaternary ammonium compounds, benzyl-C12-C18-alkyldimethyl, chlorides<br><br>Benzenemethanaminium, N,N-dimethyl-N-octadecyl-, chloride (1:1)** |
| **Structural Formula** | **See Appendix** |

**Summary**

The alkyl dimethyl benzyl ammonium chloride (ADBAC) category consists of three substances that are used as antimicrobial agents in a variety of industries. Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16) and Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18) are mixtures of alkyldimethylbenzylammonium chlorides in which the alkyl chain length varies from C12 to C18. Benzyldimethyloctadecyl ammonium chloride is not a mixture but a discrete substance with a C18 alkyl chain length. These substances are solids with negligible to low vapor pressure and are dispersible in water. They are expected to possess low mobility in soil. These substances are not expected to be persistent in the environment; however, at high concentrations they may be toxic to microorganisms which will slow the rate of biodegradation. Volatilization is considered low since these substances are quaternary ammonium salts. The rate of hydrolysis is expected to be negligible. The rate of atmospheric photooxidation is moderate; however, this is not a relevant environmental degradation pathway since these substances are not expected to exist in the vapor phase in the atmosphere. The constituents of the alkyl dimethyl benzyl ammonium chloride category are expected to have low persistence (P1) and low bioaccumulation potential (B1).

The acute oral toxicity in rats is moderate for ADBAC C12-16 and low for ADBAC C12-18 and the acute dermal toxicity of ADBAC C12-16 and ADBAC C12-18 is low in rabbits. In a 13-week feeding study with ADBAC C12-16 in rats, effects included mortality, decreased food consumption and body weights, ileus and histopathological effects in the gastrointestinal system at ~ 248 and ~ 308 mg/kg-bw/day in males and females, respectively; the NOAELs for systemic

toxicity are ~ 62 and ~ 77 mg/kg-bw/day in males and females, respectively. In a 104-week feeding study with ADBAC C12-16 in rats, reduced body weight and food consumption were observed at ~ 88 and ~ 116 mg/kg-bw/day in males and females, respectively; the NOAELs for systemic toxicity are ~ 44 and ~ 57 mg/kg-bw/day in males and females, respectively. In a 13-week feeding study with ADBAC C12-16 in mice, mortality was observed at ~ 696 and ~ 820 mg/kg-bw/day in males and females, respectively; the NOAELs for systemic toxicity are ~ 174 and ~ 210 mg/kg-bw/day in males and females, respectively. In a 78-week feeding study with ADBAC C12-16 in mice, reduced body weight and body weight gain were observed at ~ 229 and ~ 289 mg/kg-bw/day in males and females, respectively; the NOAELs for systemic toxicity are ~ 73 and ~ 92 mg/kg-bw/day in males and females, respectively. In a 13-week dermal study with ADBAC C12-16 in rats no treatment-related effects were observed; the NOAEL for systemic toxicity is 20 mg/kg-bw/day (highest dose tested). In a two-generation dietary reproductive toxicity study with ADBAC C12-16 in rats, reduced body weights and body weight gains were observed in both adults and pups at ~ 145.5 mg/kg-bw/day; the NOAEL for parental systemic and reproductive toxicity is ~ 72.6 mg/kg-bw/day. In a prenatal oral gavage developmental toxicity study with CASRN ADBAC C12-16 in rats, clinical signs of toxicity included audible respiration in dams at 30 mg/kg-day; the NOAEL for maternal toxicity is 10 mg/kg-day. No treatment-related effects were observed on developmental parameters at the highest dose tested; the NOAEL for developmental toxicity is 100 mg/kg-day. In a prenatal oral gavage developmental toxicity study with ADBAC C12-16 in rabbits, clinical signs of toxicity included hypoactivity and labored or audible respiration at 9 mg/kg-day; the NOAEL for maternal toxicity is 3 mg/kg-day. No treatment-related developmental effects were observed; the NOAEL for developmental toxicity is 9 mg/kg-day. In a prenatal oral gavage developmental toxicity study with ADBAC C12-18 in rats, mortality was observed in dams at 50 mg/kg-day; the NOAEL for maternal toxicity is 15 mg/kg-day. No treatment-related developmental effects were observed; the NOAEL for developmental toxicity is 50 mg/kg-day (highest dose tested). In a prenatal dermal developmental toxicity study with benzyldimethyl-octadecyl ammonium chloride in rats, no maternal or developmental effects were observed at the highest dose tested; the NOAEL for maternal and developmental toxicity is ~ 33 mg/kg-bw/day. ADBAC C12-16 and benzyldimethyl-octadecyl ammonium chloride did not induce gene mutations in bacteria *in vitro* and ADBAC C12-16 did not induce gene mutations in mammalian cells or unscheduled DNA synthesis in rat hepatocytes *in vitro*. ADBAC C12-16 did not induce sister chromatid exchanges in human lymphocytes *in vitro* or micronuclei in polychromatic erythrocytes in mice *in vivo*.

The acute toxicity to fish 96-h $LC_{50}$ is 0.28 – 0.93 mg/L for ADBAC C12-16 and 1.4 mg/L for ADBAC C12-18. For ADBAC C12-16, the acute toxicity to aquatic invertebrates 48-h $EC_{50}$ is 0.0058 – 0.14 mg/L and the toxicity to aquatic plants 72-h $EC_{50}$ is 0.014 – 0.058 mg/L for biomass and 0.049 – 0.078 mg/L for growth rate. For ADBAC C12-16, the chronic 28-d $LC_{50}$ value for fish is 0.094 mg/L and the chronic 28-d $EC_{50}$ (reproduction) value for aquatic invertebrates is ~ 0.059 mg/L.

No data gaps were identified under the HPV Challenge Program.

The sponsor, Consumer Specialty Products Association for the ADBAC Joint Venture, submitted a Test Plan and Robust Summaries to EPA for the Alkyldimethylbenzylammonium Chloride (ADBAC) Category on March 9, 2011. EPA posted the submission on the ChemRTK Web site on March 28, 2011 (http://www.epa.gov/chemrtk/pubs/summaries/adbac/c16856tc.html). EPA did not post comments on the submission to the website. The ADBAC category consists of the following three chemicals:

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| Alkyl (C12-16) dimethylbenzyl ammonium chloride [CA Index Name: quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides] | CASRN 68424-85-1 |
| Alkyl (C12-18) dimethylbenzyl ammonium chloride [CA Index Name: quaternary ammonium compounds, benzyl-C12-18-alkyldimethyl, chlorides] | CASRN 68391-01-5 |
| Benzyldimethyloctadecyl ammonium chloride [CA Index Name: benzenemethanaminium, N,N-dimethyl-N-octadecyl-, chloride (1:1)] | CASRN 122-19-0 |

## Category Justification

The ADBAC category consists of three quaternary ammonium compounds. All three members of the ADBAC category fit into the EPA Group II Quaternary Ammonium (Quat) Cluster, which consists of non-halogenated benzyl-substituted Quats.[1] Based on similarities in structure, physical-chemical properties, environmental fate and toxicity, EPA agrees that the chemicals in this category can be grouped and evaluated together.

Alkyl dimethyl benzyl ammonium chloride (ADBAC) is regulated by the EPA Office of Pesticide Programs (OPP) and the reregistration eligibility decision can be viewed at: http://www.epa.gov/oppsrrd1/REDs/adbac_red.pdf

## 1. Chemical Identity

### 1.1 Identification and Purity

The constituents of the ADBAC category are solids with negligible to low vapor pressure that are dispersible in water. The chemicals included in this category are FIFRA registered antimicrobial chemicals with germicidal, fungicidal, and algicidal activity. They are used extensively as bactericides, fungicides, sanitizers, deodorants, and disinfectants in the restaurant, dairy, food, laundry, and medical industries.

### 1.2 Physical-Chemical Properties

The available physical-chemical properties of the ADBAC category are summarized in Table 1.

[1] U.S. EPA, Office of Pesticides and Toxic Substances. 1988. Clustering of Quaternary Ammonium Compounds. PR Notice 88-2.

**Table 1. Physical-Chemical Properties of the Alkyl Dimethyl Benzyl Ammonium Chloride Category[1]**

| Property | Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16) | Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18) | Benzyldimethyloctadecyl ammonium chloride |
|---|---|---|---|
| CASRN | 68424-85-1 | 68391-01-5 | 122-19-0 |
| Molecular Weight | 359.6 (typical) | 377.8 | 424.2 |
| Physical State[6] | Solid | Solid | Solid |
| Melting Point | Decomposes at ~150°C without a clear melting point | Decomposes at ~150°C without a clear melting point | Decomposes at ~150°C without a clear melting point |
| Boiling Point | Decomposes before boiling | Decomposes before boiling | Decomposes before boiling |
| Vapor Pressure | $4.5\times10^{-6}$ mm Hg at 20°C (measured) | $3.0\times10^{-6}$ mm Hg at 20°C (measured) | $<1.0\times10^{-10}$ mm Hg at 25°C (estimated)[2] |
| Dissociation Constant ($pK_a$) | Not applicable | Not applicable | Not applicable |
| Henry's Law Constant | $<1.0\times10^{-10}$ atm-$m^3$/mol (estimated)[2] | $<1.0\times10^{-10}$ atm-$m^3$/mol (estimated)[2] | $<1.0\times10^{-10}$ atm-$m^3$/mol (estimated)[2] |
| Water Solubility | 409 g/L (pH 5.5) at 20°C[3]<br>431 g/L (pH 6.5) at 20°C[3]<br>403 g/L (pH 6.9) at 20°C[3]<br>379 g/L (pH 8.2) at 20°C[3]<br>All 4 values (measured)<br>Dispersible[3] | 500-1,000 g/L (pH 5, 7 and 9 at room temp) (measured)[3]<br>Dispersible[3] | Dispersible |
| Log $K_{ow}$ | 3.91 (estimated)[2]<br>Not applicable due to dispersibility[4,5] | 2.93 (estimated)[2]<br>Not applicable due to dispersibility[4,5] | 5.87 (estimated)[2]<br>Not applicable due to dispersibility[4,5] |

[1]The Consumer Specialty Products Association for the ADBAC Joint Venture. 2011. Test Plan and Robust Summary for Alkyldimethylbenzylammonium Chloride (ADBAC). Available online at http://www.epa.gov/hpv/pubs/summaries/adbac/c16856tc.html as of September 11, 2012.

[2]U.S. EPA. 2012. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.10. U.S. Environmental Protection Agency, Washington, DC, USA. Estimated Log Kow values are for the representative structures as indicated in the Appendix. Available online at http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm as of September 11, 2012.

[3]Measured water solubility values were reported. However, this substance has surfactant-like properties and is better described as dispersible.

4Tolls J; Sijm D. 2000. Estimating properties of surface active chemical. In: Handbook of Property Estimation for Chemicals. Boethling RS; Mackay D (eds.). Lewis Publishers: Boca Raton, FL, pp 419–446.

[5]These substances are surfactants, and the experimental determination of the log $K_{ow}$ could yield erroneous results due to the emulsifying actions of the surfactants in octanol-water systems.

| Table 1. Physical-Chemical Properties of the Alkyl Dimethyl Benzyl Ammonium Chloride Category[1] | | | |
|---|---|---|---|
| **Property** | **Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16)** | **Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18)** | **Benzyldimethyloctadecyl ammonium chloride** |

[6]The Registration Eligibility Decision (RED) document for Alkyl dimethyl benzyl ammonium chloride (ADBAC) identifies this substance as a yellow liquid but this is likely for a formulated product in ethanol/water solution and not the neat substance. http://www.epa.gov/oppsrrd1/REDs/adbac_red.pdf

## 2. General Information on Exposure

### 2.1 Production Volume and Use Pattern

The ADBAC Category chemicals had an aggregated production and/or import volume in the United States between 1 and 11 million pounds during calendar year 2005 (U.S. EPA, 2010).

- CASRN 68424-85-1: 1 to < 10 million pounds ;
- CASRN 68391-01-5: < 500,000 pounds ;
- CASRN 122-19-0: < 500,000 pounds ;

CASRN 68424-85-1:
Non-confidential information in the IUR indicated that the industrial processing and uses of the chemical include other basic organic chemical manufacturing as corrosion inhibitors and anti-scaling agents. Non-confidential commercial and consumer use of this chemical was “other.”

CASRN 68391-01-5 and 122-19-0:
No industrial processing and use, and commercial and consumer uses were reported for the chemicals.

### 2.2 Environmental Exposure and Fate

The constituents of the ADBAC category are expected to have low mobility in soil. The substances in this category are registered as antimicrobial chemicals and are potentially toxic to microorganisms which degrade organic substances under aerobic conditions when present at high concentrations. At an initial concentration of 5 mg/L, quaternary ammonium compounds, benzyl-C12-18-alkyldimethyl, chlorides was degraded 72% after 28 days using an activated sludge inoculum and the modified Sturm (OECD TG 301B) test. It achieved 60% biodegradation within the 10-day window following the initial 10% biodegradation and thus was classified as readily biodegradable. However, at an initial concentration of 10 mg/L, only 5% degradation was observed over the course of the 28-day incubation period using the same test. Benzenemethanaminium, N,N-dimethyl-N-octadecyl-, chloride (1:1) was not biodegraded using the MITI (OECD TG 301C) test. Although experimental details were not provided, the MITI test typically uses an activated sludge inoculum at 30 mg/L and a high concentration (100 mg/L) of the test substance. The same publication reported that a C16 ADBAC

(benzenemethanaminium, N-hexadecyl-N,N-dimethyl-, chloride, CASRN 122-18-9) was degraded 5% after 10 days using the MITI test. A substance identified as Hyamine 3500-80, which consists of quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides (80.8% ethanol/water solution), was degraded 84% after 28 days at an initial concentration of 5 mg/L and 82.6% after 28 days at an initial concentration of 10 mg/L, as measured by $CO_2$ evolution using an activated sewage sludge and a method similar to OECD TG 301B. Both benzyl-C12-16-alkyldimethyl, chlorides and benzenemethanaminium, N,N-dimethyl-N-octadecyl-, chloride (1:1) were inherently biodegradable using the modified SCAS (OECD TG 302A) test. The SCAS test was initially developed to estimate the removal of surfactants in sewage treatment facilities, and it provides a higher potential for biodegradability than the ready tests because it uses a higher biomass-to-chemical substance ratio and a re-inoculation of sewage inoculum. According to the EPA Registration Eligibility Decision (RED) document for ADBAC, a formulated product was degraded 60% after 13 days as measured by $CO_2$ evolution and the modified Sturm test. ADBAC (40% C12, 50% C14, and 10% C16) did not degrade in a flooded sandy loam incubated at 24–27°C and stored under dark conditions for 30 days. The results of these tests suggest that the substances in this category have varying rates of biodegradation depending upon the circumstances of the test, but that they are not expected to be persistent in the environment at low concentrations and are likely to be highly removed from sewage treatment facilities. Volatilization is considered low since these substances are quaternary ammonium salts. The rate of hydrolysis is expected to be negligible. A bioconcentration study using freshwater fish (bluegill) indicates that bioaccumulation of ADBAC in freshwater fish is not likely to occur. Maximum bioconcentration factors (BCFs) were 33x for edible tissues (muscle, skin), 160x for nonedible tissues (viscera, head, carcass), and 79x for whole fish tissues. The rate of atmospheric photooxidation is moderate; however, this is not a relevant environmental degradation pathway since these substances are not expected to exist in the vapor phase in the atmosphere. The constituents of the alkyl dimethyl benzyl ammonium chloride category are expected to have low persistence (P1) and low bioaccumulation potential (B1).

The environmental fate of the ADBAC category is summarized in Table 2.

**Conclusion:** The alkyl dimethyl benzyl ammonium chloride (ADBAC) category consists of three substances that are used as antimicrobial agents in a variety of industries. Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16) and Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18) are mixtures of alkyldimethylbenzylammonium chlorides in which the alkyl chain length varies from C12 to C18. Benzyldimethyloctadecyl ammonium chloride is not a mixture but a discrete substance with a C18 alkyl chain length. These substances are solids with negligible to low vapor pressure and are dispersible in water. They are expected to possess low mobility in soil. These substances are not expected to be persistent in the environment; however, at high concentrations they may be toxic to microorganisms which will slow the rate of biodegradation. Volatilization is considered low since these substances are quaternary ammonium salts. The rate of hydrolysis is expected to be negligible. The rate of atmospheric photooxidation is moderate; however, this is not a relevant environmental degradation pathway since these substances are not expected to exist in the vapor phase in the atmosphere. The constituents of the alkyl dimethyl benzyl ammonium chloride category are expected to have low persistence (P1) and low bioaccumulation potential (B1).

**Table 2. Environmental Fate Characteristics of the Alkyl Dimethyl Benzyl Ammonium Chloride Category[1]**

| Property | Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16) | Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18) | Benzyldimethyloctadecyl ammonium chloride |
|---|---|---|---|
| CASRN | 68424-85-1 | 68391-01-5 | 122-19-0 |
| Photodegradation Half-life | 2.9 hours (estimated)[2] | 3.1 hours (estimated)[2] | 2.6 hours (estimated)[2] |
| Hydrolysis Half-life | Stable | Stable | Stable |
| Biodegradation | 100% after 7 days (inherently biodegradable, OECD TG 302A);<br>82.6–84% after 28 days (non-standard test);<br>No degradation after 30 days in a sandy loam[3] | 72% after 28 days (readily biodegradable, OECD TG 301B);<br>5% after 28 days (not readily biodegradable, OECD TG 301B);<br>72% after 28 days (readily biodegradable, OECD TG 301B);<br>95% after 28 days (readily biodegradable, OECD TG 301B) | 94% (inherently, biodegradable, OECD TG 302A);<br>0% after 10 days (not readily biodegradable, OECD TG 301C);<br>5% after 10 days (not readily biodegradable, OECD TG 301C)[4,5] |
| Bioaccumulation Factor | BCF = 33–160 (measured in bluegills)[3];<br>BAF = 192.8 (estimated)[2] | BAF = 52.2 (estimated)[2] | BAF = 879.3 (estimated)[2] |
| Log $K_{oc}$ | 6.0 (estimated)[2] | 5.4 (estimated)[2] | 7.0 (estimated)[2] |
| Fugacity (Level III Model)[2] | | | |
| Air (%) | <0.1 | <0.1 | <0.1 |
| Water (%) | 2.6 | 7.2 | 1.8 |
| Soil (%) | 38.6 | 51.9 | 30.6 |
| Sediment (%) | 58.8 | 40.9 | 67.5 |
| Persistence[6] | P1 (low) | P1 (low) | P1 (low) |
| Bioaccumulation[6] | B1 (low) | B1 (low) | B1 (low) |

[1]The Consumer Specialty Products Association for the ADBAC Joint Venture. 2011. Test Plan and Robust Summary for Alkyldimethylbenzylammonium Chloride Category. Available online at http://www.epa.gov/hpv/pubs/summaries/adbac/c16856tc.html as of September 11, 2012.

[2]U.S. EPA. 2012. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.10. U.S. Environmental Protection Agency, Washington, DC, USA. Available online at http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm as of September 11, 2012.

| Table 2. Environmental Fate Characteristics of the Alkyl Dimethyl Benzyl Ammonium Chloride Category[1] | | | |
|---|---|---|---|
| **Property** | **Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16)** | **Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18)** | **Benzyldimethyloctadecyl ammonium chloride** |

[3]U.S. EPA. 2006. Environmental Fate Assessment of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) for the Registration Eligibility Decision Document. EPA-HQ-OPP-2006-0339-0025. Available online at http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2006-0339-0025 as of September 12, 2012.
[4]Van Ginkel CG. 1995. Biodegradability of Cationic Surfactants. In: Biodegradation of Surfactants. Karsa DR; Porter MR, eds. Glasgow, UK: Blackie. pp. 183–203.
[5]Data for benzenemethanaminium, N-hexadecyl-N,N-dimethyl-, chloride, CASRN 122-18-9.
[6]Federal Register. 1999. Category for Persistent, Bioaccumulative, and Toxic New Chemical Substances. *Federal Register* 64, Number 213 (November 4, 1999) pp. 60194–60204. According to the EPA RED for ADBAC and reference 3 in Table 2: The data indicate that ADBAC is hydrolytically and photolytically stable under abiotic and buffered conditions. Aquatic metabolism studies indicate that ADBAC is also stable to microbial degradation. However, a report on the biodegradability of ADBAC concluded that the degree of ADBAC biodegradability is variable and is influenced by the chemical concentration, alkyl chain length, the presence of anionic moieties, and the quantity and characteristics of the microbial population. Accordingly, ADBAC is considered biodegradable under aerobic and anaerobic conditions and, therefore, environmentally acceptable.

## 3. Human Health Hazard

A summary of health effects data submitted for SIDS endpoints is provided in Table 3. The table also indicates where data for tested category members are read-across (RA) to untested members of the category.

### *Acute Oral Toxicity*

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***
Rats (5/dose, mixed sex, strain not specified) were administered a single oral dose of ADBAC C12-16 (purity: 80% in ethanol/water) via gavage at 0.25, 0.32, 0.40, 0.50, 1.0, 2.0, 4.0, 8.0 or 16.0 mL/kg (approximately 200, 256, 320, 400, 800, 1600, 3200, 6400 or 12,800 mg/kg) and observed for 14 days. Animals dosed with ≥ 1600 mg/kg received undiluted test substance, while propylene glycol was used as a vehicle at all other dose levels. No mortality was observed at ≤ 256 mg/kg. The mortality at 320 mg/kg was 20%. All animals died at ≥ 400 mg/kg. Deaths occurred within 24 hours of dosing.
**$LD_{50}$ ~ 344 mg/kg**

***Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18; CASRN 68391-01-5)***
Wistar rats (5/sex/dose) were administered a single oral dose of ADBAC C12-18 (purity: 50% w/w) in water via gavage at 50, 500 or 5000 mg/kg and observed for 14 days. No animals died at 50 mg/kg. The mortality rates at 500 and 5000 mg/kg were 20 and 100%, respectively. All deaths occurred within 2 days of dosing.
**$LD_{50}$ = 850 mg/kg**

### *Acute Dermal Toxicity*

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***
Rabbits (4/sex/dose, strain not specified) were dermally administered ADBAC C12-16 (purity: 80% in ethanol/water) at doses of 3, 4 or 5 mL/kg (approximately 2400, 3200 or 4000 mg/kg) on abraded or intact skin under semi-occlusive conditions for 24 hours and observed for 14 days following dosing. The mortality rates were 12.5%, 75% and 87.5% at 2400, 3200 and 4000 mg/kg, respectively. Deaths occurred within 1 – 12 days.
**$LD_{50}$ ~ 2848 mg/kg**

***Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18; CASRN 68391-01-5)***
New Zealand White rabbits (5/dose, mixed sex) were dermally administered ADBAC C12-18 (purity: 50% w/w) on abraded skin at 500, 1000, 2000, 2520 or 3960 mg/kg under occlusive conditions for 24 hours and observed for 14 days following dosing. The mortality rates were 0%, 20%, 60%, 40% and 40% at 500, 1000, 2000, 2520 and 3960 mg/kg, respectively.
**$LD_{50}$ = 2300 mg/kg**

### *Repeated-Dose Toxicity*

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***
(1) Sprague-Dawley CD rats (males and females, number per dose not specified) were administered ADBAC C12-16 (purity: 79.7 – 80.51% in ethanol/water) in the diet at 0, 100, 500, 1000, 4000 or 8000 ppm (males: approximately 0, 6, 31, 62, 248 or 496 mg/kg-bw/day; females: approximately 0, 8, 38, 77, 308 or 616 mg/kg-bw/day) daily for 95 days (males) or 96 days (females). No animals in the 8000 ppm group survived past day 8 of treatment. Only three male and four female rats survived to terminal sacrifice from the 4000 ppm group. Decreased food consumption and body weights, clinical signs of toxicity, gross necropsy findings (principally ileus consisting of distended fluid and gas-filled viscera) and histopathological effects (related to gastrointestinal changes) were observed for animals in the 4000 and 8000 ppm groups. A slight trend toward decreased food consumption and reduced body weight was observed for males in the 1000 ppm group; the magnitude and statistical significance of these effects were not provided. No treatment-related findings in any in-life, clinical chemistry, hematology, gross pathology, organ weights, or histopathology evaluations were observed in males or females from any other dose group.
**LOAEL (males) ~ 248 mg/kg-bw/day** (based on mortality, decreased body weights, ileus and histopathological findings in the gastrointestinal system)
**LOAEL (females) ~ 308 mg/kg-bw/day** (based on mortality, decreased body weights, ileus and histopathological findings in the gastrointestinal system)
**NOAEL (males) ~ 62 mg/kg-bw/day**
**NOAEL (females) ~ 77 mg/kg-bw/day**

(2) Male and female Sprague-Dawley CD rats (number per dose not specified) were administered ADBAC C12-16 (purity: 81.09% in ethanol/water) in the diet at 0, 300, 1000 or 2000 ppm (males: approximately 0, 13, 44 or 88 mg/kg/day; females: approximately 0, 17, 57 or 116 mg/kg/day) daily for 104 weeks. An increased incidence of loose feces was noted in all males treated with the test substance. Treatment-related effects in body weights and food consumption were seen in both male and female rats in the 2000 ppm group. The mean absolute body weights of the 2000 ppm group for males and female rats were significantly decreased at most measurements periods (p value not provided). No treatment-

related effects were observed in survival, the type or incidence of palpable masses, clinical pathology (including hematology, clinical chemistry, and urinalysis), organ weights, gross and microscopic anatomic pathology (including reproductive histology) or ophthalmology.
**LOAEL (males) ~ 88 mg/kg-bw/day** (based on reduced body weight)
**LOAEL (females) ~ 116 mg/kg-bw/day** (based on reduced body weight)
**NOAEL (males) ~ 44 mg/kg-bw/day**
**NOAEL (females) ~ 57 mg/kg-bw/day**

(3) In a range-finding study, CD-1 mice (males and females, number per dose not specified) were administered ADBAC C12-16 (purity: 79.7 – 80.51% in ethanol/water) in the diet at 0, 100, 500, 1000, 4000 or 8000 ppm (males: approximately 0, 18, 85, 174, 696 or 1392 mg/kg-bw/day; females: approximately 0, 21, 102, 210, 820 or 1680 mg/kg-bw/day) daily for 93 days (males) or 94 days (females). No males in the 4000 ppm group, and no males or females in the 8000 ppm group survived beyond the 11$^{th}$ day of treatment. One female in the 4000 ppm group survived to terminal sacrifice. Clinical signs of toxicity were restricted to the animals that died, and were related to general cachexia and gross necropsy observations of increased amounts of liquid or semisolid material throughout the gastrointestinal tract. Death was attributed to ileus and shock. Treatment with 1000 ppm or less of the test substance in the diet resulted in no overt toxic responses. Minor decreases in body weights, relative to controls, were observed in females in the 1000 ppm group and may have been treatment-related. The magnitude and statistical significance of these effects on body weight were not provided. No changes were observed in any measurements, including food consumption, gross pathology and histopathology, throughout the study in males or females from the 100, 500 or 1000 ppm groups.
**LOAEL (males) ~ 696 mg/kg-bw/day** (based on mortality)
**LOAEL (females) ~ 820 mg/kg-bw/day** (based on mortality)
**NOAEL (males) ~ 174 mg/kg-bw/day**
**NOAEL (females) ~ 210 mg/kg-bw/day**

(4) Male and female CD-1 mice (number per dose not specified) were administered ADBAC C12-16 (purity: 81.09% in ethanol/water) in the diet at 0, 100, 500 or 1500 ppm (males: approximately 0, 15, 73 or 229 mg/kg-bw/day; females: 0, 18, 92 or 289 mg/kg-bw/day) daily for 78 weeks. No treatment-related mortality, clinical signs of toxicity, increases in palpable masses, changes in food consumption or hematological parameters, observations at necropsy, or differences in organ weights were observed. No histopathological findings were observed in any tissues, including reproductive tissues. Treatment-related findings included depressed body weights and body weight gains in the 1500 ppm group throughout the study.
**LOAEL (males) ~ 229 mg/kg-bw/day** (based on reduced body weight and body weight gain)
**LOAEL (females) ~ 289 mg/kg-bw/day** (based on reduced body weight and body weight gain)
**NOAEL (males) ~ 73 mg/kg-bw/day**
**NOAEL (females) ~ 92 mg/kg-bw/day**

(5) Sprague-Dawley CD rats (males and females, number per dose not specified) were administered ADBAC C12-16 (purity: 81.09% in ethanol/water) via the dermal route at 0 (water only), 2, 6 or 20 mg/kg-bw/day under semi-occlusive conditions for 6 hours/day, 5 days/week for 13 weeks. No systemic toxicity was observed, as measured by clinical signs, food consumption, body weight, body weight gain, ophthalmic changes, hematology and clinical chemistry measurements, gross pathology and histopathology. Slight local irritation (hyperkeratosis) was observed for males in all groups (including controls) and for females in all treatment groups.
**LOAEL = Not established**

**NOAEL(systemic) = 20 mg/kg-bw/day** (highest dose tested)

***Reproductive Toxicity***

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***
In a two-generation reproductive toxicity study, Sprague-Dawley CD rats (28/sex/dose) were administered ADBAC C12-16 in the diet at 0, 300, 1000 or 2000 ppm (approximately 0, 22.5, 72.6 or 145.5 mg/kg-bw/day) daily for 10 weeks prior to mating, during mating for three weeks, and through gestation, parturition and lactation. At weaning, 28 $F_1$ weanlings/sex/group were selected to produce the $F_2$ generation. The same exposure regime used for the $F_0$ generation was followed for the $F_1$ generation. $F_0$ females at 2000 ppm displayed reductions in body weight during weeks 5, 6, 9 and 10 of the pre-mating period. Body weight gain was reduced in $F_0$ females at 2000 ppm for one week during the pre-mating period. Food consumption in $F_0$ females at 2000 ppm was reduced for the first four exposure weeks. $F_0$ females showed reduced body weights on day 0 of gestation. Increased body weights and body weight gains were observed during lactation for $F_0$ females at 2000 ppm. $F_1$ males at 2000 ppm exhibited reduced body weight gain in the second week during the pre-mating period and reduced food consumption during 2 of the 10 weeks of the pre-mating period. Food consumption was reduced during days 7 – 11 and 14 – 17 in $F_1$ females at 2000 ppm. No treatment-related mortality was observed in adults. At postmortem, no treatment-related lesions were observed in reproductive tissues at necropsy in $F_0$ and $F_1$ males and females at 2000 ppm. Reduced pup body weights per litter were observed for $F_1$ pups on postnatal day 21 (weaning) and for both $F_1$ and $F_2$ pups on postnatal day 28 (post-weaning) at 2000 ppm. $F_1$ and $F_2$ pup body weight gains were reduced during lactation days 14 – 21 (pre-weaning) and 21 – 28 (post-weaning). No treatment-related mortality and no lesions were observed in pups of either generation.
**LOAEL (parental systemic toxicity) ~ 145.5 mg/kg-bw/day** (based on reduced body weights and body weight gains)
**NOAEL (parental systemic toxicity) ~ 72.6 mg/kg-bw/day**
**LOAEL (reproductive toxicity) ~145.5 mg/kg-bw/day** (based on reduced pup body weights and body weight gains)
**NOAEL (reproductive toxicity) ~ 72.6 mg/kg-bw/day**

***Developmental Toxicity***

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***
(1) Pregnant Sprague-Dawley CD rats (25/dose) were administered ADBAC C12-16 (purity: 81.09% in ethanol/water) via gavage at 0 (control), 10, 30 or 100 mg/kg-day on gestation days 6 – 15. Treatment-related clinical signs included audible respiration at 30 and 100 mg/kg-day and perioral wetness at 100 mg/kg-day. One dam in the 100 mg/kg-day group exhibited dehydration, unkempt appearance, loose feces and urine stains. One dam in the 30 mg/kg-day group exhibited urine stains, gasping, perinasal encrustation and loose feces. No clinical signs were observed at 10 mg/kg-day. Reduced food consumption was observed during gestation days 6 – 9 at 30 and 100 mg/kg-day. There were no treatment-related effects on gestational body weight and weight gain, corrected body weight, gravid uterine weight or pregnancy rate. There were no statistically significant differences between treated and control animals in gestational parameters (including total number of implantations and number of viable and nonviable implants per litter). There were no statistically significant differences between treated and

control fetal body weights per litter and no differences in the incidences of external, visceral and skeletal malformations and variations.
**LOAEL (maternal toxicity) = 30 mg/kg-day** (based on audible respiration)
**NOAEL (maternal toxicity) = 10 mg/kg-day**
**NOAEL (developmental toxicity) = 100 mg/kg-day** (based on no adverse-treatment related effects at the highest dose tested)

(2) Pregnant New Zealand White rabbits (16/dose) were administered ADBAC C12-16 (purity: 81.09% in ethanol/water) via gavage at 0 (control), 1, 3, or 9 mg/kg-day on gestation days 6 – 18. Treatment-related effects observed in dams were limited to hypoactivity and labored or audible respiration at 9 mg/kg-day. No effects were observed on maternal mortality, food consumption or body weights. No treatment-related differences were observed on developmental parameters including mean fetal body weight and incidences of external, visceral and skeletal malformations and variations.
**LOAEL (maternal toxicity) = 9 mg/kg-day** (based on labored breathing and hypoactivity)
**NOAEL (maternal toxicity) = 3 mg/kg-day**
**NOAEL (developmental toxicity) = 9 mg/kg-day** (based on no adverse-treatment related effects at the highest dose tested)

***Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18; CASRN 68391-01-5)***
Pregnant Wistar rats (22 – 37/dose) were administered ADBAC C12-18 (purity: 50%) via gavage at 0 (control), 5, 15 or 50 mg/kg-day on gestation days 6 – 15. Three mortalities occurred at 50 mg/kg-day. No treatment-related effects on gestation or maternal body weight were observed. There were no treatment-related effects on fetal body weight and no treatment-related skeletal or visceral variations or malformations.
**LOAEL (maternal toxicity) = 50 mg/kg-day** (based on mortality)
**NOAEL (maternal toxicity) = 15 mg/kg-day**
**NOAEL (developmental toxicity) = 50 mg/kg-day** (based on no adverse treatment-related effects at the highest dose tested)

***Benzyldimethyloctadecyl ammonium chloride (CASRN 122-19-0)***
Pregnant CFY Sprague-Dawley rats (10-20/dose) were administered 0.5 mL of Benzyldimethyloctadecyl ammonium chloride (purity not specified) in distilled water via the dermal route at concentrations of 0 (control), 1.6, 3.3 or 6.6% (w/v) (approximately 0, 8.25, 16.5 or 33 mg/kg-bw/day) under open conditions once per day on gestation days 6 – 15. No treatment-related systemic effects were observed in dams on clinical condition, body weight and food and water consumption. A dose-related increase was observed in the degree of erythema and edema. No treatment-related effects were observed on developmental parameters including litter size, post-implantation loss, litter and mean fetal weights and embryonic and fetal development. There were no treatment-related effects on the incidence of malformations or anomalies observed in fetuses.
**NOAEL (maternal toxicity) ~ 33 mg/kg-bw/day** (based on no adverse treatment-related effects at the highest dose tested)
**NOAEL (developmental toxicity) ~ 33 mg/kg-bw/day** (based on no adverse treatment-related effects at the highest dose tested)

***Genetic Toxicity – Gene Mutations***

***In vitro***

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***
(1) *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were exposed to ADBAC C12-16 in dimethyl sulfoxide (DMSO) at 0.15 – 5000 µg/plate with and without metabolic activation. Positive, negative, and vehicle (DMSO) controls were included. Cytotoxicity was observed at concentrations ≥ 15 µg/plate with and without metabolic activation. . The response of the controls was not specified.
**ADBAC C12-16 was not mutagenic in this assay.**

(2) Chinese hamster ovary (CHO) cells were exposed to ADBAC C12-16 (purity: 80% in ethanol/water) at 1.0 – 20.0 µg/mL without metabolic activation and at 1.0 – 40.0 µg/mL with metabolic activation. Positive controls were used, but the results of controls were not specified. Cytotoxicity was observed at ≥ 20.0 µg/mL without activation and at ≥ 50.0 µg/mL with activation..
**ADBAC C12-16 was not mutagenic in this assay.**

***Benzyldimethyloctadecyl ammonium chloride (CASRN 122-19-0)***
In a study conducted by the NTP, *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 were exposed to benzyldimethyloctadecyl ammonium chloride at concentrations up to 3333 µg/plate with and without metabolic activation. Precipitation was observed at ≥ 1666 µg/plate. Positive and negative controls were included and responded appropriately. This information was obtained from NTP study No. 114305 at: http://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=114305
**Benzyldimethyloctadecyl ammonium chloride was not mutagenic in this assay.**

***Genetic Toxicity – Chromosomal Aberrations***

***In vivo***

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***
In a micronucleus assay, NMRI mice (15/sex) were administered ADBAC C12-16 (purity: 80.2% in ethanol/water) via gavage at 400 mg/kg-bw. Two additional groups (5/sex/group) served as the positive and negative controls. Animals were sacrificed at 24, 48 or 72 hours after treatment. The controls yielded appropriate results. One mouse in the 72-hour test substance group was found dead on day 3 after treatment. The PCE count was reduced in the animals receiving the test substance and in the positive control group. The number of micronuclei in polychromatic erythrocytes was similar in the negative control and the test group.
**ADBAC C12-16 did not induce micronuclei in polychromatic erythrocytes in this assay.**

***Genetic Toxicity – Other***

***In vitro***

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***
(1) In a sister chromatid exchange assay, human lymphocytes were exposed to ADBAC C12-16 at 2 – 24 µg/mL with metabolic activation and at 1 – 24 µg/mL without metabolic activation. Positive and negative controls were included. Eagle's minimal essential medium with HEPES buffer served as the

vehicle control. Cytotoxicity was observed at 20 µg/mL with activation and at 16 µg/mL without activation. The response of the controls was not specified.
**ADBAC C12-16 did not induce sister chromatid exchanges in this assay.**

(2) In an unscheduled DNA synthesis assay, male rat primary hepatocytes were treated with ADBAC C12-16 (purity: 80% in ethanol/water) at 0.053 – 10.6 µg/mL. Positive and negative controls were used. Cytotoxicity was observed at concentrations ≥ 8.50 µg/mL. Concentrations below 8.50 µg/mL were not cytotoxic and resembled the solvent controls in cellular morphology. ADBAC C12-16 did not induce unscheduled DNA synthesis at the analyzed concentrations of 0.319 – 6.37 µg/mL. The response of the controls was not provided.
**ADBAC C12-16 did not induce unscheduled DNA synthesis in this assay.**

(3) In an unscheduled DNA synthesis assay, female rat primary hepatocytes were treated with ADBAC C12-16 (purity: 80% in ethanol/water) at 0.538 – 11.8 µg/mL. Positive and negative controls were used. Cytotoxicity was observed at > 4.31 µg/mL. ADBAC C12-16 did not induce unscheduled DNA synthesis at the analyzed concentrations of 0.538 – 6.46 µg/mL. The response of the controls was not provided.
**ADBAC C12-16 did not induce unscheduled DNA synthesis in this assay.**

**Conclusion:** The acute oral toxicity in rats is moderate for ADBAC C12-16 and low for ADBAC C12-18 and the acute dermal toxicity of ADBAC C12-16 and ADBAC C12-18 is low in rabbits. In a 13-week feeding study with ADBAC C12-16 in rats, effects included mortality, decreased food consumption and body weights, ileus and histopathological effects in the gastrointestinal system at ~ 248 and ~ 308 mg/kg-bw/day in males and females, respectively; the NOAELs for systemic toxicity are ~ 62 and ~ 77 mg/kg-bw/day in males and females, respectively. In a 104-week feeding study with ADBAC C12-16 in rats, reduced body weight and food consumption were observed at ~ 88 and ~ 116 mg/kg-bw/day in males and females, respectively; the NOAELs for systemic toxicity are ~ 44 and ~ 57 mg/kg-bw/day in males and females, respectively. In a 13-week feeding study with ADBAC C12-16 in mice, mortality was observed at ~ 696 and ~ 820 mg/kg-bw/day in males and females, respectively; the NOAELs for systemic toxicity are ~ 174 and ~ 210 mg/kg-bw/day in males and females, respectively. In a 78-week feeding study with ADBAC C12-16 in mice, reduced body weight and body weight gain were observed at ~ 229 and ~ 289 mg/kg-bw/day in males and females, respectively; the NOAELs for systemic toxicity are ~ 73 and ~ 92 mg/kg-bw/day in males and females, respectively. In a 13-week dermal study with ADBAC C12-16 in rats no treatment-related effects were observed; the NOAEL for systemic toxicity is 20 mg/kg-bw/day (highest dose tested). In a two-generation dietary reproductive toxicity study with ADBAC C12-16 in rats, reduced body weights and body weight gains were observed in both adults and pups at ~ 145.5 mg/kg-bw/day; the NOAEL for parental systemic and reproductive toxicity is ~ 72.6 mg/kg-bw/day. In a prenatal oral gavage developmental toxicity study with CASRN ADBAC C12-16 in rats, clinical signs of toxicity included audible respiration in dams at 30 mg/kg-day; the NOAEL for maternal toxicity is 10 mg/kg-day. No treatment-related effects were observed on developmental parameters at the highest dose tested; the NOAEL for developmental toxicity is 100 mg/kg-day. In a prenatal oral gavage developmental toxicity study with ADBAC C12-16 in rabbits, clinical signs of toxicity included hypoactivity and labored or audible respiration at 9 mg/kg-day; the NOAEL for maternal toxicity is 3 mg/kg-day. No treatment-related developmental effects were observed; the NOAEL for developmental toxicity is 9 mg/kg-day. In a prenatal oral gavage developmental toxicity study with ADBAC C12-18 in rats, mortality was observed in dams at 50 mg/kg-day; the NOAEL for maternal toxicity is 15 mg/kg-day. No treatment-related developmental effects

were observed; the NOAEL for developmental toxicity is 50 mg/kg-day (highest dose tested). In a prenatal dermal developmental toxicity study with benzyldimethyl-octadecyl ammonium chloride in rats, no maternal or developmental effects were observed at the highest dose tested; the NOAEL for maternal and developmental toxicity is ~ 33 mg/kg-bw/day. ADBAC C12-16 and benzyldimethyl-octadecyl ammonium chloride did not induce gene mutations in bacteria *in vitro* and ADBAC C12-16 did not induce gene mutations in mammalian cells or unscheduled DNA synthesis in rat hepatocytes *in vitro*. ADBAC C12-16 did not induce sister chromatid exchanges in human lymphocytes *in vitro* or micronuclei in polychromatic erythrocytes in mice *in vivo*.

**Table 3. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program - Human Health Data**

| **Endpoint** | **Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; 68424-85-1)** | **Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18; 68391-01-5)** | **Benzyldimethyl-octadecyl ammonium chloride (122-19-0)** |
|---|---|---|---|
| **Acute Toxicity Oral $LD_{50}$ (mg/kg)** | **~ 344** | **850** | No Data<br>~ 344<br>(RA) |
| **Acute Toxicity Dermal $LD_{50}$ (mg/kg)** | **~ 2848** | **2300** | No Data<br>2300<br>(RA) |
| **Repeated-Dose Toxicity NOAEL/LOAEL Diet (mg/kg-bw/day)** | **(rat; 13-wk)**<br>**NOAEL ~ 62(m)/77(f)**<br>**LOAEL ~ 248(m)/308(f)**<br><br>**(mouse; 13-wk)**<br>**NOAEL ~ 174(m)/210(f)**<br>**LOAEL ~ 696(m)/820(f)**<br><br>**(rat; 104-wk)**<br>**NOAEL ~ 44(m)/57(f)**<br>**LOAEL ~ 88(m)/116(f)**<br><br>**(mouse; 78-wk)**<br>**NOAEL ~ 73(m)/92(f)**<br>**LOAEL ~ 229(m)/289(f)** | No Data<br>(rat; 13-wk)<br>NOAEL ~ 62(m)/77(f)<br>LOAEL ~ 248(m)/308(f)<br><br>(mouse; 13-wk)<br>NOAEL ~ 174(m)/210(f)<br>LOAEL ~ 696(m)/820(f)<br><br>(rat; 104-wk)<br>NOAEL ~ 44(m)/57(f)<br>LOAEL ~ 88(m)/116(f)<br><br>(mouse; 78-wk)<br>NOAEL ~ 73(m)/92(f)<br>LOAEL ~ 229(m)/289(f)<br>(RA) | No Data<br>(rat; 13-wk)<br>NOAEL ~ 62(m)/77(f)<br>LOAEL ~ 248(m)/308(f)<br><br>(mouse; 13-wk)<br>NOAEL ~ 174(m)/210(f)<br>LOAEL ~ 696(m)/820(f)<br><br>(rat; 104-wk)<br>NOAEL ~ 44(m)/57(f)<br>LOAEL ~ 88(m)/116(f)<br><br>(mouse; 78-wk)<br>NOAEL ~ 73(m)/92(f)<br>LOAEL ~ 229(m)/289(f)<br>(RA) |
| **Repeated-Dose Toxicity NOAEL/LOAEL Dermal (mg/kg-bw/day)** | **(rat)**<br>**NOAEL = 20**<br>**(highest dose tested)** | No Data<br>(rat)<br>NOAEL = 20<br>(RA) | No Data<br>(rat)<br>NOAEL = 20<br>(RA) |
| **Reproductive Toxicity NOAEL/LOAEL Diet (mg/kg-bw/day) Reproductive Toxicity** | **(rat; 2-gen)**<br>**NOAEL ~ 72.6**<br>**LOAEL ~ 145.5** | No Data<br>NOAEL ~ 72.6<br>LOAEL ~ 145.5<br>(RA) | No Data<br>NOAEL ~ 72.6<br>LOAEL ~ 145.5<br>(RA) |

| Table 3. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program - Human Health Data | | | |
|---|---|---|---|
| **Endpoint** | **Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; 68424-85-1)** | **Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18; 68391-01-5)** | **Benzyldimethyl-octadecyl ammonium chloride (122-19-0)** |
| **Developmental Toxicity NOAEL/LOAEL Oral (mg/kg-day)** | | | No Data |
| **Maternal Toxicity** | **(rat) NOAEL = 10 LOAEL = 30** | **(rat) NOAEL = 15 LOAEL = 50** | (rat) NOAEL = 10 LOAEL = 30 |
| **Developmental Toxicity** | **NOAEL = 100 (highest dose tested)** | **NOAEL = 50 (highest dose tested)** | NOAEL = 100 |
| **Maternal Toxicity** | **(rabbit) NOAEL = 3 LOAEL = 9** | No Data (rabbit) NOAEL = 3 LOAEL = 9 | (rabbit) NOAEL = 3 LOAEL = 9 |
| **Developmental Toxicity** | **NOAEL = 9 (highest dose tested)** | NOAEL = 9 (RA) | NOAEL = 9 (RA) |
| **Developmental Toxicity NOAEL/LOAEL Dermal (mg/kg-bw/day) Maternal/ Developmental Toxicity** | No Data (rat) NOAEL ~ 33 (RA) | No Data (rat) NOAEL ~ 33 (RA) | **(rat) NOAEL ~ 33 (highest dose tested)** |
| **Genetic Toxicity - Gene Mutations *In vitro*** | **Negative** | No Data Negative (RA) | **Negative** |
| **Genetic Toxicity – Chromosomal Aberrations *In vivo*** | **Negative** | No Data Negative (RA) | No Data Negative (RA) |
| **Genetic Toxicity – Other SCE** | **Negative** | – | – |
| **Unscheduled DNA Synthesis** | **Negative** | | |

**Measured data in BOLD**; (RA) = read across; (m) = male; (f) = female; − indicates endpoint not addressed for this chemical

## 4. Hazard to the Environment

A summary of aquatic toxicity data submitted for SIDS endpoints is provided in Table 4. The table also indicates where data for tested category members are read-across (RA) to untested members of the category.

### *Acute Toxicity to Fish*

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***
(1) Sheepshead minnows (*Cyprinodon variegatus*; 20/concentration) were exposed to ADBAC C12-16 at nominal concentrations of 0 (control), 0.39, 0.65, 1.1, 1.8 or 3.0 mg/L under static renewal conditions for 96 hours. The corresponding mean measured concentrations were 0, 0.42, 0.68, 1.1, 1.8 and 2.8 mg/L, respectively. Mortality was 100% at 2.8, 1.8 and 1.1 mg/L and occurred within 2.5, 24 and 72 hours of treatment, respectively. Signs of abnormal behavior included surfacing of fish for unusually long periods of time. No deaths or signs of toxicity were observed at ≤ 0.68 mg/L.
**96-h $LC_{50}$ = 0.86 mg/L**

(2) Fathead minnows (*Pimephales promelas*; 20/concentration) were exposed to ADBAC C12-16 at nominal concentrations of 0 (control), 0.10, 0.18, 0.32, 0.56 or 1.0 mg/L under static renewal conditions for 96 hours. The corresponding mean measured concentrations were 0, 0.096, 0.18, 0.31, 0.57 and 1.0 mg/L, respectively. The mortality rates were 5, 20, 30, 100 and 100% at 0.096, 0.18, 0.31, 0.57 and 1.0 mg/L, respectively, after 96 hours. At 0.057 and 1.0 mg/L, all fish were dead within 24 hours of test initiation. No sublethal effects were observed in fish treated with the test substance.
**96-h $LC_{50}$ = 0.28 mg/L**

(3) Fathead minnows (*Pimephales promelas*; 20/concentration) were exposed to ADBAC C12-16 at nominal concentrations of 0 (dilution water control), 0 (humic acid control), 0.32, 0.56, 1.0, 1.8 or 3.2 mg/L under static renewal conditions for 96 hours. The corresponding mean measured concentrations were 0, 0, 0.30, 0.53, 0.98, 1.8 and 3.2 mg/L, respectively. At 1.8 and 3.20 mg/L, all fish were dead within 24 hours of test initiation. The mortality rate was 10% at 0.98 mg/L after 96 hours. No deaths were observed at ≤ 0.53 mg/L. No sublethal effects were noted.
**96-h $LC_{50}$ = 0.77 mg/L**

(4) Bluegill sunfish (*Lepomis macrochirus*; 20/concentration) were exposed to ADBAC C12-16 at nominal concentrations of 0 (control), 0.180, 0.320, 0.490, 0.560 or 0.750 mg/L under static renewal conditions for 96 hours. The corresponding mean measured concentrations were 0, 0.1973, 0.3171, 0.4555, 0.515 and 0.638 mg/L, respectively. Immobilization and erratic swimming were noted for several fish at 0.515 mg/L on day 1. The mortality rates were 50 and 100% at 0.515 and 0.638 mg/L, respectively. All deaths occurred within 48 hours of treatment. No deaths were observed at ≤ 0.4555 mg/L.
**96-h $LC_{50}$ = 0.515 mg/L**

(5) Rainbow trout (*Oncorhynchus mykiss*; 20/concentration) were exposed to ADBAC C12-16 at nominal concentrations of 0 (control), 0.75, 1.00, 1.25, 1.50 or 1.75 mg/L under static renewal conditions for 96 hours. The corresponding mean measured concentrations were 0, 0.619, 0.864, 1.029, 1.204 and 1.354 mg/L, respectively. Three hours after test initiation, three fish at 1.354 mg/L were swimming erratically on the water surface. All fish at 1.204 and 1.354 mg/L died within 72 hours of treatment. The mortality rates at 0.864 and 1.029 were 40 and 60%, respectively, after 96 hours. No deaths were seen at 0.619 mg/L.
**96-h $LC_{50}$ = 0.93 mg/L**

***Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18; CASRN 68391-01-5)***
Fathead minnows (*Pimephales promelas*; 20/concentration) were exposed to ADBAC C12-18 at nominal concentrations of 0 (dilution water control), 0 (humic acid control), 0.32, 0.56, 1.0, 1.8 or 3.2

mg/L under static renewal conditions for 96 hours. The corresponding mean measured concentrations were 0, 0, 0.30, 0.54, 0.99, 1.8 and 3.2 mg/L, respectively. The mortality rates were 95 and 100% at 1.8 and 3.2 mg/L, respectively, after 96 hours. All deaths occurred within 24 hours of treatment. No deaths were observed at ≤ 0.99 mg/L. No sublethal effects were observed at any concentration.
**96-h $LC_{50}$ = 1.4 mg/L**

***Acute Toxicity to Aquatic Invertebrates***

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***
(1) *Daphnia magna* (20/concentration) were exposed to ADBAC C12-16 at nominal concentrations of 0 (control), 0.01, 0.018, 0.027, 0.032 or 0.057 mg/L under static renewal conditions for 48 hours. Corresponding mean measured concentrations were 0, 0.0060, 0.0149, 0.0227, 0.0272 and 0.0516 mg/L, respectively. Mortality was 100% at 0.0227, 0.0272 and 0.0516 mg/L within 48, 24 and 24 hours of treatment, respectively. The mortality rates at 0, 0.0060 and 0.0149 mg/L were 10, 53 and 95% after 48 hours.
**48-h $EC_{50}$ = 0.0058 mg/L**

(2) *Daphnia magna* (20/concentration) were exposed to ADBAC C12-16 (purity: 51.0%) at nominal concentrations of 0 (control), 0.007, 0.012, 0.019, 0.031 or 0.052 mg active ingredient (a.i.)/L under static conditions for 48 hours. Analytical measurements of test substance concentrations were not conducted. Immobilization was 100% at 0.031 and 0.052 mg/L within 24 hours of treatment. The mortality rates at 0.012 and 0.019 mg/L were 5 and 85%, respectively, after 48 hours. No deaths were observed at ≤ 0.007 mg/L.
**48-h $EC_{50}$ = 0.016 mg/L**

(3) Eastern oyster embryo larvae (*Crassostrea virginica*) were exposed to ADBAC C12-16 at nominal concentrations of 0 (control), 0.0181, 0.0302, 0.0504, 0.084 or 0.14 mg/L under static conditions for 48 hours. Corresponding mean measured concentrations were 0, 0.025, 0.0408, 0.0586, 0.0897 and 0.145 mg/L, respectively. Embryo survival decreased in a concentration-dependent manner from 0.0408 to 0.145 mg/L. At 0.145 mg/L, mortality was > 95%. Development of embryos appeared to be unaffected at 0.025 mg/L. The percentage of abnormally developed larvae increased from 0.0408 to 0.145 mg/L in a concentration-dependent manner. The 48-hour $EC_{50}$ and $LC_{50}$ were determined to be 0.0476 and 0.0552 mg/L, respectively.
**48-h $EC_{50}$ = 0.0476 mg/L**

(4) Saltwater mysids (*Mysidopsis bahia*; 20/concentration) were exposed to ADBAC C12-16 at nominal concentrations of 0 (control), 0.029, 0.048, 0.080, 0.13, 0.22 or 0.37 mg/L under static renewal conditions for 96 hours. Corresponding mean measured concentrations were 0, 0.030, 0.047, 0.081, 0.13, 0.22 and 0.35 mg/L, respectively. Mortality was 100% at 0.13, 0.22 and 0.35 mg/L within 72, 72 and 48 hours of treatment, respectively. The mortality rate at 0.081 mg/L was 25% after 96 hours. Abnormal behavior included moribundity at ≥ 0.13 mg/L and lethargy at 0.35 mg/L. Neither mortality nor abnormal behavior was observed at ≤ 0.047 mg/L. The 48- and 96-hour $LC_{50}$s were determined to be 0.14 and 0.092 mg/L, respectively.
**48-h $LC_{50}$ = 0.14 mg/L**

### *Toxicity to Aquatic Plants*

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***

(1) Green algae (*Pseudokirchneriella subcapitata*) were exposed to ADBAC C12-16 (purity: 49-51% w/w in aqueous solution) at nominal concentrations of 0 (control), 0.0037, 0.013, 0.024, 0.043, 0.136 or 0.421 mg/L for 72 hours. Corresponding mean measured concentrations were < LOQ (limit of quantification), 0.0012, 0.0051, 0.011, 0.022, 0.098 and 0.382 mg/L, respectively. Detailed results were not provided.

**72-h $EC_{50}$ (biomass) = 0.014 mg/L**
**72-h $EC_{50}$ (growth rate) = 0.049 mg/L**

(2) Saltwater diatoms (*Skeletonema costatum*) were exposed to ADBAC C12-16 (purity: 80.9% w/w in ethanol/water) at nominal concentrations of 0 (control), 0.0085, 0.017, 0.034, 0.068, 0.14 or 0.30 mg a.i./L under static conditions for 96 hours. Corresponding mean measured concentrations were < LOQ, 0.0075, 0.016, 0.035, 0.066, 0.16 and 0.51 mg/L, respectively. At the end of the 96-hour exposure period, no viable algal cells remained at 0.16 and 0.51 mg/L. No effects were observed at ≤ 0.035 mg/L. The 96-hr $E_bC_{50}$ and $E_rC_{50}$ were 0.058 and 0.089 mg/l, respectively.

**72-h $EC_{50}$ (biomass) = 0.058 mg/L**
**72-h $EC_{50}$ (growth rate) = 0.078 mg/L**

### *Chronic Toxicity to Fish*

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***

Fathead minnows (*Pimephales promelas*) embryos were exposed to ADBAC C12-16 at nominal concentrations of 0 (control), 0.027, 0.074, 0.135, 0.180, 0.270 or 0.490 mg/L under static renewal conditions for 34 days (28 days post-hatching). Corresponding mean measured concentrations were 0, 0.0322, 0.0759, 0.1342, 0.1868, 0.2732 and 0.4887 mg/L, respectively. Hatching success was reduced only at 0.4887 mg/L. Significant mortality was observed at concentrations ≥ 0.0759 mg/L (p value not provided). No reduction in growth was observed at any of the test concentrations.

**28-d $LC_{50}$ = 0.094 mg/L**

### *Chronic Toxicity to Aquatic Invertebrates*

***Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; CASRN 68424-85-1)***

Neonate saltwater mysids (*Mysidopsis bahia*) were exposed to ADBAC C12-16 in dimethylformamide at nominal concentrations of 0 (negative control), 0 (solvent control), 0.005, 0.01, 0.02, 0.04 or 0.08 mg a.i./L under flow-through conditions for 28 days. Corresponding mean measured concentrations were < LOQ, < LOQ, 0.004, 0.008, 0.016, 0.031 and 0.059 mg/L, respectively. The LOEC for survival was 0.059 mg/L, at which juvenile and adult survival rates were 67 and 81%, respectively. Significant reductions in reproduction were observed at concentrations ≥ 0.016 mg/L ($p < 0.05$). At 0.059 mg/L, the rate of reproduction was reduced by 50%, compared to the pooled control value. No effects on body length and dry weight were observed.

**28-d $EC_{50}$ (reproduction) ~ 0.059 mg/L**

**Conclusion:** The acute toxicity to fish 96-h $LC_{50}$ is 0.28 – 0.93 mg/L for ADBAC C12-16 and 1.4 mg/L for ADBAC C12-18. For ADBAC C12-16, the acute toxicity to aquatic invertebrates 48-h $EC_{50}$ is 0.0058 – 0.14 mg/L and the toxicity to aquatic plants 72-h $EC_{50}$ is 0.014 – 0.058 mg/L for biomass and 0.049 – 0.078 mg/L for growth rate. For ADBAC C12-16, the chronic 28-d $LC_{50}$ value for fish is 0.094 mg/L and the chronic 28-d $EC_{50}$ (reproduction) value for aquatic invertebrates is ~ 0.059 mg/L.

**Table 4. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program - Aquatic Toxicity Data**

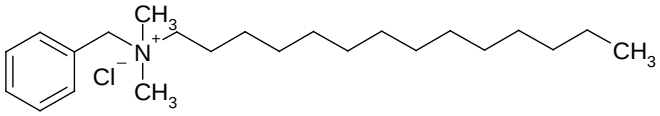
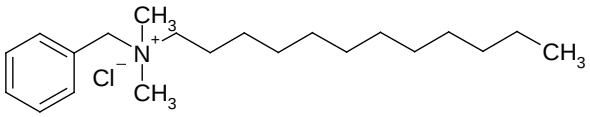
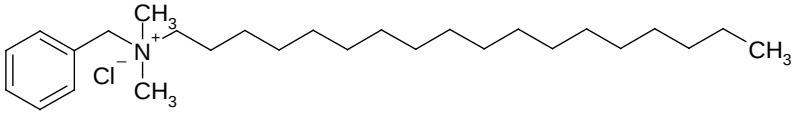
| Endpoint | Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC C12-16; 68424-85-1) | Alkyl (C12-18) dimethylbenzyl ammonium chloride (ADBAC C12-18; 68391-01-5) | Benzyldimethyl-octadecyl ammonium chloride (122-19-0) |
|---|---|---|---|
| **Fish**<br>**96-h $LC_{50}$ (mg/L)** | **0.28 – 0.93** | **1.4** | No Data<br>0.28 – 1.4<br>(RA) |
| **Aquatic Invertebrates**<br>**48-h $EC_{50}$ (mg/L)** | **0.0058 – 0.14** | No Data<br>0.0058 – 0.14<br>(RA) | No Data<br>0.0058 – 0.14<br>(RA) |
| **Aquatic Plants**<br>**72-h $EC_{50}$ (mg/L)**<br>**Biomass**<br>**Growth rate** | <br><br>**0.014 – 0.058**<br>**0.049 – 0.078** | <br>No Data<br>0.014 – 0.058<br>0.049 – 0.078<br>(RA) | <br>No Data<br>0.014 – 0.058<br>0.049 – 0.078<br>(RA) |
| **Chronic Toxicity to Fish**<br>**28-d $LC_{50}$ (mg/L)** | **0.094** | No Data<br>0.094<br>(RA) | No Data<br>0.094<br>(RA) |
| **Chronic Toxicity to Invertebrates**<br>**28-d $EC_{50}$ (mg/L)**<br>**Reproduction** | **~ 0.059** | No Data<br>~ 0.059<br>(RA) | No Data<br>~ 0.059<br>(RA) |

**Bold = measured data (i.e., derived from testing)**; (RA) = read across

## 5. References

U.S. Environmental Protection Agency (2010) Non-confidential 2006 IUR Records by Chemical, including Manufacturing, Processing and Use Information. Inventory Update Reporting (IUR); Version 6: Updated May 12, 2010. Available online at http://www.epa.gov/cdr/tools/previouslycollected.html

# APPENDIX

| Sponsored Chemical | | |
|---|---|---|
| **Chemical Name** | **CASRN** | **Structures[1,2]** |
| Quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides | 68424-85-1 |  <br>SMILES: CCCCCCCCCCCCCC[N+](C)(C)Cc1ccccc1.[Cl-] |
| Quaternary ammonium compounds, benzyl-C12-18-alkyldimethyl, chlorides | 68391-01-5 |  <br>SMILES: CCCCCCCCCCCCC[N+](C)(C)Cc1ccccc1.[Cl-] |
| Benzenemethanaminium, N,N-dimethyl-N-octadecyl-, chloride (1:1) | 122-19-0 |  <br>SMILES: CCCCCCCCCCCCCCCCCC[N+](C)(C)Cc1ccccc1.[Cl-] |

[1]Structures drawn were based on the most representative chain length distribution of CASRN 68424-85-1: C12 (40%), C14 (50%), C16 (10%).

[2]Structures drawn were based on the most representative chain length distribution of CASRN 68391-01-5: C12 (57%), C14 (18%), C16 (7%), C18 (18%).