Risk Characterization for Carcinogens that have a Mutagenic Mode of Action

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Executive Summary

In March 2005 the United States Environmental Protection Agency (USEPA) published the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposures to Carcinogens* ("Supplemental Guidance") (USEPA, 2005a) to provide additional focus on childhood exposures to carcinogens, as recommended in the *Guidelines for Carcinogen Risk Assessment* (USEPA, 2005b). The Supplemental Guidance document evaluated cancer risks from early-life exposure and compared them to cancer risks associated with exposures occurring later in life. This evaluation was done to determine if additional safety factors should be used when childhood cancer risks are quantitatively evaluated. Although the Supplemental Guidance evaluated childhood cancer risk associated with both chemical and ionizing radiation exposures, this issue paper only addresses the findings associated with chemical exposures.

To have a mutagenic mode of action (MOA) the carcinogen or a metabolite of the carcinogen must be DNA reactive and/or have the ability to bind to DNA. The Supplemental Guidance a states that, "For a mutagenic MOA for cancer, mutagenicity is an obligatory early action, i.e., generally a very early key event for the MOA, of the chemical (or its metabolite). This is contrasted with other MOAs wherein mutations are acquired subsequent to other key events (e.g., cytotoxicity, regenerative proliferation)."

The Supplemental Guidance recommends that *in some cases*, when carcinogens have a mutagenic MOA, it may be appropriate to apply a default safety factor called an age-dependent adjustment factor (ADAF) to risk calculations when evaluating cancer risk associated with exposure for children ages 0 to 16 years. For children ages 0 to 2 years the default ADAF is 10; for children ages 2 to 16 years the default ADAF is 3. The Supplemental Guidance states that these default ADAFs should be used for chemicals that are carcinogenic via a mutagenic MOA if chemical-specific data to evaluate differences between adults and juveniles are not available.

This paper does not discuss how to determine if a carcinogenic chemical has a mutagenic MOA. The main focus of this paper is on which chemicals USEPA considers carcinogenic with a mutagenic MOA, how to calculate the cancer risk for these chemicals in a HHRA, and the uncertainties associated with the evaluation of carcinogens with a mutagenic MOA. This paper is intended as a supplement to the *U.S. Navy Human Health Risk Assessment Guidance.*

In order to determine a mutagenic MOA the strengths and limitations of specific studies used must be evaluated, especially for cases where there are conflicting scientific opinions on whether a mutagenic MOA determination is relevant. The determination of a chemical's carcinogenic MOA should be made by experienced toxicologists. For additional information on this process, or to obtain an up-to-date list of carcinogens that USEPA has determined as having a mutagenic MOA, contact the Navy and Marine Corps Public Health Center. Detailed information on how the USEPA evaluates chemicals to determine their MOA can be found in the following resources:

- "Determining a Mutagenic Mode of Action under EPA's 2005 Cancer Guidelines and Supplemental Guidance," Tri-Service Environmental Work Group presentation on 29 August 2007, Portsmouth, Virginia by Resha M. Putzrath, PhD., DABT, Health Science Coordinator, U.S. EPA Office of the Science Advisor.

- *Framework for Determining a Mutagenic Mode of Action for Carcinogenicity: Using EPA's 2005 Cancer Guidelines and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (External Peer Review Draft). U.S. Environmental Protection Agency, September 2007. EPA 120/R-07/002-A.

- *Genetic Toxicology and Cancer Risk Assessment*. edited by Wai Nang Choy, Published by Taylor & Francis Group, LLC, 2001.

- The International Programme on Chemical Safety (IPSC) conceptual framework for evaluating a mode of action for chemical carcinogenesis, Sonich-Mullin C, et al. Regul. Toxicol. Pharmacol, 2001 Oct; 34(2):146-52.

Use of default ADAFs for these chemicals has been adopted in some USEPA Regions, but remains controversial within other scientific communities. USEPA Regions III and VI have incorporated ADAFs into their calculation of risk-based screening concentrations for chemicals with a mutagenic MOA (USEPA 2007a, 2007b). Some key uncertainties involved in using the default ADAFs include the following:

1. Limited studies are available to assess early-life susceptibility to mutagens. Repeated dosing or lifetime exposure studies are needed to assess early life susceptibility, and currently these studies only exist for six carcinogens with a mutagenic MOA.

2. The ADAFs were derived from studies where exposures were much higher than those typically observed in the environment. The mutagenic potential of a chemical may be much less, and may be overcome by DNA repair mechanisms at typical exposure levels.

The USEPA Scientific Advisory Board (SAB) is currently completing a preliminary review of USEPA's draft assessment of ethylene oxide (CAS Number: 75-21-8), which is the first assessment where ADAFs have been incorporated (USEPA, 2006a). Results of this review will likely have an impact on the application of ADAFs to other mutagenic chemicals.

Key Issues and Concepts

☑ Children are assumed to be at increased risk for tumor development following exposure to mutagens due to their rapid growth, fueled by rapid cell replication. It is thought that a child's DNA repair mechanisms may not be able to keep up with the rapid cell replication.

☑ Repeated dosing or lifetime exposure studies are needed to assess early life susceptibility, and currently these studies only exist for a limited number of chemicals.

☑ USEPA Supplemental Guidance recommends that default ADAFs be applied to risk calculations when evaluating cancer risk associated with exposure to mutagens for children ages 0 to 16 years. For children ages 0 to 2 years the default ADAF is 10; for children ages 2 to 16 years the default ADAF is 3.

☑ USEPA Regions III (USEPA, 2007a) and VI (USEPA, 2007b) have incorporated default ADAFs into their calculation of residential risk-based screening concentrations for chemicals that USEPA has determined are carcinogenic via a mutagenic MOA.

☑ Guidance for identifying the mutagenic MOA is still under development by USEPA (2007c).

☑ Results of the SAB's review of the draft assessment of ethylene oxide will likely have an impact on the application of the ADAFs to other chemicals with a mutagenic MOA.

1.0 Introduction

In the *Guidelines for Carcinogen Risk Assessment* (USEPA, 2005b), the United States Environmental Protection Agency (USEPA) recognized that variation exists among people in their susceptibility to carcinogens. One subgroup with potentially increased susceptibility is children, who may be at increased risk due to:

- Their rapid growth and development after birth,

- Their immature metabolic system, and

- Differences in their diet and behavior patterns that may lead to increased exposure to environmental carcinogens.

To address this potential increased susceptibility of children, USEPA released the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposures to Carcinogens* ("Supplemental Guidance") (USEPA, 2005a), which evaluated different approaches to assess risks resulting from early-life exposure to carcinogens.

Early-life susceptibility to chemical carcinogens with different modes of action (MOAs) was addressed in the Supplemental Guidance. However, due to the limited number of studies addressing early-life exposures, at this time the USEPA determined that a quantitative evaluation was only possible for those chemicals identified as having a mutagenic MOA. The Supplemental Guidance recommends that *in some cases*, when chemicals have a mutagenic MOA, it may be appropriate to apply a default safety factor called an age-dependent adjustment factor (ADAF) to risk calculations when evaluating cancer risk associated with exposure for children ages 0 to 16 years. The Supplemental Guidance states that these default ADAFs should be used for chemicals with a mutagenic MOA if chemical-specific data to evaluate differences between adults and juveniles are not available. The following sections summarize the studies used to quantify the potential increased risk based on early-life exposure, the risk characterization approach for chemicals with a mutagenic MOA, and key uncertainties associated with this approach.

2.0 Studies Supporting Early Life Susceptibility to Mutagens

At the time when the USEPA's Supplemental Guidance was released, experimental studies in animals showing comparative tumor incidence across ages were available for twelve chemicals exhibiting a mutagenic MOA. Out of these twelve chemicals, six (benzidine, diethylnitrosamine, 3-methylcholanthrene, safrole, urethane, and vinyl chloride) had data from repeated or lifetime exposures; the EPA decided to base its quantitative evaluation of age-dependent tumor incidence on these six chemicals. Analysis of these repeated and lifetime exposure studies showed that the assumption that cancer risk was equal when the product of concentration and time was constant did not hold for carcinogens with a mutagenic MOA, and that per unit time of exposure, early-life exposures were more effective in inducing tumors than were adult exposures (USEPA, 2005a).

Development of the default ADAFs was based only on results of the repeated exposure studies because it was concluded that the lifetime exposure study design had less ability to distinguish potential increased susceptibility from early-life exposures. Four chemicals had data from a repeated exposure study design (benzidine, 3-methylcholanthrene, safrole, and vinyl chloride), and were therefore considered for ADAF development (USEPA, 2005a). In these repeated exposure studies, one group of animals was exposed only during the early life period, and was then followed through adulthood to assess tumor incidence; a second group of animals was exposed only through adulthood.

Based on these results a default ADAF of 10 (an approximation of the weighted geometric mean) was chosen for the age group 0-2 years. According to the USEPA, this is the age bracket when toxicokinetic

and toxicodynamic differences between children and adults are greatest (USEPA, 2005a). Data were not available to calculate a specific ADAF for children age 2-16; therefore USEPA selected an intermediate level of adjustment, chosen as half the difference between 1 (no adjustment for adults) and 10 (adjustment for children age 0-2) on a logarithmic scale to derive a default ADAF of 3 for this age group.

2.1   Chemicals that are Carcinogenic Via a Mutagenic Mode of Action

At this time, the USEPA has not finalized guidance that can be used to determine if a chemical is carcinogenic via a mutagenic MOA (USEPA, 2007c). Despite the lack of specific guidance, to date the USEPA has identified the following chemicals as possible carcinogens that act via a mutagenic MOA:

- benz(a)anthracene (CAS No. 56-55-3)
- benzidine (CAS No. 92-87-5)
- benzo(a)pyrene (CAS No. 50-32-8)
- benzo(b)fluoranthene (CAS No. 205-99-2)
- benzo(k)fluoranthene (CAS No. 207-08-9)
- chrysene (CAS No. 218-01-9)
- coke oven emissions (coal tar) (CAS No. 8007-45-2)
- dibenz(a,h)anthracene (CAS No. 53-70-3)
- 1,2-dibromo-3-chloropropane (CAS No. 96-12-8)
- indeno(1,2,3-cd)pyrene (CAS No. 193-39-5)
- 4,4-methylene bis(2-chloroaniline) (CAS No. 101-14-4)
- N-nitrosodiethylamine (CAS No. 55-18-5)
- N-nitrosodimethylamine (CAS No. 62-75-9)
- vinyl chloride (CAS No. 75-01-4).

In the case of vinyl chloride, the USEPA does not recommend the use of default ADAFs. As explained in the vinyl chloride toxicity profile in the USEPA's Integrated Risk Information System (IRIS) database, they recommend that the potential for added risk from early-life exposure to vinyl chloride be accounted for by applying an uncertainty factor of two in the quantitative cancer risk estimates (USEPA, 2007d). Therefore when exposure to vinyl chloride occurs only during adulthood, the oral slope factor of 0.72 $(mg/kg\text{-}day)^{-1}$ should be used in the quantitative risk evaluation. However, if exposure to vinyl chloride is continuous from birth, the twofold uncertainty factor should be applied so that the appropriate cancer slope factor becomes 1.4 $(mg/kg\text{-}day)^{-1}$.

It is important to note that the determination of a chemical's MOA (e.g., mutagenic, enzyme induction, etc.) should only be made by experienced toxicologists. That is, the USEPA's draft guidance (2007c) is not intended to be used in a human health risk assessment (HHRA) being performed at a Naval site. These draft guidelines are intended to provide USEPA's risk assessors with a "consistent, objective, transparent, and scientifically sound" approach to evaluate the weight-of-evidence to determine if a chemical is carcinogenic via a mutagenic MOA (USEPA, 2007c).

3.0   Risk Characterization Approach in HHRAs

A sample equation presented in the USEPA Region III Memo showing cancer risk calculations for a carcinogen with a mutagenic MOA using default ADAFs is presented below (USEPA, 2006b). For comparison, calculation of the cancer risk without using default ADAFs is also presented. ADAFs are shown in bold typeface in the first set of calculations. To keep the calculations comparable, the soil concentration (0.022 mg/kg), exposure factors, and cancer slope factor (7.3 $[mg/kg\text{-}day]^{-1}$) were kept constant.

3.1 Sample Calculation: Carcinogenic Risk from Incidental Soil Ingestion of a Chemical with a Mutagenic Mode of Action

General Equation:

$$\frac{C \times IR \times EF \times ED \times CF \times \mathbf{ADAF} \times CSF}{ATc \times BW}$$

Where:

C =	Chemical concentration in soil (0.022 mg/kg)
IR =	Daily ingestion rate of soil (200 mg/day for ages 0 – 6 years; 100 mg/day for ages 7 – 30 years)
EF =	Exposure frequency (350 days/year)
ED =	Exposure duration (2 years for ages 0 – 2 years; 4 years for ages 2 – 6 years; 10 years for ages 6 – 16 years; and 14 years for ages 16 – 30 years)
CF =	Conversion factor (1E-06 kg/mg)
ADAF =	Age dependent adjustment factor (10 for ages 0 – 2 years; 3 for ages 2 – 6 years; 3 for ages 6 – 16 years; and 1 for ages 16 – 30 years)
CSF =	Cancer slope factor (7.3 [mg/kg-day]$^{-1}$)
AT_c =	Averaging time for carcinogenic effects (70 years or 25,550 days)
BW =	Body weight (15 kg for ages 0 – 6 years; 70 kg for ages 6 – 30 years)

Age 0-2 years:

$$\frac{0.022 \text{ mg/kg} \times 200 \text{ mg/kg} \times 350 \text{ days/year} \times 2 \text{ years} \times 1\text{ E-06 kg/mg} \times \mathbf{10} \times 7.3 \text{ (mg/kg-day)}^{-1}}{365 \text{ days/year} \times 70 \text{ years} \times 15 \text{ kg}}$$

Risk (Age 0-2) = 5.9E-07

Age 2-6 years:

$$\frac{0.022 \text{ mg/kg} \times 200 \text{ mg/kg} \times 350 \text{ days/year} \times 4 \text{ years} \times 1\text{ E-06 kg/mg} \times \mathbf{3} \times 7.3 \text{ (mg/kg-day)}^{-1}}{365 \text{ days/year} \times 70 \text{ years} \times 15 \text{ kg}}$$

Risk (Age 2-6) = 3.5E-07

Age 6-16 years:

$$\frac{0.022 \text{ mg/kg} \times 100 \text{ mg/kg} \times 350 \text{ days/year} \times 10 \text{ years} \times 1\text{ E-06 kg/mg} \times \mathbf{3} \times 7.3 \text{ (mg/kg-day)}^{-1}}{365 \text{ days/year} \times 70 \text{ years} \times 70 \text{ kg}}$$

Risk (Age 6-16) = 9.4E-08

Age 16-30 years:

$$\frac{0.022 \text{ mg/kg} \times 100 \text{ mg/kg} \times 350 \text{ days/year} \times 14 \text{ years} \times 1\text{ E-06 kg/mg} \times \mathbf{1} \times 7.3 \text{ (mg/kg-day)}^{-1}}{365 \text{ days/year} \times 70 \text{ years} \times 70 \text{ kg}}$$

Risk (Age 16-70) = 4.4E-08

Total Risk
5.9E-07 + 3.5E-07 + 9.4E-08 + 4.4E-08 = 1.0E-06

3.2   Sample Calculation: Carcinogenic Risk from Incidental Soil Ingestion of a Chemical That Does Not Have a Mutagenic Mode of Action

General Equation:

$$\frac{C \times IR \times EF \times ED \times CF \times CSF}{ATc \times BW}$$

Age 0-6 years:

$$\frac{0.022 \text{ mg/kg} \times 200 \text{ mg/kg} \times 350 \text{ days/year} \times 6 \text{ years} \times 1\text{ E-06 kg/mg} \times 7.3 \text{ (mg/kg-day)}^{-1}}{365 \text{ days/year} \times 70 \text{ years} \times 15 \text{ kg}}$$

Risk (Age 0-6)   = 1.8E-07

Age 6-30 years:

$$\frac{0.022 \text{ mg/kg} \times 100 \text{ mg/kg} \times 350 \text{ days/year} \times 24 \text{ years} \times 1\text{ E-06 kg/mg} \times 7.3 \text{ (mg/kg-day)}^{-1}}{365 \text{ days/year} \times 70 \text{ years} \times 70 \text{ kg}}$$

Risk (Age 6-30) = 7.5E-08

Total Risk
1.8E-07 + 7.5E-08 = 2.6E-07

3.3 Comparison of Risk Calculation Results

A comparison of the cancer risk calculated with (1.0E-06) and without (2.6E-07) incorporation of the default ADAFs show that incorporation of the default ADAFs resulted in a cancer risk that was approximately four times higher.

4.0 Uncertainties in Evaluation of Early Life Susceptibility to Mutagens

The USEPA acknowledges that "The practice of risk assessment with respect to accounting for early-life exposures to toxicants continues to develop, and specific components of this Supplemental Guidance may become outdated or may otherwise require modification in individual settings" (USEPA, 2007c). HHRAs should strive to use, to the extent practicable, the best available science. The USEPA's recommendation of using ADAFs for carcinogens with a mutagenic MOA in some cases is the first time that default safety factors have been considered in HHRA based on the MOA. As such, there are many uncertainties inherent with this practice, including:

- At this point in time, the database used to evaluate early life susceptibility to mutagens is limited.

- Development of the default ADAFs was based only on results of the repeated exposure studies because it was concluded that the lifetime exposure study design had less ability to distinguish potential increased susceptibility from early life exposures. Only four chemicals had data from a repeated exposure study design (benzidine, 3-methylcholanthrene, safrole, and vinyl chloride)[1].

- Within the repeated exposure study database used to develop the default ADAFs, there is a large range of tumor incidence ratios of early-life to adult exposures. The wide range of ratios indicates that in some cases *fewer* cancers were elicited by early-life exposures when compared to adults and in other cases more cancers were elicited by early-life exposures.   However, the trend was that more cancers were elicited by early-life exposures. (USEPA, 2005a).

- The Supplemental Guidance does not present the criteria for determining whether or not a chemical is carcinogenic via a mutagenic MOA.

- The default ADAFs were derived from studies where exposures were much higher than those typically observed in the environment. The mutagenic potential of a chemical may be much less, and may be overcome by DNA repair mechanisms at typical exposure levels.

- Periods of increased cell replication can vary for different tissues (e.g., mammary tissues in rats have increased development in weeks 6-8 of life), which can make the window of susceptibility different for different individual chemicals, depending on their target tissue(s). For this reason using the same default ADAFs for all age groups, for all chemicals with a mutagenic MOA may not be appropriate.

5.0 Recommendations

The application of default ADAFs in the risk characterization is not required by the USEPA Headquarters or the Navy. Remedial Project Managers (RPMs) may use their discretion in deciding if the procedures included in the USEPA's Supplemental Guidance are appropriate to use in a HHRA being performed at one of their sites. The Supplemental Guidance does not establish any binding requirements or policies. As stated by USEPA in the preface to the Supplemental Guidance,

> "*Therefore, the Supplemental Guidance has no binding effect on EPA or on any regulated entity. Where EPA does use the approaches in the Supplemental Guidance in developing risk assessments, it will be because EPA has decided in the context of that*

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[1] The study design for the remaining chemicals was either lifetime exposure, or in some instances there were no studies that specifically addressed early life exposures. For those chemicals that didn't have study data focused on early life exposures, they were determined to have a mutagenic MOA based on other tests. The Executive Summary section lists documents that can be referenced for the criteria used to determine a mutagenic MOA

risk assessment that the approaches from the Supplemental Guidance are suitable and appropriate." (USEPA, 2005a).

Given the current state of the science, it is difficult to provide clear criteria to help Navy RPMs decide if default ADAFs should be used to quantitatively evaluate risk at a site. The following recommendations should be considered:

- Since default ADAFs are intended to evaluate exposure to certain carcinogens during childhood, ADAFs should only be considered for use when the conceptual site model (CSM) includes complete exposure pathways to children within the age range of 0 – 16 years (e.g., residential exposure).

- Default ADAFs are only appropriate for use with chemicals that are carcinogens via a mutagenic MOA when chemical-specific data for a susceptible lifestage is not known. Carcinogens with MOAs other than mutagenic, and when the MOA is unknown, should not use default ADAFs or any other adjustments. At this time, the USEPA recommends that default ADAFs are only appropriate *in certain situations*, for the following chemicals (and any others identified by USEPA after the publish date of this paper):

  - ✓ benz(a)anthracene
  - ✓ benzidine
  - ✓ benzo(a)pyrene
  - ✓ benzo(b)fluoranthene
  - ✓ benzo(k)fluoranthene
  - ✓ chrysene
  - ✓ coke oven emissions (coal tar)

  - ✓ dibenz(a,h)anthracene
  - ✓ 1,2-dibromo-3-chloropropane
  - ✓ indeno(1,2,3-cd)pyrene
  - ✓ 4,4-methylene bis(2-chloroaniline)
  - ✓ N-nitrosodiethylamine
  - ✓ N-nitrosodimethylamine

- Although vinyl chloride has been identified by USEPA as having a mutagenic MOA, default ADAFs should **not** be used to quantify potential risk from exposure during childhood. Quantitative risk from early childhood exposure to vinyl chloride should be evaluated using an uncertainty factor of 2, consistent with the recommendations in the vinyl chloride IRIS profile (USEPA, 2007d).

- Due to uncertainties regarding use of the default ADAFs, it is recommended that when risks are calculated using default ADAFs that they should also be calculated without the default ADAFs. As appropriate these results can be compared and discussed in the uncertainty section of the risk characterization.

- Whenever default ADAFs are used to quantify risk in an HHRA, the uncertainties associated with the application of ADAFs should be presented in the uncertainty section of the risk characterization.

- The determination of a chemical's carcinogenic MOA should be made by experienced toxicologists. For additional information on this process, or to obtain an up-to-date list of carcinogens that USEPA has determined have a mutagenic MOA, contact the Navy and Marine Corps Public Health Center at 757-953-0940.

6.0 References

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