STANDARD PRACTICE FOR WILDLIFE TOXICITY REFERENCE VALUES

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This technical guide and a current listing of wildlife toxicity assessments are available at the following website:

http://chppm-www.apgea.army.mil/tox/herp/wta.htm

FORWARD

This technical guide provides our Center's standard practice for the development and documentation of wildlife toxicity reference values, which are used to assist in the evaluation of risks that military-related chemicals may pose for environmental quality. Informed and defensible environmental health risk management is limited by the quality of the risk assessments used to support them. Therefore, this technical guide is designed to improve the analyses behind these risk management decisions. It is written primarily for risk assessors.

This technical guide should not be construed as official Department of the Army policy unless so designated by other authorizing documents. This document provides guidance and technical reference material based on scientific information current at the time of publication. As available information and supporting data are continuously being advanced, users are cautioned to ascertain existence of any updated information.

The Surgeon General is responsible for providing policy and technical expertise on human health and ecological aspects of pollution resulting from Army activities and operations (Army Regulation 200-1 (AR 200-1) Environmental Protection and Enhancement and AR 40-5 Preventive Medicine). The Surgeon General has delegated this responsibility through the U.S. Army Medical Command to the U.S. Army Center for Health Promotion and Preventive Medicine. This guide was developed pursuant to this authority.

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USACHPPM TECHNICAL GUIDE NO. 254

STANDARD PRACTICE FOR WILDLIFE TOXICITY REFERENCE VALUES

1. Introduction

1.1 Purpose

This U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254 (USACHPPM TG 254) outlines a Standard Practice that establishes a methodology for—

- Generating defensible wildlife **toxicity reference values** (TRVs)[1] for chemicals of interest in Army ecological risk assessment (ERA) programs.
- Preparing the documentation to support such TRVs. A wildlife TRV is similar to a human health **reference dose** (RfD)[2].

1.2 Audience

Ecological risk assessors and toxicologists are the target audience for this Standard Practice. Army risk managers and staff responsible for coordination of ERA programs should ensure that their project teams consider this Standard Practice during project design and implementation.

1.3 Application

This TG is primarily intended for use by this Center to generate wildlife TRVs for military-related substances that are more defensible than those typically used in many U.S. Army risk assessments. If a TRV relevant to a particular ERA has been generated by this Center using this methodology, then its use is expected unless an alternative can be reasonably defended. This Center will apply the methodology in a phased approach, focusing upon the highest priority chemicals first. Other U.S. Army and military entities are encouraged to use this Standard Practice within their ERA programs.

1.4 Limitations of Use/Exceptions

By definition, the procedures described herein result in measures of toxicity (i.e., TRVs) that evaluate the likelihood of effects in *individual* organisms that may be relevant to a *population* of organisms in the wild. This TG does not specifically address how the measures, or resulting risk estimates, relate to **demographic rates** (or outcomes) for any particular population of interest. These methods create a biased risk estimate for use in screening-level evaluations. Assessing risk to populations involves using these methods and other lines of evidence[3] before any risk management action to protect populations can be recommended based upon scientific information.

Methodological exceptions to this Standard Practice may be warranted in some circumstances. These circumstances are—

- When the procedures are not consistent with promulgated Federal or state law.
- When the ERA documents persuasive scientific evidence, or argument, to bear on the specific issue in question.

1.5 TG Revisions

This TG will be reviewed on a regular basis. If the Standard Practice is determined to be inconsistent with current procedures and/or regulations, it will be revised and reissued with an appropriate revision number.

This TG may also be revised, as appropriate, when the ongoing U.S. Environmental Protection Agency (USEPA) collaborative effort to develop guidance for ecological soil screening levels (EcoSSLs) is finalized.

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[1] Definitions of terms in bold-faced font are provided in Appendix B.
[2] This document uses the term 'wildlife' to specifically refer to *vertebrate* organisms other than fish that live in the wild.
[3] For example: site-specific fieldwork, evaluations of reproductive success, demographic (population) modeling, and/or biological monitoring.

1.6 Background

An integral component of a wildlife ERA is the development of some quantitative measure of the toxicity of a chemical to the animals (or receptors) of concern. Toxicity measures that are employed in Army programs have not been consistent or, in some cases, necessarily defensible.

2. Methodology

In general, TRVs are needed to represent levels of exposure that are associated with low risk for entire **taxonomic** classes (e.g., mammals) or for selected foraging **guilds** (e.g., carnivorous mammals). This TG focuses upon the development of chemical-specific TRVs for these receptor groups.

The methodology for generating defensible wildlife TRVs and for preparing acceptable documentation to support such TRVs consists of two phases.

a. **Phase 1 – Toxicity Profile**
   (1) Perform data collection and literature search.
   (2) Identify relevant studies.
   (3) Prepare a toxicity profile.

b. **Phase 2 – TRV Report**
   (1) Derive TRVs and document selection rationale.
   (2) Assign confidence levels to the TRVs.
   (3) Prepare the TRV report.

The outcome of these two phases are combined into a comprehensive "wildlife toxicity assessment" for the chemical(s) under review. Each wildlife toxicity assessment report shall contain a list of the primary author(s), contact information, and a report date.

2.1 Data Collection/Literature Search

The literature search will provide—

- Qualitative information on the toxicological characteristics of the chemical(s) under consideration.
- A set of relevant studies that may be used in the development of TRVs.

All appropriate sources should be searched for specific toxicological information for mammals, birds, and herpetofauna. Presently, there is no single source that provides a comprehensive review for substances of concern. Potential sources include:

- TOXLINE (National Library of Medicine),
- ATSDR Toxicity Profiles
- BIOIS (Biological Abstracts),
- Hazardous Substances Data Bank (National Library of Medicine),
- Integrated Risk Information System (IRIS),
- ECOTOX database (USEPA, Duluth),
- Medline (National Library of Medicine),
- Registry of Toxic Effects of Chemical Substances (RTECs), and
- Toxicological *Benchmarks for Wildlife* (Oak Ridge National Laboratory) [Sample et al. 1996].

A thorough examination of the toxicological literature is necessary to support and defend any toxicity measure used in risk assessments. Although up-to-date toxicity information is important, useful updates for ERAs are infrequent. To ensure that all potentially relevant information is collected, the literature search should be inclusive of all intra-class foraging guilds (e.g., small mammalian herbivores and mammalian invertivores).

Unpublished data that are scientifically defensible can be used if the data (or study) is provided in the final wildlife toxicity assessment report.

When toxicity data are unavailable for a class of animals (e.g., birds), data from other classes of animals will not be used to derive a quantitative measure of toxicity[4]. Physiological differences between taxonomic classes are assumed to be too great to make any **extrapolation** useful in predicting effects to another taxonomic class of animal (e.g., using mammal data for birds). This science-policy choice is based on three points.

a. As the taxonomic distance increases between any two groups of organisms, physiological differences increase and the uncertainty associated with toxicity extrapolations across those taxa increases [Suter 1993]. This has been recognized by the USEPA who state that

[4] An appropriate exception is when the mechanism of toxicity is clearly known and an understanding of the physiological differences allows for extrapolation.

"whatever methods are employed...it is important to apply the methods in a manner consistent with sound ecological principles and the availability of an appropriate database" [USEPA 1998, p. 26878].

b. Extrapolations between two species may be more credible if factors such as similarities in food preferences, body mass, physiology, and seasonal behavior are considered [Sample et al. 1996, USEPA 1998].

c. Extrapolation requires context, and employing the use of large (3 or 4 orders of magnitude) uncertainty factors is unrealistic as identified in current guidance [Chapman et al. 1998, USEPA 1998, USACE 1996].

In these cases, the following strategies can be used to assist in an ERA although they do not produce TRVs. Other strategies than those listed here may be appropriate; however, they should be based upon site-specific conditions.

a. Acknowledge the uncertainty due to the lack of appropriate data. Qualify the extent and direction in which inter-class physiological differences are expected to influence any toxicity estimate.

b. Apply methods using Quantitative Structure-Activity Relationships (QSARs) to estimate the toxicity when there is information on a structurally similar organic substance that has a suspected similar mode of action. This alternative is useful when assessments have historically used a chemical presumed to be the most toxic of a class of chemicals. For example, using the benzo(a)pyrene TRV for other similar polycyclic aromatic hydrocarbons (PAHs) when no useful toxicity data are available for other PAHs.

c. Employ alternative lines of evidence for assessing ecological risk. Examples are:

- Measures of the likelihood of exposure given availability and quality of habitat;
- Measures of spatiotemporal scale of the extent of contamination;
- Measures of species diversity/abundance, toxicity tests; and
- Measures of fitness, and reproductive performance.

Predominantly, the data collection/literature search effort will result in identifying relevant controlled toxicity studies. Tissue investigations and field evaluations rarely provide appropriate cause-and-effect data that are helpful in deriving TRVs. However, this information should be provided and discussed in the toxicity profile, if applicable.

2.2 Identification of Relevant Studies

After the data collection/literature search effort is completed, the studies that are relevant to the development of TRVs applicable to wildlife need to be identified.

The paragraphs below discuss the criteria used to select toxicity data relevant to TRV development. The available studies in the literature may not satisfy all of these criteria; therefore, those studies that satisfy as many of these criteria as possible will be considered relevant. In most cases, it is expected that a small set of studies will be identified that are 'nearly equivalent' in terms of their relevance.

a. The toxic effects identified are most clearly linked to factors suspected to greatly influence population sustainability (i.e., demographic rates: birth, death, and dispersal rates). Prior knowledge of factors most relevant in population-specific regulation is needed. More often than not, this information will not be available specific to the animals of concern. In this case, choosing the **endpoints** that are protective of the other endpoints is recommended (i.e., considering sensitive endpoints). Toxicological endpoints should be evaluated in terms of their relevance to the health and ecology of the whole organism(s). Several endpoints that satisfy these criteria are-

(1) Mortality.
(2) Reproduction.
(3) Development.
(4) Growth.
(5) Behavior relevant to reproduction, feeding, and predator avoidance.
(6) Decreased resistance to disease (stress).

Other indirect acting endpoints may also be important. Examples may include factors that influence energy allocation that may indirectly influence reproductive performance and success. In the absence of sound ecological knowledge for the species' of concern, these endpoints must be considered as nearly equivalent.

This criterion is designed to focus TRV development on the types of wildlife health effects that are most relevant to risk management goals. It assumes that the goal is to protect against a decline in a wildlife population. Therefore, the most important toxic endpoints are those listed above in the order of their theoretical relative importance to population sustainability. This criterion is consistent with USEPA guidance [USEPA 1997, pp. 1-9].

b. The exposure duration in each study should be clearly identified. Typically, chronic exposures should be most protective, thus most relevant. However, given the differences in species response, methods, observed effects, dispersal characteristics and habitat use in the field, and all potential toxicological endpoints, all exposure periods should be considered. The following guidelines are used to determine the exposure duration of a toxicity study:

(1) Chronic exposures are considered to be those equal to or greater than 10% of the life span of the test organism. An exception to this criterion is when exposure occurs during a sensitive life stage such as gestation. Classifying such tests as "chronic" is considered reasonable for endpoints specific to that life stage (e.g., embryo development and clutch size).

(2) Subchronic exposures are considered to be those repetitive exposures less than 10% of the life span of the test organism, yet greater than 14 days.

(3) Acute exposures are considered to be those of a single or repetitive exposure less than 14 days or 10% of the life span of the test organism.

These exposure duration definitions were developed primarily from USEPA regulations concerning regulatory toxicity testing under the Toxic Substances Control Act (TSCA) and the Risk Assessment Guidance for Superfund [USEPA 1998b, 1998c, and 1989]. Also considered were references provided in the USEPA Great Lakes Water Quality Initiative Technical Support Document for Wildlife Criteria [USEPA 1995a, pp.11-12] and the work of Sample et al. [1996].

For mammalian tests, defining tests that are greater than 10% of the test organism's life span as chronic is consistent with USEPA regulations for conducting toxicity studies under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and TSCA. Exposure during a sensitive life stage (e.g., gestation and embryo development) is considered a reasonable criterion to classify a test as chronic because of the potential for impaired reproduction and development. This is consistent with the method of Sample et al. [1996]. For subchronic mammalian tests, the USEPA defines a 90-day exposure duration as a standard for mice and rats, yet describes those exposures as approximately 10% of the life span of the animal [USEPA 1998b and 1998c]. Tests that are single exposures of extremely short duration (< 14 days) are considered acute.

c. The effect levels in the study should be those most clearly associated with no-to-low adverse effects. The type of effect levels that satisfy this criterion are--

(1) No-observable-adverse-effect-level **(NOAEL).**

(2) Lowest-observable-adverse-effect-level **(LOAEL).**

(3) Effect Dose **(Ed_x)**, where x is less than 50.

The effect levels most useful for an ERA are those at the low end of the dose-response function.

d. The exposure pathway in the study most closely matches the pathway that will contribute the most to the exposure in the field. This will be a professional judgment

determination. For example, for oral exposures a feeding study may be preferred to a gavage study if the dose in food was well characterized and more applicable to the exposure route and matrix in the field.

e. The overall validity of the study design (e.g., exposure conditions and chemical form) relative to the appropriate exposure pathways in the environment will ensure the best possible toxicological risk estimate.

f. The quality of the study must be assessed and determined to meet general, minimal requirements appropriate for inclusion. Criteria that must be considered include--

(1) The variability in response (i.e., power of the statistical comparisons) must be assessed to be relevant and par to other studies considered for a specific substance and class of vertebrates.

(2) Bioavailability of the substance in the field and the one used in the toxicity studies must be comparable.

(3) Dose (administered) was quantified appropriately with a minimal amount of variability.

(4) Repeatability of study. Sufficient information must be presented to allow for a given study and its results to be repeated.

(5) Corroboration with other similar data.

A statement that describes the quality of all included relevant studies (or minimal criteria) should be presented in the toxicity profile after a characterization of effects, yet before the table or scatter diagram is completed.

The final step during relevant study identification is to determine if the relevant studies collected provide the data necessary to meet the minimum data set requirement. The minimum data requirements are—

- Data exist from at least three studies of sufficient quality to be deemed relevant (using the above criteria) which collectively provide data for three or more species within the taxonomic class.
- Data exist for at least two different taxonomic orders.
- At least two chronic LOAELs and at least one chronic NOAEL are available.

These minimum data set requirements for test organisms is consistent with the number of species required for the certification of substances for Food and Drug Administration (FDA) approval for human applications [FDA 1966]. Given the current state of the toxicological database, and the general variation in toxic response between species within a class, these requirements are considered reasonable. The minimum requirement for endpoint selection is based on professional judgment and experience with the literature. Section 2.4.4 discusses procedures for dealing with cases where the minimum data requirements are not met.

2.3 The Toxicity Profile

The toxicity profile is the written documentation of the collected information regarding the toxicological characteristics of the chemical(s) of interest before the selection or development of the TRVs. The toxicity profile must be designed to provide all the necessary documentation needed for the final TRV report to be clear and transparent. This is needed in order to defend risk management decisions.

A toxicity profile consists of two components:

a. Documentation of the literature search and how the relevant studies were selected.

b. Presentation of the data relevant to the development of TRVs (including a table and a scatter diagram of effects).

The toxicity profile should summarize the basic physicochemical characteristics of the chemical(s) and basic environmental fate and transport information. Such information is useful for the understanding of the potential exposure and toxicity of the chemical(s).

The documentation of the data collection and literature search should include—

a. The dates of the search.

b. A description of the search strategy used (including key words for computer searches) and the results.

c. An account of the relevant references obtained from which information was collected.

d. A listing of the literature sources actually reviewed.

The main portion of the profile should be the presentation of the available toxicity data. The extent of the discussion should provide all the information known about the nature of exposure and toxicity that is necessary for a risk assessor to understand the general characteristics of the chemical(s), yet be limited in scope (e.g., identify major target organs and endpoints, including details of the method of exposure, but not necessarily effects at higher exposures to non-target tissues). Major sources of information and data should be cited.

Major section headings should be organized first by class (e.g., mammals), then by route of exposure (e.g., oral, inhalation, dermal), and then by exposure duration (acute, subchronic, chronic). Exceptions can be made for appropriate mesocosm/microcosm or field studies.

All studies identified as relevant to the development of TRVs must be identified, and the rationale for their selection must be documented. The documentation should include a presentation of how each study satisfies the criteria used to identify them as relevant. The rationale behind the selection of particular studies and data to be used to develop TRVs needs to be documented so that it can stand up to peer review. Also, a discussion should be included summarizing the relevance of the available data with regard to population-level effects.

The profile shall include a scatter diagram that presents the quantitative data in the relevant studies specific to each taxonomic class. The scatter diagram will contain all reliable data regarding a specific route of exposure (e.g., oral), categorized based on endpoint (e.g., mortality, reproductive, developmental, systemic, and behavioral). Each data point presented in the scatter diagram will also be presented in table format including toxic endpoint, species, concentration and reference. All test species will be identified, as well as the effect levels (e.g., NOAELs and LOAELs). The scatter diagram approach is one of the best ways to summarize the data relevant to the development of TRVs. In this type of graphical representation, patterns of variability among species, endpoints, and exposure can be clearly evaluated.

To be consistent, the form and appearance of this presentation should generally follow the example provided in Appendix C.

2.4 TRV Derivation

At this point in the process, the toxicity profile is completed and all of the available data within a taxonomic class that are relevant to the development of TRVs have been presented. The toxicity profile will provide data that can be used to develop TRVs that will be protective of the entire taxonomic class and, in some cases, TRVs that are more specific to a **guild association**.

The USACHPPM Wildlife TRV Report will develop TRVs for each taxonomic class where sufficient data exists. Such class-specific TRVs are most useful as screening-level tools. This will allow project-specific screening-level assessments to be conducted with limited data analysis. In order to proceed through the ERA process with limited resources, the screening approach is suggested as a way to feasibly evaluate the potential hazards of many substances in an efficient manner [USACE 1996, Tri-Services 1996, and USEPA 1997 and 1998]. This approach helps to reduce the generally long list of potential chemicals of concern at many sites to a more manageable list. It is biased to support decision criteria requiring a high level of confidence to determine whether or not to further investigate potential risks.

When more site-specific TRVs are needed for a particular project (i.e., TRVs for a guild association or particular species), the data provided in the toxicity profile section of the wildlife TRV report should be used to develop such a TRV, if the appropriate data are available. Depending upon available resources, each wildlife TRV report produced by USACHPPM may provide one or more guild association TRVs in addition to the class-specific TRVs. The Standard Practice does not result in species-specific TRVs that may be needed for some assessments (see Sample and Arneal 1999 for an approach based upon **allometry**).

2.4.1 TRV Development Approaches

The available data (as documented in the toxicity profile) will determine which of the three following procedures are to be used. Regardless of the procedure, two TRVs are developed for use—a low and a high. A bracketed range provides the risk assessor with a level of confidence between which no observed adverse effects may occur and where low adverse effects may occur. It also allows for flexibility while considering the magnitude of uncertainty by not defining a bright line threshold. A range can be used to discriminate the relative importance of exposures that exceed the low TRV (e.g., when the HQ > 1). Although procedurally different, this concept is based on the collaborative work of the U.S. Navy, USEPA Region 9, California EPA, and others [PRC 1997].

a. Benchmark dose approach. Data that show a clear dose/response relationship in a unimodal design are best used to derive two TRVs based on the benchmark dose approach.

b. NOAEL/LOAEL approach. Data that do not have clear dose response relationships within well-designed and conducted parameters should be used to derive two TRVs, one based on an NOAEL and one based on an LOAEL.

c. Approximation approach. Where data are scarce and cannot be used for the aforementioned procedures, then the second approach will be approximated with the use of **uncertainty factors** (UFs) to derive TRVs that estimate an NOAEL and/or an LOAEL.

Each of these approaches describes development of pathway-specific toxicity values that can be used to evaluate an exposure consistent with the pathway of interest. For some organisms (e.g., terrestrial amphibians or pulse-feeding reptiles), a pathway-specific exposure TRV may not be appropriate since total exposure to the media would best describe exposure and would most likely be represented in the literature. In these cases, media concentrations (i.e., soil concentrations) can be derived using the same logic presented in each of the above procedures.

2.4.2 Benchmark Dose Approach

The benchmark dose approach uses the dose response curve to select the dose that corresponds to a 10% response (the ED_{10} or benchmark dose) and a dose that corresponds to the lower bound on the ED_{10} (the LED_{10}; based on the lower 95% confidence limit). These two doses (the ED_{10} and the LED_{10}) are then selected as the TRVs.

The benchmark dose represents the dose level that is associated with the effect level of concern. Since the precise shape of the dose/response relationship is critical at low estimates (Moore and Caux 1997), a 10% benchmark response is recommended as the "threshold for adverse effects" [USEPA 1998 and 1997] for the assessment endpoint. This infers that there is a 90% chance that no adverse effects will occur at exposures at the specified daily intake levels. The benchmark dose should ultimately be defined as an effective dose (e.g., ED_{10}) on the dose-response curve where, if exposures exceed the dose, it is suspected that adverse changes in the assessment endpoint will begin to become unacceptable. In this procedure, a study is chosen from those determined relevant based on endpoint, design, model, and overall quality. The endpoint selection should be one that is either suggestive of a population-relevant endpoint (see Section 2.2) or, when that is not known, is protective of the other endpoints.

The use of this approach is expected if available toxicological data can support it (i.e., if the data from the relevant studies identified in the toxicity profile can be used to develop a reasonable dose response curve). The curve should be developed using methods that are consistent with the current regulatory guidance on developing dose response curves and benchmark doses for use in risk assessment [USEPA 1995b] and the Benchmark Dose Software (BMDS, currently version 1.2) available from the USEPA National Center for Environmental Assessment found at the following address: www.epa.gov/ncea/bmds.htm.

The USEPA states that the "advantages of curve-fitting approaches include using all of the available experimental data and the ability to interpolate to values other than the data points measured" [USEPA 1998, p.26876]. These curves are more defensible and more useful in predicting and communicating risk. The shape of the dose response curve can be used to determine the presence or absence of an effects threshold, to evaluate incremental risks, and used as input for effects models (e.g., demographic models) [USEPA 1998].

The disadvantages of using dose-response curves are that the number of data points needed to complete the analysis are often not available, it is time intensive, and it is not always practical for toxicants that have a complex dose response relationship [USEPA 1998]. If

sufficient and appropriate data exists, however, the USEPA guidance supports the use of this approach [USEPA 1998 and 1995b].

2.4.3 NOAEL/LOAEL Approach

This approach produces two TRVs for the wildlife group of interest: the LOAEL for the most sensitive and ecologically relevant endpoint and the NOAEL for that same endpoint. These TRVs will be selected from the scatter diagram provided in the toxicity profile.

When the minimum data set requirements are met (Section 2.2) for the wildlife group of interest, then the TRVs are chosen from the studies identified as relevant in the toxicity profile using the following procedure. Selections should be made or reviewed by a toxicologist familiar with the literature.

a. Choose the LOAEL-based TRV by selecting the lowest documented LOAEL that either is suggestive of a population-relevant endpoint (Section 2.2) or, when that is not known, the LOAEL that is protective of the other endpoints.

b. Choose the NOAEL-based TRV by selecting the highest NOAEL (that is lower than the selected LOAEL) within the same endpoint as the selected LOAEL. If an NOAEL from the same endpoint is unavailable, then the highest NOAEL (that is less than the selected LOAEL) within all relevant endpoints should be selected.

The use of the NOAEL and LOAEL in screening-level assessments is consistent with USEPA guidance [USEPA 1997]. Selecting the highest NOAEL that is less than the lowest LOAEL, assuming that both toxic endpoints are relevant, is consistent with USEPA guidance [USEPA 1997, pp. 1-10] and ensures against unnecessary overprotection (i.e., where the lowest possible NOAEL is selected).

Chronic effect levels should almost always be included; however, an acute or subchronic exposure period may include important toxicological endpoints for some species and may better represent interspecific sensitivities. If the exposure duration of concern in an ERA is not the chronic scenario, then the choice of the exposure duration for the selection of the TRV should be left to the professional judgment of the project toxicologist.

Deviations from this procedure are acceptable if the reported toxicity data are not consistent with other work (e.g., outlier data) or if the endpoints are of questionable ecological relevance (e.g., enzyme induction).

When the minimum data requirements are met, the toxicity profile and its scatter diagram represent all the available data within a class of animals (including sensitive species); therefore, no UFs are needed to modify the values in setting the TRVs. All relevant class-specific data for each substance (including sensitive species) would be included in the toxicity profile (e.g., all mammal data). This format allows the variability in the data to be used to determine the taxonomic differences in toxicity instead of ambiguous UFs. This approach is consistent with guiding principles of toxicity data extrapolation [Chapman et al. 1998].

If the minimum data requirements are not met for the wildlife group, then the approximation approach should be used to develop the TRVs.

2.4.4 Approximation Approach

If the minimum data requirements are not met, then this approach is used. When the data set requirements are not satisfied, it means that the available toxicity data are insufficient to characterize toxicity for a class of animals with the desired degree of certainty. Therefore, it becomes necessary to use UFs in the development of TRVs until more toxicity data are available.

In this approach, the most relevant study identified in the toxicity profile that is most reliable in terms of quality and applicability should be used to develop TRVs that approximate the NOAEL and LOAEL-based TRVs described previously. These TRVs are developed by dividing the effect level of interest by appropriate UFs where multiple UFs are multiplied before dividing.

Extrapolation from a single study or from data that are unreliable given an understanding of the design (e.g., power of the statistical comparisons) may be not be appropriate. Professional judgment by a toxicologist is recommended to determine if the development of TRV approximations from limited data are justified.

The UFs used to develop TRVs need to account for potential differences in response between species, and differences in response due to exposure duration (e.g., acute vs. chronic) and endpoint (e.g., lethality vs.

NOAEL). A general UF of 10 to protect against potential interspecies differences should be used for screening-level assessments.

The UFs in Table 1 should be used to account for differences in exposure duration and endpoint. Most of these factors are based on the work of Ford et al. [1992], and are also presented in the current Tri-Service guidelines [Tri-Services 1996]. The factor for the chronic LOAEL to chronic NOAEL conversion is 10, whereas Ford et al. [1992] would apply a factor of 5. The USEPA identifies an approach that would apply a factor of 10 [USEPA 1997, pp. 1-10], based on an evaluation by Dourson and Stara [1983]. Note that where Ford et al. [1992] uses a combined UF of 16 to account for interspecies variability, this procedure uses a UF of 10 (see paragraph above). The rationale behind this change is that Chapman et al. [1998] recommends that any particular factor used in extrapolation should be limited to an order of magnitude.

Table 1. Uncertainty factors accounting for differences in response due to exposure duration and endpoint

Type of data available	UF to approximate a TRV that is	
	NOAEL-based†	LOAEL-based*
Chronic NOAEL	1	na
Chronic LOAEL	10	1
Subchronic NOAEL	10	na
Subchronic LOAEL	20	4
Acute NOAEL	30	na
Acute LOAEL	50	10
LD50	100	20

(†) Ford et al. 1992, except for the chronic LOAEL
(*) The factors for approximating an LOAEL-based TRV are derived using the other factors, assuming the chronic LOAEL is 5 times the chronic NOAEL.
(na) not appropriate

These UFs may be updated as new or as class- or chemical-specific information becomes available.

2.5 Confidence Level Assignment

All measures of effect contain some degree of uncertainty. The data available to develop TRVs are usually limited and not equal in their ability to describe risk. An assigned level of confidence should be used to communicate this fact, as it can be helpful to risk assessors and risk managers in—

- Determining the accuracy of the risk estimate.
- Judging overall uncertainty.
- Deciding where to focus additional resources to increase certainty.

The purpose of this step is to ensure that a qualifying estimate of the reliability for each TRV is documented and available.

The confidence levels should be qualitative (high, medium, and low) estimates of accuracy in the toxicity estimates. They should be based on professional judgment reflecting the confidence that the toxicologist has that the TRV selected will be accurate in predicting benchmarks of toxicity. Factors considered may include the range of interspecific variation in response, completeness of the database, and overall quality of the experiments from which the conclusions were based.

This step is consistent with the methods used by the USEPA in RfD derivation in human health risk assessment applications.

2.6 The TRV Report

The wildlife TRV report for a chemical shall describe the derivation of the TRV that, at a minimum, shall consist of the following components:

a. Discussion of how the data were used to generate the TRVs.

b. Documentation of the rationale behind all decisions made in the development of the TRVs.

c. Documentation of the confidence associated with each measure.

APPENDIX A

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APPENDIX B

GLOSSARY

Allometry — The USEPA [1998, p. 26880] provides the following discussion: "Allometry is the study of change in the proportions of various parts of an organism as a consequence of growth and development. Processes that influence toxicokinetics (e.g., renal clearance, basal metabolic rate, and food consumption) tend to vary across species according to allometric scaling factors that can be expressed as a nonlinear function of body weight."

Demographic Rates — Demographic rates refer to survival rate, birth rate, death rate, dispersal rate (i.e., immigration and emigration), and recruitment rate.

ED_x Values — An effective dose (ED) is one that elicits a response in a percentage (x) of animals tested. For example, consider a test where 10 out of 100 animals experience reduced growth after they are exposed to chemical X at a concentration approximately equal to 25 units per day for their lifetime. This result, lifetime exposure of 25 units per day of chemical X, can be expressed as the ED_{10} for growth effects.

Endpoints — Adverse effects that are likely to occur in a terrestrial vertebrate as a result of exposure to a contaminant. These effects need to be considered in an ecological context where effects likely to alter reproductive performance (e.g., courtship, nest defense, etc.), subsequent reproductive success (e.g., mortality) or other factors (e.g., interspecific competition, dispersal) are important in the life history of the species, the population, or the community.

Guild or Guild Association — In a general sense, a guild (or guild association) is a group of species with similar functional roles within a community [Simberloff and Dayan 1991]. In this document, guild refers more specifically to a group of species that have similar foraging (i.e., feeding) behavior and are related taxonomically (currently defined as within the same class). The implicit assumptions are: (1) species with similar foraging behavior are likely to be exposed to chemicals in similar ways and (2) the more taxonomically related species are, the more similar they are in terms of sensitivity to a toxin. Guild associates are the individual species within a particular guild.

NOAEL and LOAEL — These are acronyms for two toxicological endpoints. The NOAEL (no-observed-adverse-effect-level) is a concentration associated with no observed adverse effects in the tested organisms. The LOAEL (lowest-observed-adverse-effect-level) is a concentration associated with the lowest observed level of adverse effects in the tested organism.

Reference Dose (RfD) — An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from an NOAEL, LOAEL, or benchmark dose, with UFs generally applied to reflect limitations of the data used. Generally used in USEPA's noncancer health assessments.

Taxonomy and Taxon — Taxonomy is the science of classification as applied to organisms. A taxon is any group of organisms to which any rank of taxonomic classification is applied. Taxonomic nomenclature are based on a hierarchy of phylogeny (or similarity) of groups. Examples include species, genus, family, order, class, and phylum.

Toxicological Data Extrapolation — The procedure that estimates dose-response relationships for organisms that have not or cannot be tested themselves. It entails the process of inferring toxicity characteristics from a set of empirical toxicity data for an organism or taxon to other organisms or taxons.

Toxicity Reference Value (TRV) — A chemical concentration expressed as an administered dose (e.g., oral, inhalation or dermal dose) or as a media concentration for terrestrial amphibians that is used in conjunction with an exposure prediction to estimate health hazard or ecological risk.

Uncertainty Factor (UF) — A numerical value used to adjust an estimate of toxicity or risk. It is an approach for dealing with uncertainty related to assessing chemical risks.

APPENDIX C

WILDLIFE TOXICITY ASSESSMENT REPORT FOR 2,4,6-TRINITROTOLUENE (SAMPLE DOCUMENT)

Wildlife Toxicity Assessment for 2,4,6-TRINITROTOLUENE (TNT)

OCTOBER 2000

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

USACHPPM Project No: 39-EJ-1138-00
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Department of the Army
U.S. Army Center for Health Promotion and Preventive Medicine

Wildlife Toxicity Assessment for 2,4,6-Trinitrotoluene

CAS No. 118-96-7 **25 September 2000**

1. INTRODUCTION

This Wildlife Toxicity Assessment is the result of a thorough investigation of the scientific literature regarding the toxicological characteristics of 2,4,6-trinitrotoluene (TNT) that may be important for the health of wildlife (mammals, birds, reptiles and amphibians) exposed to the substance. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

This document is designed to support ecological risk assessment activities. The measures of toxicity derived in this document are intended to be used in screening-level assessments. By definition, the measures of toxicity presented herein evaluate the likelihood of effects in *individual* organisms that may be relevant to a *population* of organisms in the wild. This Wildlife Toxicity Assessment does not specifically address how the measures, or any resulting risk estimates, relate to demographic rates or outcomes for any particular population of interest. Assessing risk to populations involves using these methods and other lines of evidence before risk management actions to protect populations can be recommended based upon scientific information. Therefore, the toxicity measures in this document should not be used to demonstrate unacceptable population risks that require remedial action without further site-specific study.

2. TOXICITY PROFILE

2.1 Literature Review

Given the predominant military use of trinitrotoluene, many studies were found from U.S. Army sources. These military sponsored studies, and subsequent reports, were found through TOXLINE and DTIC searches. However, the most appropriate ones were found though traditional cross-referencing techniques and through individual queries to project investigators within the Army. Several databases were searched and Appendix A contains details of this search.

2.2 Environmental Fate and Transport

The distribution of TNT at many U.S. military sites is substantial. At least 17 Army installations have reported soil concentrations ranging from 0.08 to 64,000 micrograms per gram (µg/g) (Hovatter et al. 1997). Of those that had detectable concentrations, 5 installations had samples in which surface soils exceeded 10,000 µg TNT/g soil dry weight (Walsh and Jenkins 1992).

A summary of physical and chemical properties is provided in Table 1. An important route for the contamination of surface water, ground water, and surface soils with TNT has historically been due to large aqueous effluents of rinse water ("pink water," Walsh and Jenkins 1992, ATSDR 1995). Some sources have reported wastewater emissions ranging from 61 – 210 pounds/day (Rosenblatt et al. 1973). Due to its relatively low vapor pressure, and relatively high water solubility, TNT does not actively partition from surface waters to the atmosphere (ATSDR 1995). Photolysis studies, comparing river waters and distilled water, have shown that the rate of TNT photolysis is directly related to increases in pH and organic matter content (Spanggord et al. 1980). Generally, TNT is not expected to hydrolyze or bioconcentrate in aquatic systems under normal environmental conditions (HSDB 1997).

Table 1. Summary of Physical-Chemical Properties of 2,4,6-Trinitrotoluene

CAS No.	118-96-7
Molecular weight	227.13
Color	yellow-white
State	Monoclinic needles
Melting point	80.1°C
Boiling point	240°C
Odor	Odorless
Solubility	130 mg/L in water at 20°C; soluble in acetone, benzene, alcohol and ether
Partition coefficients	
Log K_{OW}	1.60; 2.2 (measured), 2.7 (estimated)
K_{OC}	300 (estimated), 1,100 (measured)
Vapor pressure (at 20°C)	1.99E-04 mm Hg
Henry's Law constant (at 20°C)	4.57E-07 atm m^3/mole
Conversion factors	1 ppm = 9.28 mg/m^3 1 mg/m^3 = 0.108 ppm

Source: ATSDR (1995)

Soil contamination of TNT can result from spills, disposal of solid waste, open incineration and detonation of explosives, or leaching from poorly engineered impoundments (Burrows et al. 1989). Retrieval and subsequent destruction of unexploded ordinance (UXO) can result in soil contamination as well (includes open burning/open detonation, OB/OD areas). Based primarily upon the physical and chemical properties of TNT (i.e., octanol-water partition coefficient (K_{ow}) and water solubility), TNT is not expected to bioaccumulate or biomagnify in terrestrial systems (HSDB 1997).

Based on the measured and estimated soil organic carbon adsorption coefficient (K_{oc}) of 300 – 1100, TNT is not expected to significantly partition to sediment (from surface waters) or sorb to soil particles (HSDB 1997, ATSDR 1995). However, the biotransformation of TNT in soil can be significant, and can be readily reduced under anaerobic conditions. These anaerobic reactions occur through microbial reduction, primarily through successive reduction of the nitro groups (Burrows et al. 1989). Several bacteria have been identified in these reactions. They include species of *Pseudomonas, Escherichia, Bacillus, Citrobacter, Enterobacter, Klebseilla, Veillonella,* and *Clostridium* (Burrows et al. 1989). Fungi are also capable of reducing TNT (Burrows et al. 1989, ATSDR 1995). Microbial transformation of TNT leads to a variety of reduction products, including 2-amino and 4-amino dinitrotoluene and azoxydimers (Burrows et al. 1989, HSDB 1997), though some oxidation products have been identified (Won et al. 1974). Biological transformation by bacterial and fungal species occurs slowly in the environment, with slightly higher rates in the presence of other carbon sources. However, biological degradation may not extend to cleavage of the TNT ring (the successive reductions of each of the nitro groups to amines followed by oxidative deamination to a phenol that releases an ammonia or nitrite has been described (HSDB 1997)). Accurate mass balance without the use of radio-labeled compound is difficult with TNT based on its crystal forming tendencies, low organic solubility, and relatively low water solubility (M. Major, USACHPPM, pers. comm.).

Another process that can affect the fate and transport of TNT in the environment is photolysis. Photolysis has been reported to produce “pink water” from TNT-contaminated surface water (ATSDR 1995). Numerous transformation products have been identified in pink water, the predominant ones including 1,3,5-trinitrobenzene, 4,6-dinitroanthranil, 2,4,6-trinitrobezaldehyde, 2,4,6-trinitrobenzonitrile, in addition to several azo and azoxy derivatives formed by the coupling of nitroso and hydroxyamine products (Jerger et al. 1976, Spanggord et al. 1980).

2.3 Summary of Mammalian Toxicology

2.3.1 Mammalian Toxicity

2.3.1.1 Mammalian Oral Toxicity- Acute

Oral lethal dose to 50% of the exposed population (LD_{50}) values of 660 milligrams per kilogram (mg/kg) in male and female mice and 1320 and 795 mg/kg in male and female rats, respectively, have been reported (Dilley et al. 1982a). These animals developed seizures (grand mal), followed by mild convulsions 1 – 2 hours after exposure. All deaths occurred within 24 hours after exposure; red urine and lethargy were other signs of exposure (Dilley et al. 1982a). Animals that survived the convulsions were still alive 14 days following the exposure (Dilley et al. 1982b). Variation in response for dogs was considered significant (Voegtlin et al. 1921). Cyanosis was evident 12 hours following administration of 100 mg/kg TNT. Severe incoordination and tremors followed. However, the authors note that some dogs receiving 100% of the 100 mg/kg dose did not exhibit the same symptoms as those receiving 50% or less (Voegtlin et al. 1921). Most species showed signs of ataxia after dosing (Voegtlin et al. 1921, Dilley et al. 1982b).

Cats injected intraperitoneally with 0.10 to 0.15 grams per kilogram (g/kg) TNT died within 5.5 hours (Bredow and Jung 1942). Injections of 0.04 g/kg caused convulsions, paralysis of the hindlimbs, decrease in body temperature, and enhanced saliva secretion. Methemoglobin was also present in the blood. Cats given daily subcutaneous injections of 50 mg/kg TNT died within 4 to 9 days (Lillie 1943). Each showed signs of splenic congestion. Livers had fat accumulation (steatosis) and Kupffer cell hemosiderosis.

White-footed mice (*Peromyscus leucopus*; 10/group/sex) were exposed to one of five treatments of 0, 0.042, 0.083, 0.165, and 0.330% TNT in feed for 14 days (Johnson et al. 2000a). These treatments were calculated by the authors to be equivalent to 66, 145, 275, and 602 mg TNT/kg body weight per day (bw/d) for males and 70, 142, 283, and 550 mg/kg/d for females for the 0.042, 0.083, 0.165, and 0.330% TNT, respectively. Indicators suggesting hemolysis were evident in the 0.330% treatment for both sexes, where only males had suppressed splenic phagocyte hydrogen peroxide production for the 0.165 and the 0.330% treatments, and a reported reduction in phagocytosis for males in all TNT exposures. However, the authors note that the significance of the latter endpoint (i.e., inhibited phagocytosis for males and not females) is questionable.

Oral LD_{50} estimates for cotton rats (*Sigmodon hispidus*) exposed to TNT in corn oil were 607 and 767 mg TNT/kg bw for males and females, respectively (Reddy et al. 2000). Animals exhibited an increased respiratory rate within 90 minutes after dosing. Orange-colored urine and urinary bladder distension was observed in all animals at necropsy. No other abnormal histological observations were reported.

A 7-day gavage exposure representing 1/8, 1/4, and 1/2 the LD_{50} for male (75.9, 151.8, and 303.5 mg TNT/kg bw/d) and female cotton rats (96, 192, and 384 mg TNT/kg bw/d) was conducted using corn oil (Reddy et al. 2000). Histopathology of major organs as well as hematology, hepatic metabolizing enzymes, and clinical chemistry of the sera were evaluated. Splenic weights were increased in the 192 (females only) and the 384 mg/kg/d treatments; and liver weights were increased in the 151.8 (males only) and 303.5 mg/kg/d treatments. These two high dose groups also showed hematological results consistent with erythrolytic anemia. Hemosiderin laden macrophages were noted in the spleen of rats receiving the lowest dose. Subtle testicular lesions were noted in the two high dose groups.

2.3.1.2 Mammalian Oral Toxicity- Subchronic

Subchronic exposures to rats, mice, and dogs have produced consistent hematologic effects (Von Oettingen et al. 1944, Dilley et al. 1982b, Levine et al. 1990a, b). Exposures of 13 weeks were sufficient to produce anemia (consisting of reduced number of red blood cells, reduced hemoglobin and hematocrit) in all of these species. Increases in immature red blood cells (reticulocytes), reduction in blood, hematocrit, and corpuscle volumes were evident after only 15 days in dogs administered TNT in gelatin capsules of dosages ranging 5 – 33 mg (Voegtlin et al. 1921). TNT exposure is reported to result in direct hemolysis within circulating blood, leading to an increase in spleen weight. Dilley et al. (1982a, b) reported similar findings including pathological assessment of the spleen that suggested hemolytic anemia in beagles. Other important effects included increased liver weight (including hepatocytomegaly), intestinal inflammation (and mucoid stools), enlarged kidneys, and splenic congestion in mice, rats, and dogs (Dilley et al. 1982b, Levine et al. 1990a, b). Most animals in the highest dose group of all species displayed some degree of hemosiderosis of the spleen (Dilley et al. 1982b). Rats and dogs had dose-related increased serum cholesterol and lower iron and serum glutamic-pyruvic transaminase (SGPT) levels following the 13-week exposure period; mice seemed to be more resistant to treatment (Dilley et al. 1982b). Increased serum cholesterol was consistent with doses in rats and dogs (Levine et al. 1984, Dilley et al. 1982b). Other endpoints consistent with anemia were decreased erythrocyte numbers, hemoglobin and hematocrit values, and occasionally bone marrow hyperplasia.

Testicular atrophy was most pronounced in rats (Dilley et al. 1982b), and consisted of dose-related degeneration of the germinal epithelium lining the seminiferous tubules and hyperplasia of interstitial Leydig cells (in high dose group, 300 mg/kg/d; Levine et al. 1984). The No Observable Effect Levels (NOELs) for these three species were: dogs, 0.20; rats, 1.42; and mice, 7.76 mg/kg/d, suggesting that dogs were the most sensitive (Dilley et al. 1982b). Dilley et al. (1982b) also mention that the effects appear to be totally reversible (up to a 4-week exposure) following a 4-week recovery period.

A single study investigating the functional response of splenic phagocytes to TNT in NMRI mice was conducted (through chemiluminescent analysis) from exposure TNT metabolites (2,4-diamino-dinitrotoluene, 2,4,6 triaminotoluene, 2-amino-6-nitrotoluene, 4-amino-3,5-dinitrotoluene, and 2-amino-4,6-dinitrotoluene) *in vitro* (Thierfelder and Masihi 1995). This assay quantifies intracellular-activated oxygen species. Relatively high doses of metabolites were associated with reduced response relative to controls. Specifically, > 1 milligram per liter (mg/L) of 2,4-diaminotrinitrotoluene, >50 mg/L for 4-amino-3,5-dinitrotoluene, and > 100 mg/L for 2-amino-4,6-dinitrotoluene caused a plateau of 57 – 65% inhibition (Thierfelder and Masihi 1995).

Results of a 90-day feeding study using white-footed mice (*Peromyscus leucopus*) provided evidence that Nearctic mice may be more resistant than Palearctic (Old-World: *Mus)* species. McCain (1998) exposed 100 male and female *P. leucopus* to concentrations of 660, 1320, and 2640 parts per million (ppm) TNT in feed. The calculated dosage was about 165, 330, and 660 mg/kg/d, respectively. The highest concentration used in this study (2640 ppm; 660 mg/kg/d) was equivalent to the LD_{50} of 660 mg/kg reported by Dilley et al. (1982b) in *Mus*, yet none died during the study. Initial animal weight reduction consistent with reduced palatability was reported, yet all groups gained weight over time. McCain (1998) found only exposures to 1320 and 2640 ppm associated with adverse physiological changes (organ weight, incidence of chromaturia, hemosiderin, etc.), and established a No Observable Adverse Effect Level (NOAEL) of 660 ppm (165 mg/kg/d).

2.3.1.3 Mammalian Oral Toxicity- Chronic

Effects from chronic exposures were consistent with those of sub-chronic exposures. Two studies using Fisher 344 rats (Furedi et al. 1984) and beagle dogs (Levine et al. 1990a) reported dose-dependent indicators suggesting hemolytic anemia (e.g., reduced hemoglobin, hematocrit, and erythrocyte counts, increased quantities of reticulocytes). These effects were different from controls at doses ≥ 8.0 (i.e., and 32 mg/kg/d for dogs; Levine et al. 1990a) and for all TNT treatments for rats (i.e., 0.4, 2.0, 10.0, and 50.0 mg/kg/d; Furedi et al. 1984). Exposures for the rat study lasted 106 weeks and 26 weeks for dogs. Compensatory responses to anemia were minimal in rats (e.g., erythrocytic macrocytosis and reticulocytosis; Furedi et al. 1984). Methemoglobinemia was apparent in both studies in animals of the higher dose groups. Reduction in body weight was apparent in rats exposed to 10 mg/kg/d or greater, and at 8 mg/kg/d or greater for dogs (Furedi et al. 1984, Levine et al. 1990a). Dose-related hepatomegaly (and increased kidney weights) was evident in rats receiving > 2.0 mg/kg/d; this was only evident in the high dose group for dogs. Splenomegaly was evident in rats and dogs in the higher dose groups. Hemosiderosis in Kupfer's cells was seen in various dogs at most dose levels (Levine et al. 1990a). Renal injury was supported by gross and tissue morphological examinations (in high dose groups; Furedi et al.

1984). Increased pigment deposition occurred in the kidneys (as did evidence of bone marrow fibrosis) of rats exposed to 2.0 mg/kg/d or greater (Furedi et al. 1984). It was reported that the observed enteritis of the small intestine was related to TNT treatment in dogs (Levine et al. 1990a). Urinary bladder carcinomas were evident in some rats (2 males and 4 females of 1794 and 1754 rats, respectively) exposed for 106 weeks (Furedi et al. 1984). Given the rate of occurrence for these types of neoplasias, this finding was considered biologically significant. An NOEL was determined to be 0.4 mg/kg/d for rats (Furedi et al. 1984); none was found for dogs (Levine et al. 1990a). TNT was found to be mutagenic (without S9 activation) in *Salmonella typhimurium;* the reduced metabolites were less potent mutagens (Tan et al. 1992).

2.3.1.4 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

Primary target organs for TNT include the nervous system (primarily from acute effects) and blood (Table 2, Figure 1). Since TNT causes erythrolysis, the primary blood conditioning organs may also be affected (e.g., liver and kidney). These conditions were found in *Peromyscus* (McCain 1998), beagle dogs (Dilley et al. 1982b, Levine 1990a), rats (Furedi et al. 1984), and laboratory mice (*Mus*; Dilley et al. 1982b). Several studies were found that were current, well designed, and appropriate for the development of Toxicity Reference Values (TRVs) for mammals. The work of Dilley et al. (1982b), Levine et al. (1984, 1990a) and Furedi et al. (1984) are particularly valuable since they include chronic, subchronic and acute exposures, and use several species identified above. Two Orders and three families of *Mammalia* are represented that include: Carnivora: Canidae; Rodentia: Cricetidae, Muridae. Two wildlife species were also evaluated. Effects from exposure are consistent, yet slightly variable in magnitude of effect. Each study identifies several NOAELs and Low Observable Adverse Effect Levels (LOAELs) for various endpoints of effect, and the investigations are inclusive of other potential organ systems. It is for these reasons that this review is sufficient to derive class-specific TRVs for TNT.

With few exceptions, data from acute studies where gavage methods were employed were deemed irrelevant and not used for comparison (TRV derivation) purposes. Exceptions included acute or gavage studies that included other species not previously evaluated (e.g., Reddy et al. 2000). All other reports that evaluated TNT in feed were of sufficient quality and importance to include in this evaluation. These studies were consistent in quality and reporting of the methods.

2.3.2 Mammalian Oral Toxicity– Other

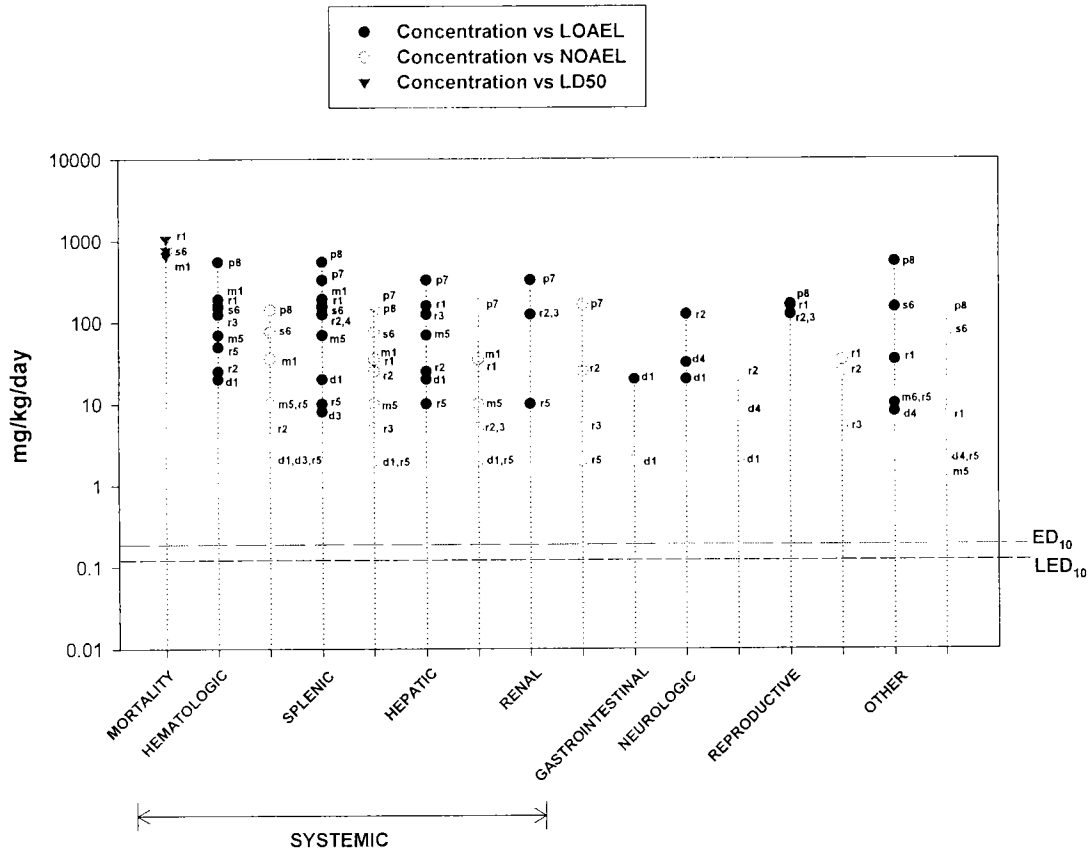
No other data relevant to oral exposures for mammals were found.

Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
McCain 1998	Mouse (*Peromyscus leucopus*)	90 d	165	330	Increased kidney, liver, spleen weights; presence of hemosiderin in spleen; chromaturia; increased extramedullary hematopoiesis in spleen
Johnson et al. 2000	Mouse (*Peromyscus leucopus*)	14 d	142	550 (?)	Indicators of erythrolytic anemia (increased spleen weight, histopathology); decreased intracellular hydrogen peroxide of splenic phagocytes); phagocytosis results of uncertain biological significance
Reddy et al. 2000	Cotton rat (*Sigmodon hispidus*)	7 d	76 (?)	152 (?)	Erythrolytic anemia; changes in spleen and liver pathology, hematology; changes in hepatic glutathione S-transerfase for females, not males of uncertain biological significance; male dose protective of female dose for all other endpoints.
Dilley et al. 1982b	Rat (Sprague-Dawley)	13 wk	1.4	160	Anemia and leukocytosis
			34.7	160	Increased cholesterol, decreased body weight (10-20%), increased spleen weight, hemosiderosis, lymphocytosis; testicular atrophy
			7	34.7	Decreased food consumption
	Mouse (Wistar)	13 wk	35.7	193	Decreased hematocrit/RBC, liver necrosis
	Dog (Beagle)	13 wk	2	20	Mucoid stools (red), diarrhea, anemia, increased liver weight, bilirubin, and cholesterol; lethargy
Levine et al. 1984	Rat (Fisher 344)	13 wk	5	25 (?)	Anemia, increased serum cholesterol
			25	125	Lipofuscin-like pigment in renal cortex, splenic enlargement with congestion, slight lethargy and ataxia; reduced food intake and body weight; atrophic seminiferous tubules, degenerated germinal epithelium
Levine et al. 1990a	Dog (Beagle)	6 mos	2 (?) 8 (?)	8 (?) 32 (?)	Anemia, methemoglobinemia, increased platelets, slight ataxia; chromaturia
			2 (?)	8 (?)	Decrease in body weight (16.4%; females at 32)
Levine et al. 1990b	Rat (Fisher 344)	13 wk	5	125	Increased spleen weight with diffuse congestion
Furedi et al. 1984	Rat (Fisher 344)	24 mos	10 (?)	50 (?)	Bone marrow fibrosis
			2 (?)	10 (?)	Increased cholesterol, enlarged liver; 14% decrease in body weight gain; splenic congestion, extramedullary hematopoiesis
Furedi et al. 1984	Mouse (B6C3F1)	24 mos	10 (?)	70 (?)	Mild anemia, increased liver weight, reduced serum globulin levels; 10-15% decrease in body weight gain; enlarged spleen and lymph nodes

Figure 1.

TNT HEALTH EFFECTS TO MAMMALS



1= Dilley et.al. 1982b
2 = Levine et.al. 1984
3 = Levine et.al. 1990a
4 = Levine et.al. 1990b
5 = Furedi et al. 1984
6 = Reddy et al. 2000
7 = McCain 1998
8 = Johnson et al. 2000a

RAT (*Rattus*) = r
MOUSE (*Mus*) = m
DOG (*Canis*) = d
New World Mouse (*Peromyscus*) = p
New World Rat (*Sigmodon*) = s

2.3.3 Mammalian Inhalation Toxicity

No inhalation studies conducted using animals were found.

2.3.4 Mammalian Dermal Toxicity

No dermal studies conducted using animals were found; however, information suggesting the importance of dermal exposures for humans has been reported (Hathaway 1977, Woollen et al. 1986). In addition, studies investigating the potential for TNT to transverse mammalian skin *in vitro* from a soil matrix have demonstrated that dermal exposures to TNT in soil may add to total systemic dose (Reifenrath 1994).

2.4 Summary of Avian Toxicology

2.4.1 Avian Toxicity - Oral

2.4.1.1 Avian Oral Toxicity - Acute

Three experimental trials for the acute lethal dose (ALD) were recently performed on Northern Bobwhite (*Colinus virginianus*) (Gogal et al. *in draft*). Both male and female birds were gavaged with single oral doses of 4508, 3005, and 2003 mg TNT/kg bw and observed for 14 days. All birds except one female exposed to 3005 mg/kg died within 5 days. The female dosed at 2003 mg/kg exhibited extreme ataxia, yet survived until necropsy. Reddish-brown stool was observed 24-48 hrs following dosing, characteristic of hematuria seen in mammals. A single oral dose of 2003 mg/kg was determined to be the lowest concentration resulting in death to Northern Bobwhite.

2.4.1.2 Avian Oral Toxicity - Subchronic

Adult male and female Northern Bobwhite (*Colinus virginianus*; N = 50) were provided TNT in feed at concentrations of 3300, 1560, 863, and 160 mg TNT/kg feed for a 90-day exposure (Gogal et al. *in draft.*). Initially, 4/10 birds died from exposure to the 3300 mg/kg treatment, yet none thereafter. Histopathology and sensitive indicators of immune function were evaluated. The effects included a dose-dependant non-significant decreasing trend in: total red blood cell counts, packed cell volume, total plasma protein, blood prolymphocytes, blood lymphocytes, an increase in late apoptotic/necrotic blood leukocytic cells, and slight hemosiderosis in the liver. It was noted by the authors that significant erythrolytic anemia does not seem to be the major target of toxicity in quail, most likely due to the refractory nature of the avian hematological and vascular system. No adverse histopathology was associated with any animal exposed to the 160 mg/kg treatment.

2.4.1.3 Avian Oral Toxicity- Chronic

No data are available for chronic exposures.

2.4.1.4 Avian Oral Toxicity- Other

No other avian studies are available for TNT.

2.4.1.5 Studies Relevant for Avian TRV Development for Ingestion Exposures

The only study found that evaluated the effects of TNT to birds was Gogal et al. (*in draft*). The 90-day results suggest that birds are much less sensitive to the hemolytic mechanisms found in mammals, yet there is evidence of some mild erythrolytic effect. Given the refractory nature of the avian hematopoietic system and the magnitude of these observations, these findings are of uncertain biological significance. Consistent with the mammalian data are the initial central nervous system (CNS)-related effects of exposure where individuals exhibited ataxia and neuromuscular effects. These effects were observed prior to death of the quail in the high dose group (3300 mg/kg). Therefore, an NOAEL of 7 mg/kg/d was suggested by the authors based upon the lack of adverse pathological and immunotoxicological observations for any individual in the low dose group (160 mg/kg). These data are summarized in Table 3 and Figure 2. An LOAEL was identified as 178 mg/kg/d based on the four deaths that occurred in the high dose group, and that possible adverse histopathology was associated with some individuals in the 3300 mg/kg group.

Table 3. Summary of Relevant Avian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL mg/kg/d	LOAEL mg/kg/d	Effects at LOAEL
		Lowest lethal dose detected (LD_{LOW}) 2003 mg/kg	na	na	Male dies during the determination of the approximate lethal dose at 2003 mg/kg; female did not.
Gogal et al. (*in draft*)	Northern Bobwhite (*Colinus virginianus*)	90 d	7	175	4/10 initial deaths in high dose group (3300 mg/kg); dose-dependant non-significant decreasing trend in: total red blood cell counts, packed cell volume, total plasma protein, blood prolymphocytes, blood lymphocytes, an increase in late apoptotic/necrotic blood leukocytic cells, and slight hemosiderosis in the liver.

na – not applicable

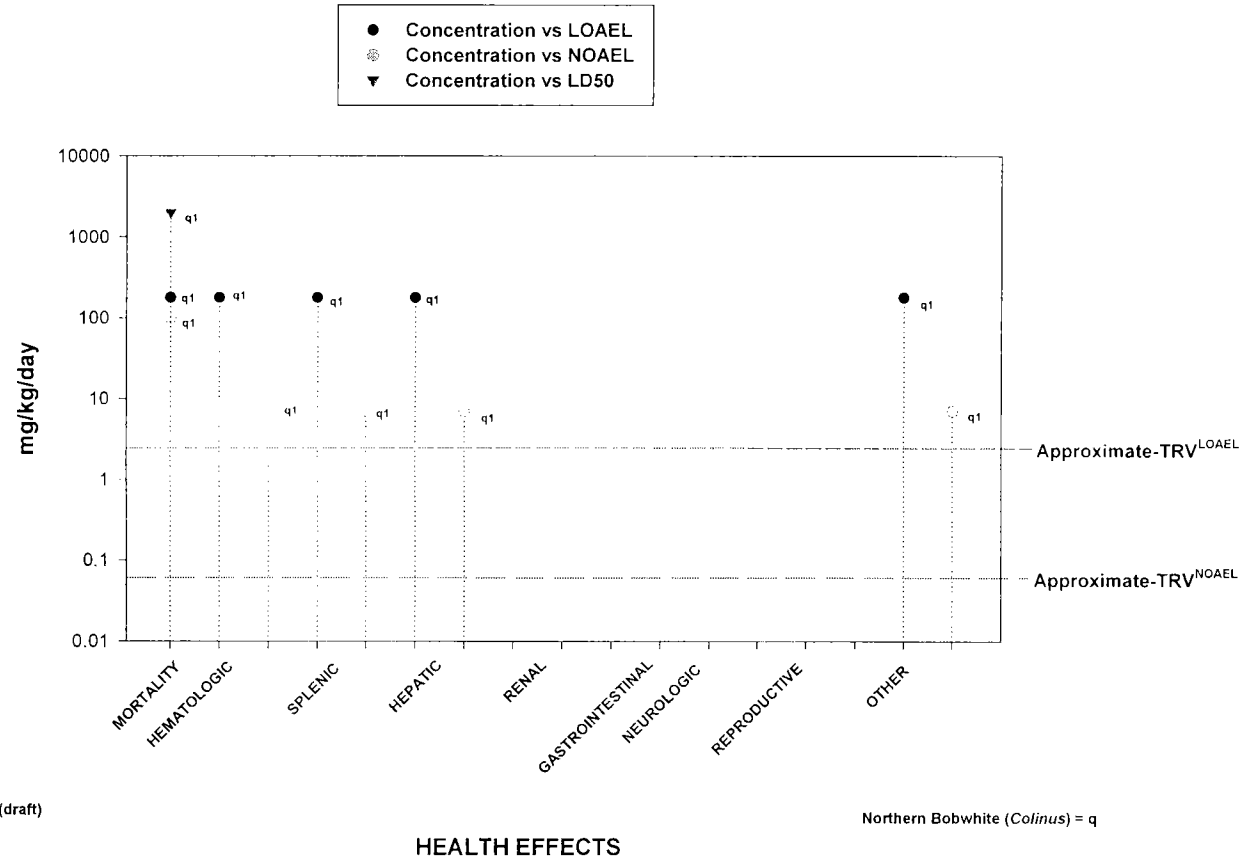
2.4.2 Avian Inhalation Toxicity

No data available.

2.4.3 Avian Dermal Toxicity

No data available.

TNT HEALTH EFFECTS TO BIRDS



1= Gogal et al. (draft)

Northern Bobwhite (*Colinus*) = q

2.5 Amphibian Toxicology

Only one study investigating 14-day exposures to TNT in soil in a terrestrial salamander was located.

2.5.1 Amphibian Microcosm Study

Tiger salamanders (*Ambystoma tigrinum*) were exposed to TNT in a soil matrix and were fed earthworms exposed to TNT in soil using a microcosm design for 14-days (Johnson et al. 2000b). Previous dermal exposures to TNT have been shown to be considerable compared to oral exposures in *Ambystomid* salamanders (Johnson et al. 1999). The TNT concentrations in soil reduced with time, ranging from 280 µg/g at the beginning to 59 µg/g at the conclusion. At which time the primary reduction products of TNT increased (39 and 62 µg/g at the beginning to 58 and 78 µg/g of 2-amino-4,6-dinitrotoluene and of 4-amino-2,6-dintitrotoluene at the conclusion, respectively). Concentrations of TNT in earthworms ranged from 0.25 – 0.62 µg/g, and from 2.1 – 2.6 µg/g of the primary reduction products mentioned previously. Immune function, histopathology, weight changes, and blood parameters were investigated. No adverse health effects were observed and the animals gained weight during exposure.

2.5.2 Relevance for Amphibian TRV Development

This study used a microcosm design that considered all pathways of exposure and potential variation in feeding regimes (Johnson et al. 2000b). Since soil concentrations of TNT were monitored, these data are used to derive a NOAEL for terrestrial salamanders, a soil concentration of 59 mg/kg that reflects all exposure pathways. Since adverse effects were not observed in the study, a LOAEL is not available.

2.6 Reptilian Toxicology

No data for reptiles are available.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Based on the information from five species, as described in Section 2.3.1.4, the dog appears to be the most sensitive mammal from oral exposures to TNT. The lowest LOAEL is 8 mg/kg/d, where Levine et al. (1990a) reported evidence of blood effects and decreased weight gain in dogs receiving 8 mg/kg/d but not at 2 mg/kg/d. The highest NOAEL within the same endpoints and species was the dose of 2 mg/kg/d reported by the same authors. Because decreased weight gain (an indicator of reduced growth and/or energy efficiency) and anemia have the potential to adversely effect future fitness, these endpoints are

considered to be ecologically relevant. In addition, this and the other studies satisfy the minimum data set requirement of the Standard Practice, Section 2.2 (USACHPPM 2000); thus, no uncertainty factors are needed to derive the TRVs. The data were appropriate for a benchmark dose derivation and are presented in Appendix B. A benchmark dose (BMD or ED_{10}) of 0.3 mg/kg/d was calculated from the model fit of the mean response at the 10% response level. A lower-bound on the benchmark dose (BMDL or LED_{10}) was calculated to be 0.2 mg/kg/d from the lower 95% confidence interval (CI) of the modeled curve. These values are selected as the class-specific TRVs (Table 4). Since these studies were well calibrated and the results are consistent with those of others, this TRV is given a high degree of confidence.

Table 4. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
LED_{10}	0.2 mg/kg/d	High
ED_{10}	0.3 mg/kg/d	High

3.1.2 TRVs for Ingestion Exposures for Mammalian Foraging Guilds

TRVs specific to particular guild associations (e.g., small herbivorous mammals) have not yet been derived. However, since the dog is the most sensitive mammal tested, the class-specific TRVs shown in Table 4 are considered to be protective of non-carnivorous mammals. More specific TRVs may be developed considering the data provided in Table 2.

3.1.3 TRVs for Inhalation Exposures for the Class Mammalia

Not available at this time.

3.1.4 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time.

3.2 Toxicity Reference Values for Birds

3.2.1 TRVs for Ingestion Exposures for the Class Aves

The only study that has evaluated the effects of TNT to birds is Gogal et al. (*in draft.*). These investigations evaluated hematological effects as well as systemic organ and sensitive immune parameters. Given the variation in response, only trends were evident. However, there were no incidences of adverse pathology associated with the low concentration treatment of 160 mg/kg. There were four mortalities in the high concentration treatment of 3300 mg/kg, and a non-significant dose-

related trend was evident in hematological and immune parameters. Though the biological significance concerning the magnitude of the hematological and immune parameters are questionable, the fact that mortality occurred initially in 4/10 animals is significant. The authors calculate an NOAEL at 7 mg TNT/kg bw/d at 160 mg TNT/kg feed dry weight treatment, and an LOAEL (serious) of 178 mg TNT/kg bw/d for the 3300 mg TNT/kg feed treatment. Since this is the only bird study, TRVs based on an approximation of the NOAEL and LOAEL were developed to represent the Class Aves. Given that the 90-d exposure regime represents <10% of the average lifespan of Northern Bobwhite it is considered a subchronic study. Therefore, an uncertainty factor (UF) of 100 was applied to account for interspecific variability (UF of 10) and to extrapolate from a single subchronic study (UF of 10). Table 5 presents the selected TRVs. A low level of confidence has been given to these TRVs because only one study is available, the single study only evaluates one species, and the study has relatively low power in its statistical comparisons.

Table 5. Selected Ingestion TRVs for the Class Aves

TRV	Dose	Confidence
NOAEL-based	0.07 mg/kg/d	Low
LOAEL-based	1.8 mg/kg-d	Low

3.2.2 TRVs for inhalation exposures for the Class Aves

Not available at this time.

3.2.3 TRVs for dermal exposures for the Class Aves

Not available at this time.

3.3 Toxicity Reference Values for Amphibians

Since the exposures were relatively brief, considering the average life span of *Ambystomid* salamanders (> 10 years), these were classified as acute exposures and an NOAEL was identified (Johnson et al. 2000b). In addition, since dermal exposures to TNT were reported to be considerable, a pathway-specific (i.e., oral) TRV would not be appropriate. However, since this study used a holistic exposure regime, a media-based value for soil could be derived. The acute (14-d) NOAEL of TNT in soil (59 µg/g) was divided by a UF of 300 to approximate a chronic NOAEL for terrestrial amphibians (a UF of 30 for an acute NOAEL to a chronic NOAEL and a UF of 10 to extrapolate across multiple species).

This resulted in an approximation of an NOAEL-based TRV of 0.2 mg TNT/kg soil dry weight intended to be protective of terrestrial amphibians. However, since an LOAEL was not identified, an approximation of an LOAEL-based TRV could not be derived. Table 6 presents the selected TRVs. A low confidence level has been assigned to the available TRV because a study observing adverse effects was not available, the only study is of limited length of exposure, and no other terrestrial amphibian data is available.

Table 6. Selected Soil TRVs for Terrestrial Amphibians

TRV	Dose	Confidence
NOAEL-based	0.2 mg/kg soil (dry weight)	Low
LOAEL-based	Not available	—

3.4 Toxicity Reference Values for Reptiles

Not available at this time.

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APPENDIX A
LITERATURE REVIEW

The following databases were searched using the following keywords July 13, 1999:

TOXLINE & MEDLINE

Conditions: Two-word search; 1965 to present.

Trinitrotoluene and mammals - Trinitrotoluene = 911
Mammals = 471106
Combination = 158
Of these, 5 were appropriate and included.

Trinitrotoluene and birds - Trinitrotoluene = 911
Birds = 17894
Combination = 1
After review of the title, the single query result was not appropriate for this document.

Trinitrotoluene and wildlife - Trinitrotoluene = 911
Wildlife = 11830
Combination = 7
Of these, none were deemed appropriate for this document.

Trinitrotoluene and salamanders – Trinitrotoluene = 911
Salamanders = 398
Combination = 0

Trinitrotoluene and toads – Trinitrotoluene = 911
Toad = 411
Combination = 0

Trinitrotoluene and reptiles – Trinitrotoluene = 911
Reptiles = 4886
Combination = 0

Trinitrotoluene and snake – Trinitrotoluene = 911
Snake = 5825
Combination = 0

WinSPIRS 2.0

Conditions: Two-word conditional search; 1979-1997.

Trinitrotoluene and amphibian - Trinitrotoluene = 281
Amphibian = 2031
Combination = 0

Trinitrotoluene and salamander - Trinitrotoluene = 281
Salamander = 711
Combination = 0

Trinitrotoluene and frog - Trinitrotoluene = 281
Frog = 4412
Combination = 0

BIOSIS

Conditions: Two-word conditional search; 1984-1997.
Trinitrotoluene and wildlife - Trinitrotoluene = 1182
Wildlife = 17829
Combination = 73
Of these, most concerned the effects of effluent; duplicates with TOXLINE/MEDLINE search.

Trinitrotoluene and mammal - Trinitrotoluene = 1182
Mammal = 44329
Combination = 178
Of these, most concerned the effects of effluent; duplicates with TOXLINE/MEDLINE search.

Trinitrotoluene and bird - Trinitrotoluene = 1182
Bird = 24112
Combination = 3
These were not appropriate (non-laboratory evaluations).

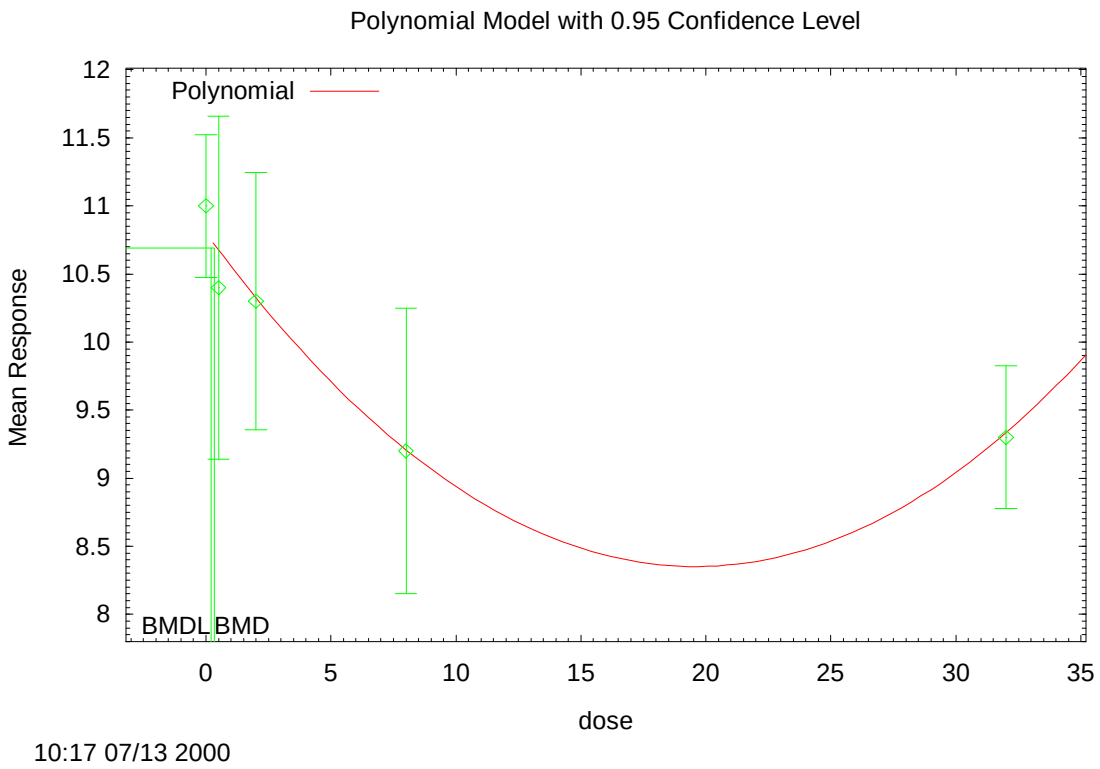
STINET – DTIC

Conditions: Two-word boolean search
Trinitrotoluene and mammal - Combination = 8
Original reports referenced (from which some peer reviewed submissions were based).

Trinitrotoluene and wildlife - Combination = 0
Trinitrotoluene and bird - Combination = 0
Trinitrotoluene and reptile - Combination = 0
Trinitrotoluene and amphibian - Combination = 0

APPENDIX B
Benchmark Dose Calculation for Mammals

The data presented below are from Levine et al. (1990a) where mean body weight (in kg = Mean Response) was measured in dogs from a 6-month feeding study. The data from the most sensitive sex was used in the calculation. Data from changes in hemoglobin and hematocrit followed the same trend and resulted in benchmark dose estimates that were statistically equivalent (One-way ANOVA on Ranks, P > 0.40).



Results of the model are presented below:

BMD = 0.324674
BMDL = 0.21622

==========

Polynomial Model. $Revision: 1.1.1.8 $ $Date: 2000/03/22 17:51:39 $
Input Data File: A:\TNT.(d)
Gnuplot Plotting File: A:\TNT.plt
Fri Jul 14 12:23:19 2000

==========

BMDS MODEL RUN

The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	0.75
beta_0 =	10.7721
beta_1 =	-0.249975
beta_2 =	0.00637527

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.647838	5.97832
beta_0	10.7721	6.80499
beta_1	-0.249975	100.578
beta_2	0.00637527	3122.43

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1	beta_2
alpha	1	-1e-007	-1e-007	-1.2e-007
beta_0	-1e-007	1	0.58	0.48
beta_1	-1e-007	0.58	1	0.98
beta_2	-1.2e-007	0.48	0.98	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	6	11	0.5	10.8	0.805	0.283
0.5	6	10.4	1.2	10.6	0.805	-0.309
2	6	10.3	0.9	10.3	0.805	0.0029
8	6	9.2	1	9.18	0.805	0.0244
32	6	9.3	0.5	9.3	0.805	-0.00146

Model Descriptions for Likelihoods Calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-7.94995	6	27.8999
A2	-4.40918	10	28.8184
fitted	-8.48827	4	24.9765
R	-16.93	2	37.86

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	25.0416	8	<.0001
Test 2	7.08154	4	0.1316
Test 3	1.07665	2	0.5837

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.950000

BMD = 0.324674
BMDL = 0.21622