

**Comments on EPA's  
*Science Issue Paper: Mode of Carcinogenic Action  
for Cacodylic Acid and Recommendations for  
Dose Response Extrapolation***

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# Table of Contents

	<u>Page</u>
Executive Summary.....	ES-1
1 Introduction .....	1
2 Endpoint Selection .....	2
3 Benchmark Dose Analysis .....	3
4 Uncertainty Factors .....	5
4.1 Intraspecies Variability .....	5
4.2 Interspecies .....	5
4.3 The FQPA Safety Factor .....	7
5 Conclusions and Recommendations.....	8
6 References .....	10

# **Comments on EPA's Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid and Recommendations for Dose Response Extrapolation**

## **Executive Summary**

Based on studies in rats, it is clear that the mode of action (MOA) for DMA<sup>V</sup>-induced bladder tumors is cytotoxicity, resulting in persistent regenerative proliferation, leading to development of bladder tumors. This MOA has no mutagenic component and has a nonlinear dose response. The toxicokinetics of DMA<sup>V</sup> is different from the toxicokinetics of DMA<sup>V</sup> in other species, including humans, rendering the rat uniquely susceptible to DMA<sup>V</sup> carcinogenicity.

In a recent report, "*Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid and Recommendations for Dose Response Extrapolation*", the EPA Office of Pesticide Programs (OPP) uses a reference dose approach to estimate the daily exposure to DMA<sup>V</sup> that is not associated with any increased cancer risk. The reference dose,  $7 \times 10^{-5}$  mg/kg/day is based on the BMDL<sub>1</sub> (*i.e.* the lower 95% confidence limit on the benchmark dose (BMD) that is associated with 1% of the maximum cell proliferation rate above background) and a composite Uncertainty Factor (UF) of 1000, composed of three 10-fold UFs - for intraspecies variability, interspecies variability, and the FQPA safety factor for special sensitivity of early life stages.

While OPP's framework is reasonable, several aspects of OPP's analysis are overly conservative and not based on the best available scientific data:

- The BMD<sub>10</sub> rather than the BMD<sub>1</sub> is more appropriate as a point of departure (POD)
- The interspecies UF should be 1-fold at the most, since the model species, the rat, is more sensitive than humans to DMA<sup>V</sup>.
- The FQPA UF should be 1-fold since there are data showing there is no increased sensitivity to DMA<sup>V</sup> in early life stages compared to adults.

A more appropriate reference dose, based on sound scientific evidence, can be calculated as  $2.9 \times 10^{-2}$  mg/kg/day. This value is greater than that proposed by OPP, but is still protective of public health. When comparing this revised cancer reference dose to EPA's estimated daily intake of DMA<sup>V</sup> due to agricultural use, the resulting margin of exposure (MOE) is over 100,000-fold.

# 1 Introduction

The Office of Pesticides Programs (OPP) of the United States Environmental Protection Agency (EPA, The Agency) recently issued, "*Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid (Dimethylarsinic Acid, DMA<sup>V</sup>) and Recommendations for Dose Response Extrapolation*" (US EPA, 2005a). In that document, the OPP provides a review of the toxicology of DMA<sup>V</sup>, and an analysis of information from animal studies on the mode of action (MOA) for DMA<sup>V</sup>-induced rat bladder tumors. OPP concluded that the MOA for DMA<sup>V</sup>-induced rat bladder tumors involves cytotoxicity to the bladder epithelium, followed by a compensatory cell proliferation that leads to hyperplasia, and ultimately, a low incidence of bladder tumors (*i.e.*, the mode of action described by Cohen *et al.*, 2002). The OPP concluded further that DMA<sup>V</sup>-induced bladder tumors had a nonlinear dose response. In particular, the OPP analysis indicated that bladder tumor formation was a high-dose phenomenon that would occur only if the reactive compound dimethylarsinous acid (DMA<sup>III</sup>) that is produced during the metabolism of DMA<sup>V</sup> were generated in sufficient quantities to cause urothelial toxicity and a compensatory proliferative response.

Studies in rats have been used to characterize the MOA of DMA<sup>V</sup> since the rat's toxicokinetic and toxicodynamic handling of this compound renders it the only species tested that is susceptible to DMA<sup>V</sup>-induced bladder tumors. The rat is the only species known to generate enough of the reactive compound DMA<sup>III</sup> to initiate the step-wise progression toward bladder tumors (Cohen *et al.*, 2002). The dose of DMA<sup>V</sup> required to cause rat bladder tumors is relatively high (approximately 8.0 mg/kg/day) (US EPA, 2001; Gur *et al.*, 1989).

Based on this finding, the OPP recommended a "reference dose approach" to characterize DMA<sup>V</sup> cancer risk in humans. In a process somewhat parallel to developing a reference dose (RfD) for noncancer endpoints, OPP identified a DMA<sup>V</sup> dose from animal studies associated with negligible adverse effects. OPP then applied several uncertainty or safety factors to arrive at a daily dose of DMA<sup>V</sup> that they estimated would be protective of public health. In developing a reference dose associated with DMA<sup>V</sup> cancer risk, OPP made the following recommendations:

- Endpoint selection: Based on studies in rats that demonstrate the MOA for bladder tumors involves a statistically significant increase in cell proliferation induced by DMA<sup>III</sup>, and that cell proliferation has a highly nonlinear dose-response, OPP recommended increased cell proliferation as the endpoint to conservatively model DMA<sup>V</sup> cancer risk in humans.

- Benchmark dose analysis<sup>1</sup> and point of departure calculation: Relying on cell proliferation data in the bladder urothelium observed in rat studies, OPP recommended a benchmark dose (BMD) analysis to establish a BMDL<sup>2</sup>. The OPP recommended the BMDL<sub>1</sub> be used as a point of departure (POD).
- Uncertainty Factors (UF) application:
  - A UF of 10 was applied for intraspecies variability,
  - A UF of 10 was applied for interspecies variability,
- Food and Quality Protection Act (FQPA) safety factor application: OPP applied a 10-fold FQPA safety factor to account for the potential sensitivity of early life stages to DMA<sup>V</sup>.

We have evaluated the scientific basis for OPP's methodology of using a "reference dose approach" to characterize the human health risk associated with DMA<sup>V</sup>. Our analysis concludes OPP's decision to use a nonlinear approach (*i.e.*, reference dose approach) to describe DMA<sup>V</sup>'s dose-response relationship is supported; however, the choice of the specific POD, as well as two of the three uncertainty/safety factors, are not supported by the currently available scientific information. In this document, we provide a refined approach for developing the reference dose for DMA<sup>V</sup>, based on the same MOA framework presented by EPA, but include changes to incorporate current species- and compound-specific data. Our recommendations result in a cancer reference dose for DMA<sup>V</sup> that is higher than the value calculated by EPA, yet is still protective of public health.

## 2 Endpoint Selection

DMA<sup>V</sup>, when fed to rats at sufficiently high doses, causes tumors in the bladder urothelium. The formation of these tumors progresses through a series of well defined events. Any of these events could be used as a suitable endpoint to derive a POD. Cancer risk assessments have typically used tumor formation as the endpoint. This choice is made when an MOA is not established and tumors remain the only measurable carcinogenic effect. Quantification of tumor response at low doses is associated with

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<sup>1</sup> A benchmark dose (BMD) analysis is a more quantitative alternative for establishing a starting point (*i.e.*, POD) when evaluating the human health risks compared to the more conventional NOAEL/LOAEL approach. A BMD analysis uses a model to compute the amount of change associated with a specified adverse response considered to be biologically significant.

<sup>2</sup> BMDL refers to the corresponding lower limit of a one-sided 95% confidence interval on the BMD (*i.e.*, lower 95% confidence interval on the central estimate).

more uncertainty, and, thus, a tumor-based POD would require a larger margin of exposure to assure that the underlying process causing tumors is negligible at the POD.

Although tumor production and hyperplasia could both be used as endpoints for deriving the reference dose, OPP chose increased cell proliferation as "the rate limiting step for tumor formation" and correctly notes that "there must be a sufficient level of cytotoxicity and proliferation attained and sustained, to lead to hyperplasia and tumors". We agree with OPP that selection of cell proliferation is scientifically sound and health protective. From the non-tumor MOA endpoints, increased urothelial cell proliferation is the rate-limiting step and the key response that signals increased risk of tumors. Increases in cell proliferation can be quantified with a higher degree of confidence than increases in tumors. Proliferation shows a nonlinear dose response and a clear dose level below which cell division rates are unaffected. Doses that do not increase urothelial cell proliferation do not pose an increased bladder cancer risk. This endpoint is conservative since doses that cause increases in sustained cell proliferation do not necessarily cause increases in tumor incidence.

### **3 Benchmark Dose Analysis**

A BMD analysis allows for fuller use of dose-response data. The agency has addressed statistical uncertainties in the calculation through the selection of a lower confidence limit on the BMD, *i.e.* the BMDL. However, by choosing the BMDL<sub>1</sub> instead of the BMDL<sub>10</sub> the OPP has introduced unnecessary uncertainty and conservatism into its analysis.

EPA's Draft Benchmark Dose Technical Guidance Document (US EPA, 2000) defines a BMD as "an exposure due to a dose of a substance associated with a specified low incidence of risk, generally in the range of 1% to 10%, of a health effect; or the dose associated with a specified measure or change of a biological effect". For continuous data, such as cell proliferation, the response can be dichotomized and treated as a quantal variable, or the magnitude in change can be expressed as a change in the mean response. Changes in mean response can be expressed in several ways, depending on the nature of the specific endpoint in question (US EPA, 2000). In the case of the DMA<sup>V</sup> cell proliferation data, where there is a clear maximum response, OPP calculated the change in response to be a fraction of the range of responses because this endpoint had a clear maximum value. That is, OPP calculated the BMD<sub>1</sub> and BMD<sub>10</sub> values based on a percent of the maximum value of cell proliferation above baseline.

For selection of the POD, the EPA recommends using the lower 95% bound on the BMD. Using the lower bound is a conservative measure that accounts for the uncertainty inherent in a given study, and assures that the specified change of response is not exceeded (US EPA, 2000).

OPP calculated a BMD<sub>10</sub> and BMD<sub>1</sub> for cell proliferation using the data of Arnold *et al.* (1999), in which F344 rats were treated orally with DMA<sup>V</sup> for up to 10 weeks to determine the effects on the bladder urothelium. Effects on cell proliferation were determined at 10 weeks. The specific values of 0.65 mg/kg/day for the BMD<sub>10</sub> and 0.54 mg/kg/day for the BMD<sub>1</sub> were derived using the Hill model<sup>3</sup>, the statistical model that best fit the experimental data. OPP chose to use the lower confidence interval on the BMD<sub>1</sub> (*i.e.* BMDL<sub>1</sub> [0.07]), which is approximately 115 times lower than the lowest dose that caused cancer in the two-year rat bioassay.

Several reasons support the use of the BMDL<sub>10</sub> rather than the BMDL<sub>1</sub>:

1. The BMD<sub>10</sub> and BMD<sub>1</sub> values are very similar (0.65 *vs.* 0.54). This shows that the dose-response curve for induced cell proliferation is very steep, with an almost step-like transition between doses that cause no increase and doses that cause a detectable and marked increase in cell proliferation. This feature increases the confidence that the low doses causing increases in cell proliferation have been identified. These low doses can be better be characterized by the more reliably estimated BMDL<sub>10</sub>.
2. Cell proliferation at baseline is  $0.20 \pm 0.03$  (standard error), while the cell proliferation at the BMD<sub>10</sub> is 0.27<sup>4</sup>. The amount of excess cell proliferation at BMD<sub>10</sub> is modest, only approximately 35% above controls and within the range of variability that occurs among controls.
3. There is substantially less uncertainty associated with the BMD<sub>10</sub> than with the BMD<sub>1</sub>. The greater uncertainty of the BMD<sub>1</sub> estimates is reflected in the relatively large confidence interval that surrounds this estimate compared to the BMD<sub>10</sub>. The BMD<sub>10</sub>/BMDL<sub>10</sub> ratio is 2.2, whereas the BMD<sub>1</sub>/BMDL<sub>1</sub> ratio is 7.7.
4. Using the lower confidence limit on the BMD<sub>10</sub> is conservative and appropriate for calculating a POD. In fact, the BMDL<sub>10</sub> is already 30 times lower than doses that lead to

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<sup>3</sup> The Hill model determines a change in response that is a certain percent of the maximum amplitude (range between the control response and maximum response). The Hill model allows for non-linearity, contains an asymptote term for the estimation of a plateau, and is conservative because it does not allow a slope of zero, or a true threshold (US EPA, 2003). This model's ability to estimate a very steep dose-response curve appears to have been important in describing the Arnold *et al.*, (1999) data.

<sup>4</sup> Based on the Hill model, the baseline (or control) cell proliferation rate was 0.20, and the maximal response was 0.93. The difference between the baseline and maximal response (*i.e.*, 0.93-0.20), which is the maximum amplitude, was 0.73. Ten percent of the maximum change (0.73) is 0.07, so 10% of the maximum response is equivalent to 0.27 [*i.e.*, 0.20 (baseline) + 0.07 (10% of the maximum amplitude)]. Similarly 1% of the maximum response over baseline is equivalent to 0.207 [*i.e.*, 0.2 (baseline) + 0.007 (1% maximum amplitude)].

bladder tumors, and about 14 times lower than NOAEL for bladder tumors in the two-year bioassay.

## **4 Uncertainty Factors**

### **4.1 Intraspecies Variability**

The standard 10-fold uncertainty factor (UF) for variability in the human population is appropriate.

### **4.2 Interspecies**

A UF of 10 is routinely used by EPA to account for interspecies variability, which includes both toxicokinetic and toxicodynamic components. According to EPA Food Quality Protection Act (FQPA):

...for interspecies variability, a factor of 10-fold is applied as a default assumption to account for differences in sensitivity between species when animal data are used to assess human risk. Although the default 10X is generally used in the Agency, when data indicate that humans are less or more sensitive than animals, the interspecies group uncertainty factor of 10-fold may be reduced or raised (US EPA, 2002a).

In the case of DMA<sup>V</sup>, using a UF of 10 is inconsistent with EPA guidance (US EPA, 2002a) based on data demonstrating that rats are substantially more sensitive to DMA<sup>V</sup> toxicity than other animal species, including humans. Thus, for the reasons described below, the interspecies UF of 10 used by OPP to account for interspecies variability should be reduced to a value of 1 or less.

As described in OPP's analysis (US EPA, 2005a), the unique toxicokinetics of DMA<sup>V</sup> in the rat, makes the rat particularly susceptible to DMA<sup>V</sup>-induced cytotoxicity and tumorigenicity. Rat toxicokinetics of DMA<sup>V</sup> differ from those of other species in a number of respects. First, the rat metabolizes DMA<sup>V</sup> to trimethylarsenic oxide (TMAO) more extensively than do other species (Cohen et al., 2005), which indicates that more DMA<sup>III</sup> is formed as a metabolic intermediate (US EPA, 2005a, p.32). The presence of DMA<sup>III</sup> in sufficient quantities causes cytotoxicity in bladder cells, most likely through the interaction with sulfhydryl groups on macromolecules (Cohen *et al.*, 2005). As a consequence of the uniquely efficient metabolism of DMA<sup>V</sup> in the rat, the rat is the only known species in which DMA<sup>V</sup> administration results in urinary concentrations of DMA<sup>III</sup> equivalent to those that are cytotoxic to urothelial cells *in vitro* and *in vivo* (Cohen *et al.*, 2002). In general, TMAO accounts for

roughly 40% of the urinary metabolites exposed to DMA<sup>V</sup> (Yoshida *et al.*, 1997; Yoshida *et al.*, 1998; Cohen *et al.*, 2002). This is in sharp contrast to mice and hamsters, which only excrete about 5% of a DMA<sup>V</sup> dose as TMAO (Marafante *et al.*, 1987). Human metabolism of DMA<sup>V</sup> is much more like that of mice and hamsters, than that of the rat (Marafante *et al.*, 1987).

Another feature that distinguishes rats from other species is the enhanced binding of arsenic compounds to rat hemoglobin (Lu *et al.*, 2004). The increased binding compared to that of other species effectively prolongs the half-life of these compounds *in vivo* and may contribute to the high levels of TMAO generated in the rat. Lu *et al.* (2004) demonstrated that after incubation of red blood cells with graded concentrations of inorganic arsenic, at dose levels from 1  $\mu\text{M}$  to 100  $\mu\text{M}$ , the rat binds significantly more DMA<sup>III</sup> to hemoglobin than humans. This enhanced binding may be involved in higher doses of DMA<sup>III</sup> accumulating in the rat urothelium over time.

The available *in vitro* evidence suggests DMA<sup>III</sup> induces comparable cytotoxicity in rat and human bladder cells. Cohen *et al.* (2002) demonstrated that the toxicity of DMA<sup>III</sup> for the human bladder cell 1T1 (LC<sub>50</sub>= 0.8  $\mu\text{M}$ ) was comparable to the toxicity for rat bladder cell line MYP3 (LC<sub>50</sub>= 0.5  $\mu\text{M}$ ). More recently, based on differential gene expression determined with a microarray analysis, Sen *et al.*, (2005) demonstrated that *in vitro*, gene expression changes were minimal in UROtsa cells (human bladder cells) after incubation with DMA<sup>V</sup>. In contrast, MYP3 cells treated with DMA<sup>V</sup> showed marked differential gene induction compared to controls. Because the toxicity in target cells appears to be comparable or less in humans compared to rats, and there is no indication of any additional sensitivity in humans, an interspecies UF to account for toxicodynamic differences is not necessary.

Thus, while the toxicity of DMA<sup>III</sup> to urothelial cells is similar in rat and human cells *in vitro*, the quantity of DMA<sup>III</sup> that accumulates in rat bladder *in vivo* is much higher than the amount that can form in a human bladder from a similar exposure. The extensive metabolism of DMA<sup>V</sup> in the rat makes this species susceptible to DMA<sup>III</sup>-induced cytotoxicity and ultimate tumor formation.

In summary, application of an interspecies UF greater than 1 is not warranted when there is sufficient evidence that the animal model is more sensitive than humans. Based on the overwhelming amount of data indicating that rats are *more* sensitive to DMA<sup>V</sup>-induced bladder tumors, than any species, including humans, an UF of less than 1 would be appropriate to account for differential sensitivity between rats and humans, and an UF of 1 would still be sufficient for public health protection.

### 4.3 The FQPA Safety Factor

The "FQPA Factor" of 10 was designed to account for the uncertainty in the toxicity of noncancer endpoints, and was to be used if early life stages had not been fully tested for relevant noncancer endpoints (*i.e.* for neurodevelopmental toxicity, teratogenicity, *etc.*), and/or if early life stages show greater susceptibility to chemical-specific effects (US EPA. 2002b).

The issue of life-stage differences in responses to carcinogens is further discussed in EPA's recently revised guidelines "Supplemental Guidance for Assessing Susceptibility from Early Life Exposure to Carcinogens". These guidelines state:

The Supplemental Guidance addresses a number of issues pertaining to cancer risks associated with early-life exposures generally, but provides specific guidance on potency adjustment only for carcinogens acting through a mutagenic mode of action....Default adjustment factors are meant to be used only when no chemical-specific data are available to assess directly cancer susceptibility from early-life exposure to a carcinogen acting through a mutagenic mode of action (US EPA, 2005b).

Thus, according to these recent guidelines, in the absence of evidence for early life special susceptibility, EPA recommends that no special adjustment be used for non-mutagenic carcinogens, or even for ones with uncertain modes of action. Only clearly mutagenic carcinogens that act *via* direct interaction with DNA warrant adjustment.

There is a large body of scientific literature demonstrating that DMA<sup>V</sup> is not a direct acting mutagen and that any related genotoxicity is indirect (Cohen *et al.*, 2005; Nesnow *et al.*, 2002; Kligerman *et al.*, 2003). Because there is clear evidence that DMA<sup>V</sup> is not mutagenic and its indirect genotoxic effects are not integral to the MOA, an adjustment for early life sensitivity to DMA<sup>V</sup>-induced carcinogenesis is not warranted.

General principles of bladder carcinogenesis would also argue against the application of the FQPA safety factor. As the EPA July 2005 document on DMA<sup>V</sup> quite appropriately points out:

The bladder and urinary tract are anatomically complete and functionally competent throughout life, which suggests qualitatively that there are no age dependent differences in susceptibility to chemically-induced cancer among humans. Furthermore, there is no indication that children are at any increased sensitivity, as bladder cancer is very uncommon at early ages in humans and given the late age of onset of bladder cancer, there is no evidence that, in general, there is a shortened latency for tumor development after childhood exposure (US EPA, 2005a).

Moreover, teratogenicity and multigenerational studies with DMA<sup>V</sup> have demonstrated that the embryo is not especially sensitive to the effects of DMA<sup>V</sup> toxicity, with any adverse developmental outcomes occurring only at maternally toxic doses (LSRI, 1986; LSRI, 1988a; LSRI, 1988b).

Thus, given that the lack of evidence of DMA<sup>V</sup>-specific developmental toxicity at non-maternally toxic doses, the lack of DMA<sup>III</sup> direct-acting mutagenicity, and the lack of evidence of increased susceptibility of children to bladder tumors, FQPA safety factor should be reduced to 1-fold.

## 5 Conclusions and Recommendations

The OPP has conducted a progressive and scientifically sound process to develop a DMA<sup>V</sup> risk assessment by using a nonlinear "reference-dose approach". Their approach is consistent with the available data that indicate that DMA<sup>V</sup>-induced rat bladder tumors occur only at relatively high doses and have a nonlinear dose response relationship. We agree with the OPP's use of a BMD approach and the use of cell proliferation data as the endpoint of concern. Using a BMD approach based on cell proliferation is adequately protective because it is the key, rate-limiting step process. Without increases in cell proliferation, no secondary cancer risk will occur. However, rather than the BMDL<sub>1</sub> as a POD (as OPP has suggested), we recommend the BMDL<sub>10</sub>. The BMDL<sub>10</sub> will provide higher confidence that doses associated with negligible cell proliferation are appropriately characterized.

Several other aspects of OPP's analysis are based on default assumptions rather than on available, sound DMA<sup>V</sup>-specific information on the species differences in toxicokinetics, toxicodynamics, and the relative susceptibility of early life stages. This information on DMA<sup>V</sup> indicates that adjustments for interspecies differences and the FQPA (*i.e.*, early life susceptibility) are not necessary. Table 1 provides an overview of OPP's recommendations compared to alternative approaches that could be used to develop a cancer "reference dose" for DMA<sup>V</sup>.

Using OPP's assumptions, the daily intake of DMA<sup>V</sup> associated with negligible human cancer risk (*i.e.*, the cancer reference dose) would be calculated as to be  $7 \times 10^{-5}$  mg/kg/day<sup>5</sup>. Using our recommendations for a POD and uncertainty/safety factors, the revised cancer "reference dose" for

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<sup>5</sup> Calculated by applying a 10X UF for interspecies variability, a 10X UF for intraspecies variability, and a 10X FQPA safety factor to 0.07, which is the BMDL<sub>1</sub> associated with increased cell proliferation.

DMA<sup>V</sup> would be  $2.9 \times 10^{-2}$  mg/kg/day<sup>6</sup>. This value is health protective, supported by the most current science and in accordance with most recent EPA guidance. While this analysis is focused on the carcinogenicity assessment for DMA<sup>V</sup>, we note that estimated doses of DMA<sup>V</sup>, based on EPA's HED evaluation (US EPA, 2001), are well below (*i.e.* more than 100,000-fold) the cancer reference dose noted above of  $2.9 \times 10^{-2}$  mg/kg/day.

**Table 1.**

**An Alternate Approach Proposed for the Calculation of a Cancer "Reference Dose " for DMA<sup>V</sup>**

<b>Input</b>	<b>OPP Choice</b>	<b>Gradient's Proposal</b>	<b>Comments</b>
Dose-Response	Nonlinear	Nonlinear	OPP approach is appropriate. Studies in rats demonstrate the MOA for rat bladder tumors is cytotoxicity followed by regenerative hyperplasia. This MOA has a nonlinear dose-response relationship.
Endpoint	Cell Proliferation	Cell Proliferation	Cell proliferation is an early pre-cancer cursor and the rate-limiting event in DMA <sup>V</sup> 's MOA.
Point of departure	BMDL <sub>1</sub> (0.07 mg/kg/day)	BMDL <sub>10</sub> (0.29 mg/kg/day)	The BMDL <sub>10</sub> is associated with less statistical uncertainty, while still being conservative.
Intraspecies variability	10	10	The standard 10-fold interspecies UF is appropriate.
Interspecies UF	10	1	Toxicokinetic and toxicodynamic information indicates that the rat is more sensitive to DMA <sup>V</sup> than humans.
FQPA Safety Factor	10	1	There is no indication of increased sensitivity of the young to DMA <sup>V</sup> for noncancer endpoints and no indication that the young would be more susceptible than adults to bladder carcinogens.

In conclusion, while OPP's framework is reasonable, several aspects of OPP's analysis are overly conservative and not based on the best available scientific data:

- The BMD<sub>10</sub> rather than the BMD<sub>1</sub> is more appropriate as a POD.
- The interspecies UF should be 1-fold at the most, since the model species, the rat, is more sensitive than humans to DMA.

<sup>6</sup> Calculated by applying a 1X UF for interspecies variability, a 10X UF for intraspecies variability, and a 1X FQPA safety factor to 0.29, which is the BMDL<sub>10</sub> associated with increased cell proliferation.

- The FQPA UF should be 1-fold since there are data showing there is no increased sensitivity to DMA<sup>V</sup> in early life stages compared to adults.

Using a refined approach based on sound scientific principles, a more appropriate reference dose of  $2.9 \times 10^{-2}$  mg/kg/day can be calculated. This value is greater than that proposed by OPP, but is still protective of public health. When comparing this revised cancer reference dose to EPA's estimated daily intake of DMA<sup>V</sup> due to agricultural use, the resulting MOE is over 100,000-fold.

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