

# OECD GUIDANCE DOCUMENT FOR THE PERFORMANCE OF CHRONIC TOXICITY AND CARCINOGENICITY STUDIES, SUPPORTING TG 451, 452 AND 453

## TABLE OF CONTENTS

**NOTE: Only Section 3.1 on dose selection of this draft Guidance Document is currently available. This section is developed in parallel with the revision of Test Guidelines 451, 452 and 453 on carcinogenicity, chronic toxicity and combined carcinogenicity/chronic toxicity studies. Additional chapters will be developed and the Guidance Document will be updated each time a new chapter is available.**

PREFACE, ABOUT THE OECD.....

### DEFINITIONS

1.	GENERAL INTRODUCTION .....	
	1.1 Guiding principles and considerations	
	1.2 Testing strategies	
	1.3 Animal welfare issues	
	1.4 Objectives of a chronic toxicity study	
	1.5 Objectives of a carcinogenicity study	
	1.6 Objectives of a combined chronic toxicity/carcinogenicity study	
2.	CONCEPTS .....	
	2.1 Mode of Action of carcinogens.....	
	2.2 Mechanisms of toxicity and target organs.....	
	2.3 Human relevance.....	
3.	STUDY DESIGN	
	General Introduction	
3.1	DOSE SELECTION .....	
	3.1.1 Introduction.....	
	3.1.2 Objectives of a chronic bioassay	
	3.1.2.1 Screening Chemicals to Identify Carcinogens (or other toxic effects).....	
	3.1.2.2 Characterizing the Dose–Response Curve in the Observable Range.....	
	3.1.2.3 Characterizing the Dose–Response Curve to Facilitate Low-Dose (Linear) Extrapolation.....	
	3.1.2.4 Defining a Threshold or BMD Departure Point.....	
	3.1.2.5 Providing Data on Health Effects at Human Exposure Levels.....	
	3.1.2.6 Providing Data to Test Hypotheses Regarding Mode of Action.....	
	3.1.3 Consideration of animal numbers and proposed test designs, taking into account varying study objectives	
	3.1.4 Consideration of the highest dose level and dose level spacing	
	3.1.5 Relevance of effects at high dose levels	
	3.1.6 Study design strategy and decision tree.....	
	3.1.7 Relevance of effects at high dose levels	
3.2	ROUTES OF EXPOSURE	
	3.2.1 Oral	
	3.2.2 Dermal	
	3.2.3 Inhalation	

- 3.3 CHOICE OF SPECIES AND STRAIN, NUMBERS & SEX OF ANIMALS, STUDY DURATION
  - 3.3.1 Testing in rodents
  - 3.3.2 Species and strain specificity
  - 3.3.3 Study duration
  - 3.3.4 Testing in non-rodents
  - 3.3.5 Study duration
- 3.4 HOUSING, FEEDING AND PREPARATION OF ANIMALS, DOSING PROCEDURES
- 3.5 PHARMACOKINETICS
- 3.6 INVESTIGATIONS (INCLUDING HISTOPATHOLOGICAL GUIDANCE)
- 3.7 STATISTICAL AND DOSE RESPONSE ANALYSIS, INCLUDING BENCHMARK DOSE AND LINEAR EXTRAPOLATION, NOAELS AND NOELS, LOAELS AND LOELS
- 4. DOCUMENTATION AND DATA ACCEPTABILITY, USE OF HISTORICAL CONTROL DATA
- 5. REPORTING
- 6. OTHERS?

## **1. GENERAL INTRODUCTION .**

---

### **1.1 Guiding principles and considerations**

In the 1960s, chronic animal bioassays began to be routinely used to assess the qualitative potential of a chemical to cause chronic toxicity and cancer. The use of formal risk assessment procedures by government regulatory bodies began to emerge in the late 1970s and early 1980s bringing with it a strong interest in using data for quantitative as well as qualitative purposes. The need to gather data that allowed an understanding of the shape and slope of the dose-response curve focused attention on the number of doses in a bioassay and their spacing. Advances in knowledge of how chemicals perturbed or otherwise modulated biological processes in the development of tumors or other forms of toxicity provided bases for further improving the risk assessment process. Through meetings of experts a mode of action framework (3) was developed and refined by several expert groups (4, 5, 6 7). The goal was to use a broad array of relevant data to determine the predictive value of a bioassay tumor response to risk in humans. Several governmental organizations have adopted consideration of the mode of action framework into their deliberations; these include the U.K., Canada, U.S., Australia, and the WHO/FAO Joint Meeting on Pesticide Residues.

### 1.2 Testing strategies

### 1.3 Animal welfare issues

### 1.4 Objectives of a chronic toxicity study

### 1.5 Objectives of a carcinogenicity study

### 1.6 Objectives of a combined chronic toxicity/carcinogenicity study

2. CONCEPTS .....

2.1 Mode of Action of carcinogens.....

2.2 Mechanisms of toxicity and target organs.....

2.3 Human relevance.....

### 3. STUDY DESIGN

#### 3.1 DOSE SELECTION .....

##### 3.1.1 Introduction.....

1. This section of the guidance document provides guiding principles to be considered in dose selection for chronic animal bioassays. It is designed to underpin and expand the principles of dose selection for chronic toxicity and carcinogenicity studies outlined in OECD Test Guidelines 451, Chronic Carcinogenicity Study in Rodents, TG 452, Long-Term Chronic Toxicity Study in Rodents by Oral Administration and TG 453, Combined Chronic Toxicity/Carcinogenicity Studies.
2. The broadened range and complexity of scientific data used to evaluate chemical toxicity and carcinogenicity potential for humans revealed a need to revise and update these OECD Test Guidelines. A major element of these revisions is to consider factors that influence the selection of test doses. The core dose selection strategy is dependent on the primary objective or objectives of the study (Allaben et al., 1997; Rhomberg et al. 2007), namely identification of the hazardous properties of a chemical, characterisation of the dose:response relationship, identification of a threshold or Benchmark Dose departure point, the provision of information on the health effects at human exposure levels and/or provision of data to test hypotheses regarding mechanism of action. In selecting appropriate dose levels, a balance has to be achieved between hazard screening on the one hand and characterisation of low-dose responses and their relevance on the other. This is particularly relevant in the situation where a combined chronic toxicity and carcinogenicity study (TG 453) is to be carried out.
3. This Guidance Document seeks to address this topic and has been developed on the basis of two reports of the International Life Sciences Institute (ILSI). The initial report, entitled *Principles for the Selection of Doses in Chronic Rodent Bioassays* (Allaben et al., 1997), presented common views on the selection of doses for chronic carcinogenicity and toxicity studies. A second ILSI working group report, entitled *Issues in the Design and Interpretation of Chronic Toxicity and Carcinogenicity Studies in Rodents: Approaches to Dose Selection* (Rhomberg et al. 2007) referred in this guidance document as *Issues in Design & Interpretation Report*, provides additional practical guidance on factors that influence dose selection in chronic bioassays.
4. Chapter 2 of the report of Allaben et al (2007) on *Principles for Selection of Dose* presents the requirements of national and international systems regarding dose selection that existed at the time of the review. The report demonstrates the different approaches that existed at that time. The report of Rhomberg et al. (2007) incorporates concepts included in recent documents prepared by national and international organizations (OECD, ECETOC, NTP and USEPA). A stated goal of the ILSI effort was to provide approaches to data evaluations that will facilitate uniform decisions on the acceptability of the doses used in carcinogenicity bioassays. While their focus was primarily on cancer bioassays, they also asserted that these studies are of sufficient length and breadth of scope to generate data relevant to the examination of non-tumor pathology and non-cancer toxicity associated with chronic exposure.
5. It is gratefully acknowledged that ILSI made this information available to the OECD before publication in the open literature and thus enabled the accelerated development of this Guidance Document. The Guidance Document incorporates both the views expressed

in the ILSI reports and the views of OECD experts developed at a workshop on the revision of OECD Test Guidelines 451, 452 and 453 held in Washington on 26-28 February, 2008. The text in italic type in the Guidance reflects a direct quotation from the ILSI reports (1) and (2).

### 3.1.2 Objectives of a Chronic Bioassay

6. Today's chronic bioassays typically have objectives that go far beyond hazard identification. The chronic bioassay design must consider financial constraints and societal desires to keep the number of animals to the minimum needed for scientific interpretation of results. Thus, chronic organ toxicity and carcinogenicity are often co-objectives within the same study.
7. There is a growing desire for chronic studies to have data that serve a variety of needs; these include characterizing the nature of specific toxic responses, describing dose-response shape, finding inflection points, and providing insight into the roles of pharmacokinetics and mechanisms of toxic action.
8. Rhomberg et al. (2007) distinguishes 6 possible objectives of a chronic bioassay and provides for each of them separately, the theoretical best approach for dose selection:
  - Screening chemicals to identify carcinogens or those causing other toxic effects)
  - Characterizing the dose-response curve in the observable range
  - Characterizing the dose-response curve to facilitate low-dose (linear) extrapolation
  - Defining a threshold or BMD departure point
  - Providing data on health effects at human exposure levels
  - Providing data to test hypotheses regarding mode of action
9. In practice, it may be necessary for the bioassay design to be a compromise among a set of different purposes; to the extent that the ability to address one question is enhanced, the ability to address others may be diminished. For example, it may be necessary to achieve a balance between the power to detect toxicity and the ability to estimate the dose-response relationship of any observed effects.
10. When combining objectives into a single study, selection of dose must be done in a way that does not compromise the primary objective while still allowing a secondary objective to be pursued in an acceptable albeit suboptimal manner. For example, a trade-off may be qualitative hazard identification and quantitative use of data to characterize dose response. The report of Rhomberg et al. (2007) explored potential conflicts that arise from differing objectives of a bioassay. These six varied objectives are presented below:

#### 3.1.2.1 Screening Chemicals to Identify Carcinogens (or other toxic effects)

11. **Approach:** *If this were the only objective, doses should be placed high, near the MTD, in order to maximize the power of the assay, to ensure that at least one dose below an unacceptable toxic level is achieved, and to yield high sensitivity. That is, this objective stresses minimizing the chance of a false negative (failing to detect an effect that actually exists) at some increased risk of a false positive (finding a high-dose effect that is an artifact of excessively high doses and is not relevant to the dose range of interest). The use of two low doses may not always be necessary, since the lower dose serves (under this*

objective) as a hedge against discovery after-the-fact that the top dose unacceptably exceeds an MTD. Animals should be divided nearly equally among the doses to ensure that the low dose group can be reliably used as a surrogate for the high dose if necessary.

- **Advantage:** The results are relatively straightforward to interpret and widely acceptable. The data generated should provide a clear answer to the question of whether a chemical can cause a particular toxic response. A valid negative outcome provides the strongest evidence possible from an animal bioassay that the compound lacks the ability to cause the toxic effect in question, since lower doses would be even less powerful in detecting an effect.
- **Limitations:** This approach will provide minimal data regarding the shape of the dose–response curve. Although the dose–response data gained are slightly superior to those that might be obtained with a single dose, the support for low-dose extrapolation is minimal. The data will say little about possible nonlinearities in the dose–response curve or the existence of a threshold. The relevance of these high doses to potential human exposures will also be questioned. Mechanistic information gleaned from this type of study may be irrelevant. If top doses are set lower, to ensure relevance, however, questions will arise about whether the power to detect effects was compromised. In short, positives may be difficult to interpret vis-à-vis low exposure levels, because they may reflect a high-dose-only phenomenon.

### 3.1.2.2 Characterizing the Dose–Response Curve in the Observable Range

12. **Approach:** Doses should be spread out to characterize as large a portion of the dose–response curve as possible. A minimum of three doses should be used; more doses may be appropriate if nonlinearities are expected and if the effects of interest occur frequently (i.e., if power to detect rare events is not critical). Doses should be placed where they will be informative, which will in turn be dictated by what possible dose–response phenomena could be involved and the dose range over which effects large enough to be detected are expected (i.e., the “observable range”). Characterization of J- or U-shaped dose–response curves would require dose placement different from that employed in conventional bioassays. The MTD may have little relevance except as an indicator of the ability of the assay to find adverse effects. Indeed, depending on the endpoint of interest, the top dose may be well below the conventional MTD. Animal number should be distributed fairly evenly across the range of doses if very little is known about the nature of the response prior to the experiment. If prior evidence allows, it may be possible to optimize the design in terms of the location of the doses and the allocation of animals to the doses.

- **Advantage:** This approach aids in understanding how the toxicity of the chemical changes with increasing dose, providing some possible insights as to the mode of action. It allows for extrapolation to health effects at meaningful human exposures, and still retains some limited ability for hazard identification.
- **Limitations:** The assay will have less power to detect effects compared with the approach outlined for Objective 1 unless additional animals are used. For purposes of hazard identification, the likelihood of a false-negative result, even at the MTD, will be increased. The utility in defining the dose–response curve will depend on how well the dose placement anticipates the interesting and informative

*parts of the curve; if the chosen doses “miss” these points, the results may not be informative.*

### 3.1.2.3 Characterizing the Dose–Response Curve to Facilitate Low-Dose (Linear) Extrapolation

**13. Approach:** *If low-dose extrapolation is the paramount concern, and if the shape of the dose–response curve at relatively high doses is considered marginally relevant (as with the US EPA’s current “two-step” point-of-departure-and-linear-extrapolation procedure), all of the doses should be placed quite low, so that even if they have no significant response, the fitted curve is forced to be as low and flat as possible. The lowest linear “upper-bound” extrapolation consistent with any actual effects that may exist is achieved if a dose anchors the observed dose–response relationship at just under the dose where observable effects begin to occur. If nonlinearities are expected, the doses should be placed below the inflection point, if it exists. Dose location and animal number should be selected in order to minimize the standard error of low-dose estimates.*

- **Advantage:** *This approach would best facilitate extrapolation to health effects at meaningful human exposures and would provide a bounding estimate of the dose–response slope. When low-dose extrapolations are linear or upper bounds, this approach minimizes the tendency to overestimate low-dose risks.*
- **Limitations:** *The assay will provide no data at all about the shape of the dose–response curve in the observable range and will be of no value for hazard identification. Indeed, by avoiding high doses, actual positive responses might not be detected. The approach is most useful when the agent’s carcinogenicity is stipulated, and the question is solely one of obtaining a linear low-dose extrapolation. If the true dose–response curve is nonlinear, the lack of information about shape will make it difficult to choose a low-dose extrapolation approach suited to non-linear responses (such as the margin-of-exposure approach) over a low-dose linear extrapolation.*

### 3.1.2.4 Defining a Threshold or BMD Departure Point

**14. Approach:** *The number of doses should be high, with fewer animals per dose. The dose range should be wide enough to include a high dose associated with a large effect and a low dose associated with a smaller but detectable effect (preferably < the BMD). Doses and animal numbers should be concentrated in the region of the anticipated NOAEL or low response. A minimum of three doses will likely be required, with the high dose used mainly as a check on the ability of the assay to detect adverse effects (i.e., few animals needed at the high dose). The middle portion of the dose–response curve will largely be excluded. Information essential for defining a threshold or BMD departure point in a chronic toxicity study will be derived from an initial 28-day or 90-day toxicity study employing a number of doses, and that a BMD approach is applied in such a study to determine doses for the chronic study.*

- **Advantage:** *This approach helps to answer the question, How much is safe? Because a dose-response curve is generated to determine the BMD, it may also help answer the question, How much is toxic? The data generated are more likely to be relevant to human exposure levels.*
- **Limitations:** *This assay will be of little use for hazard identification; a negative result at the high dose would be of little value. The results may not provide much*



information regarding the shape of the dose–response curve, particularly if nonlinearities occur in the middle range. The assay’s power at the lower doses will also be limited if the responses of interest are rare, and, because of the small numbers of animals used at the high dose, the data collected will do little to decrease the confidence bounds at the  $BMD_{10}$  or  $BMD_{01}$  (estimates of doses producing 10% or 1% elevation of risk, respectively). If one tests doses that are too low, the responses will not be much different from those among controls. In general, the utility of the approach depends on how well the dose range of interest is anticipated.

#### 3.1.2.5 Providing Data on Health Effects at Human Exposure Levels...

15. **Approach:** Doses should be based on multiples of a defined level of the target population’s exposure of interest (e.g., 10 times or 50 times higher). If pharmacokinetic data are available, doses based on internalized doses (e.g., AUC) can be used. Animal numbers will have to be concentrated in these lower doses to maximize assay power. Determination of adverse effects (e.g., use of the MTD) may be a lower priority than maximizing low-dose sensitivity. Again, depending on the human exposure level, the middle portion of the dose–response curve may be excluded.

- **Advantage:** The data generated will be most relevant to anticipated target population exposures. The approach is most relevant for frequently occurring health effects (e.g., respiratory irritation in occupational settings) where assay power is not particularly critical.
- **Limitations:** This assay will be of no use for hazard identification. Determining the appropriate target population exposure level to use as the dose departure point will be controversial; if actual exposures (or future exposures) are higher than envisioned in the bioassay design, the results will be less applicable. The target population exposure is likely to be a distribution, perhaps a broad and highly skewed one, and so the adequacy of the study for the whole range of exposures may not be evident. The results may not provide much information regarding the shape of the dose–response curve. The assay’s power at the lower doses will again be limited if the responses of interest are rare. An issue will be the likelihood of false negatives and the loss consequences of missing possible true effects, which can be compared with the benefits of the activities leading to the target population exposures.

▪

#### 3.1.2.6 Providing Data to Test Hypotheses Regarding Mode of Action

16. **Approach:** Doses would need to be placed carefully to yield observations of subtle precursor effects or other biomarkers of toxicity without inducing confounding effects related to frank toxicity. This approach would require some previously generated information on potential modes of action. Timing will be a critical element in this investigation; the number of dose groups may need to be constrained to provide enough animals for multiple sacrifice/evaluation times. Depending on the state of knowledge regarding possible modes of action (e.g., the number of possible alternatives), sensitivity may be a less important constraint on dose group number than the need for an experimental design that can compare the various alternative modes of interest. This is a method driven by hypotheses regarding the mode of action.

- **Advantage:** *Such an assay is of potentially great use for enhancing our understanding of the way in which toxicity occurs and, by inference, what factors may influence the shape of the dose–response curve.*
- **Limitations:** *The assay may provide less information about the shape of the dose–response curve in the observable range and will be of much-reduced value for hazard identification. Consideration of dose placement is likely to be of limited value in obtaining information on mode of action (Krewski and Goddard, 1990). More important considerations might be time-dependent dosing patterns (Goddard et al., 1995), initiation/promotion protocols (Gart et al., 1986), and auxiliary data on intermediate endpoints involved in the process of carcinogenesis, possibly obtained from separate experiments (Moolgavkar et al., 1999).*

17. In reviewing the above alternatives, a key question becomes apparent: What properties of the dose–response curve are most important—the steepness of the slope? the flatness of the tails? the location of key values such as the  $ED_{10}$  or  $LED_{10}$ ? A second question involves how one determines the placement of animals into the various dose categories. Each different objective seeks to maximize the assay’s power in a different portion of the dose–response curve. The focus may be on a level of response or on the shape and slope of the overall curve. The situation is also complicated by the fact that, below a certain dose, attempts to increase assay power by rearranging animal numbers in particular dose groups become futile.

### **3.1.3 Consideration of animal numbers and proposed test designs, taking into account varying study objectives**

18. The current chronic OECD Test Guidelines 451, 452 and 453 should be viewed as being composed of a core minimum wherein there are four dose groups per sex – control and three treatment groups, each of which is exposed to different concentrations of the test material. Each group should contain at least fifty animals per sex for studies conducted under TG 451 and 453 and at least 20 animals per sex per group for TG 452. These recommendations relate to the “standard” bioassay as described in these guidelines, and the numbers of animals stipulated are appropriate to situations where the outcome measure per animal is binary – presence or absence of an endpoint such as a tumour. There may be cases where a quantitative endpoint is measured, for which design considerations would be quite different. The numbers of animals, the doses to be administered and the spacing of these doses must be chosen to meet as far as possible the study objective(s), as described in paragraph 17. There may be embellishments to this core design based on study objectives but it would be a rare event when an erosion of the core minimum would be acceptable.
19. The *Design Issues Report (REF)* suggested that perhaps a compromise between case-by-case optimization and a standard design is to select a few core selection schemes. The four schemes are presented below:

**Hazard Screening Plus Dose–Response:** *This is modeled on the current carcinogenicity bioassay. The top dose is chosen to increase the study’s statistical power to detect effects that may be rare. A second dose combines two functions: (1) hedging against the top dose being found to have been too high in retrospect, and (2) providing the opportunity for dose–response characterization of any effects found. Other lower doses can be placed so*

*as to inform dose–response, no-effect levels, or other purposes. Key questions will be balancing statistical power and toxicological relevance of the high dose and compromising among subsidiary objectives while accounting for relevant dose-related physiological changes when setting lower doses.*

***NOAEL/BMD-Seeking for Threshold Effects:*** *This is modeled on the current chronic study for non-cancer effects. The main aim is to identify no-effect (or low-effect) levels for the more sensitive adverse threshold effects. The top dose should aim at engendering frankly adverse effects, the lowest dose should aim at constituting a NOAEL, and intermediate doses should be set so as to identify the dose levels at which the high-dose responses become manifest.*

***Assessment of Safety of Human Exposure Levels:*** *This is modeled on safety assessment studies for pharmaceuticals. For agents that are not genotoxic, show low toxicity, and evince no known difference in metabolic profile between rodents and humans, one can test multiples of anticipated human exposure. Lack of adverse effects at doses sufficiently above human exposure (and the perceived implausibility of non-threshold effects) gives evidence supporting the safety of the anticipated exposures. The bioassay exposures should be selected on an appropriate basis for animal-human comparison; for instance, the application to pharmaceuticals is typically based on area under the blood concentration-time curve that results from anticipated human exposures.*

***Special-Purpose Bioassays:*** *Whenever the main emphases of the above three core schemes do not apply—or when they are dominated by another compelling purpose—it is necessary to consider a more completely case-specific design. For instance, if one is conducting a second bioassay to address dose–response properties and organ toxicity dependence of a tumor response that has previously been discovered in a screening bioassay, the dose selection should be optimized for the specific purposes at hand, and the choice of the top dose will no longer be dominated by hazard identification concerns.*

20. Rhomberg et al. (2007) in their report urge that in each of the four approaches that one not automatically set lower doses as fractions of the highest dose, such as MTD, ½ MTD and ¼ MTD. They favor selection of doses that reflect the purposes of the study and use of knowledge of how dose-dependent biological and impacted physiological factors may affect study outcomes.

### **3.1.4 Consideration of the highest dose level and dose level spacing**

21. The key points in the *Five Principles for Selection of Doses* as summarized by the recent report of Rhomberg et al. (2007) follow:
- *Dose selection must be based on sound toxicologic principles and be within a reasonable range to maximize the sensitivity of the chronic bioassay.*
  - *A chronic bioassay requires a major investment in resources and time; therefore, the objective of such a study should be broader than simply hazard identification. Approaches to dose selection should consider appropriate study designs, mechanistic data, and other information related to study design and interpretation.*

- *Human exposure should be considered in dose selection, particularly for selection of the middle and lowest doses, to characterize the shape of the dose-response curve as much as possible.*
- *Innovative approaches, additional endpoints (e.g., effects on physiological function) and other relevant information (e.g., toxicokinetics, mode of action) should be used in the selection of doses.*
- *Doses should be selected to minimize or avoid adverse nutritional, physical, organoleptic, and irritant effects.*

#### Selection of the highest dose.

22. Nutritional effects, physiological factors, physical-chemical factors and compound bioavailability can influence selection of the largest dose.
23. For nutritional and possibly other physiological reasons a maximum level is imposed – commonly 5% concentration in the diet. If oral gavage is used the dose typically will not exceed 1,000 mg/kg bodyweight per day. However, according to ICH S1CR, the limit dose for carcinogenicity studies for pharmaceuticals is 1500 mg/kg, provided that this dose results in systemic exposure that is 10 times the expected human systemic exposure. For some pharmaceuticals, exposure after a nominal dose of 1000 mg/kg may not exceed the anticipated human exposure. Palatability of a compound in either feed or water can also perturb physiological homeostasis or nutritional status. A compound's solubility limit or vapor pressure may constrain selection of the top dose. Irritation at the site of compound deposition may constrain dose or otherwise confound cross species extrapolation. Inhalation of doses that overwhelm pulmonary clearance may lead to tissue responses that are specific to the rat.
24. If the main objective of a chronic bioassay is to identify a cancer hazard there is broad acceptance that the highest dose should not cause overt toxicity and not be anticipated to shorten the test animal's life expectancy for reasons other than the development of tumors. Criteria that have evolved for the selection of an adequate high dose level include: (in particular) pharmacokinetics; saturation of absorption; results of previous repeated dose toxicity studies; and the maximum tolerable dose (MTD). Operationally the MTD dose ideally produces some minimal signs of toxicity such as slight depression of body weight gain (not more than 10%), normally without causing tissue necrosis or metabolic saturation and without substantially altering normal life span due to effects other than tumors. However it is recognized that e.g. metabolic saturation or tissue necrosis is a key event in the MOA of a number of carcinogens (see chapter 2), and it may be necessary to deviate from these principles regarding the MTD in order to fully explore the carcinogenic potential and MOA of the chemical in question

The general approach to selecting the maximum dose in a study should be based on a tiered approach. For compounds that are (or might be) genotoxic, conventional considerations of MTD would apply. For compounds that are not genotoxic, the maximum dose should be informed by considerations of MOA. For compounds such as chloroform, if the MOE based on long term studies of hepatic toxicity is acceptable, this would be protective against any carcinogenic effect. This is because non-genotoxic carcinogens produce cancer by perturbing normal physiology or biochemistry. The

chronic assay should be designed to identify and characterise these perturbations and not necessarily cancer per se.

### Dose spacing

25. Selection of dose intervals is influenced by the study objective and the available information. The dose spacing does not need to be regular. Rhomberg et al. (2007) in their report were of the view that dose placement be considered with respect to the following:

- *known or suspected nonlinearities or inflection points in the dose–response;*
- *suspensions regarding the role of hormesis or the existence of J- or U-shaped dose–response curves;*
- *pharmacokinetics, and dose ranges where metabolic induction, saturation, or nonlinearity between external and internal doses does or does not occur;*
- *precursor lesions, markers of effect, or indicators of the operation of key underlying biological processes;*
- *key (or suspected) aspects of mode of action, such as doses at which cytotoxicity begins to arise, hormone levels are perturbed, homeostatic mechanisms are overwhelmed, etc.;*
- *regions of the dose–response curve where particularly robust estimation is required, e.g., in the neighborhood of the anticipated BMD;*
- *in the region of a suspected threshold; and*
- *with consideration of anticipated human exposure levels.*

*It may be possible to place adjacent doses somewhat above and below the levels at which a key transition in underlying biological actions is believed to lie, thereby revealing its influence on response. (Transitions need not be sharp; typically, there are ranges of doses over which an underlying biological factor, such as metabolic saturation or cytotoxicity, comes increasingly into play. The aim is to place doses so that the contrast in the role on overall response of such underlying phenomena can be revealed.) The issue of where to place the lowest dose should receive comparable attention to the placement of the top dose. If the lowest dose is too low, it may be insufficiently powerful and therefore uninformative; if too high, it may lose opportunities to characterize effects as near as possible to environmental exposure levels.*

26. PBPK modeling is proposed as a valuable tool for defining doses where non-linear pharmacokinetics may occur thus allowing this to be considered in selecting dose. These topics and other factors are presented and discussed in depth in chapter 4 of the report of Rhomberg et al. (2007).

### **3.1.5 Relevance of effects at high dose levels**

### **3.1.6 Study design strategy and decision tree**

27. The Rhomberg et al. (2007) report devotes its Chapter 3 to presenting and describing a Bioassay Design Decision Process. A schematic of that Process is reprinted below. Those interested in adopting or refining a process are encouraged to read the chapter.

.....

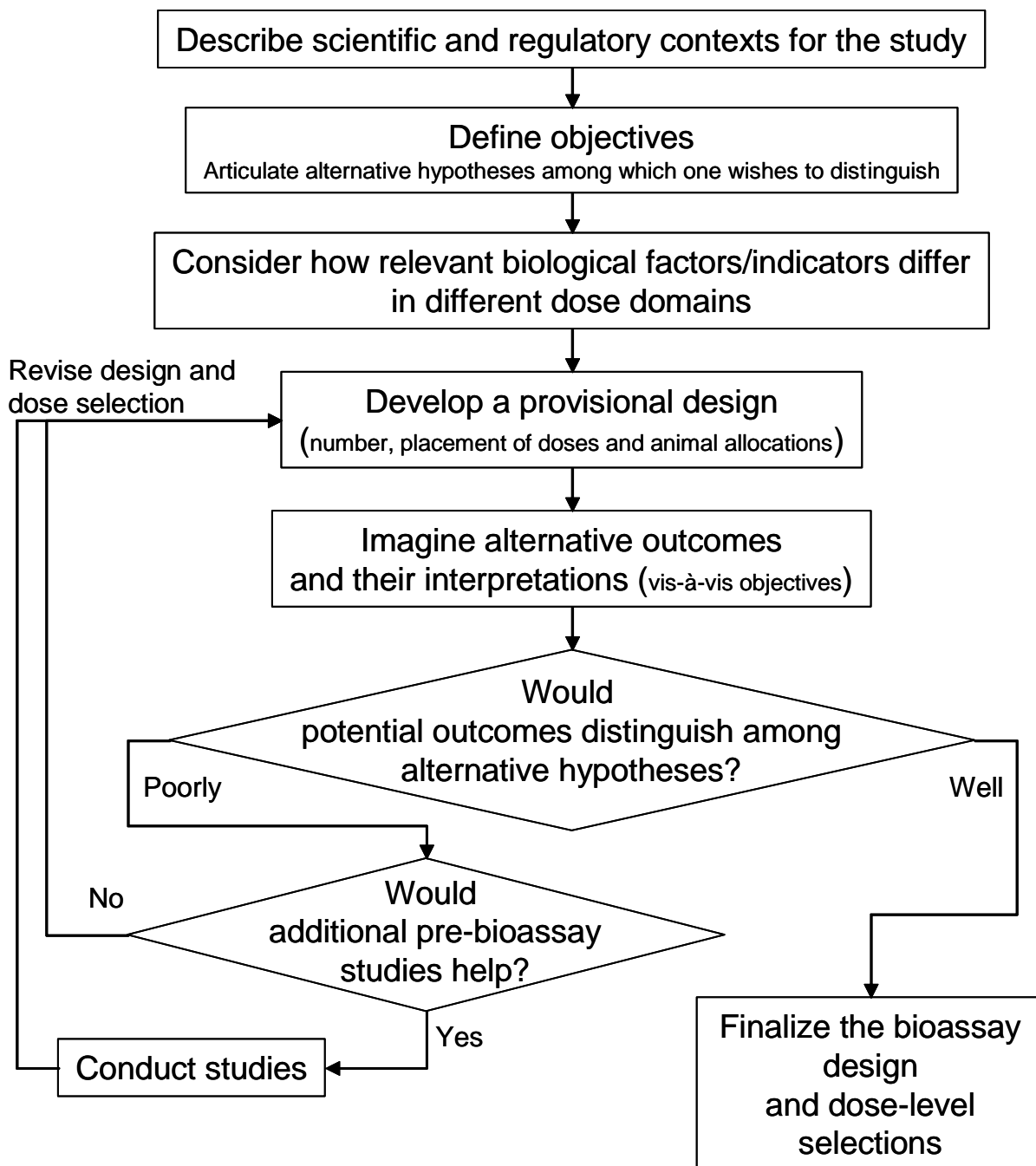


Figure 1: A Bioassay Design Decision Process

## Literature

1. Allaben, W., Ashby, J., Bucher, J., Burnham, W., Bus, J., Butterworth, B., Carakostas, M., Cardona, R., Cohen, S., DeGeorge, j., Domeyer, B., Emerson, J., Fenner-Crisp, Foran, G., P., Gaylor, D., Gee, J., Goodman, J., Grant, D., Hatton, D., Hayashi, Y., Henderson, R., Herman, J., Koeter, H., LeBuoef, R., Lorentzen, R., McConnell, E., Mitsumori, K., Olin, S., Parker, J., Renwick, A., Stevens, J., Swenberg, J., Taylor, A., Van Gemert, M., Vu, V., Walker, R., Watson, M., Yielding, K.L.(1997) Principles for the Selection of Doses in Chronic Rodent Bioassays; Jeffery A. Foran, Editor. ILSI Press, Washington D.C., USA
2. Rhomberg, L., Baetcke, K., Blancato, J., Bus, J., Cohen, S., Conolly, R., Dixit, R., Doe, J., Ekelman, K., Fenner-Crisp, P., Harvey, P., Hattis, D., Jacobs, A., Jacobson-Kram, D., Lewandowski, T., Liteplo, R., Pelkonen, O., Rice, J., Somers, D, Turturro, A., West, W. Olin, S. (2007). Issues in the Design and Interpretation of Chronic Toxicity and Carcinogenicity Studies in Rodents: Approaches to Dose Selection. *Crit. Rev. Toxicol.*37:729-837.
3. Sonnich-Mullin, C., Fielder, R., Wiltse, J., Baetcke, K., Dempsey, J., Fenner-Crisp, P., Grant, D., Hartley, M., Knaap, A., Kroese, D., Mangelsdorf, I., Meek, E., Rice, J., & Younes, M. IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis. *Reg. Toxicol. & Pharm.* (2001) 34:146-152.
4. Boobis, A.R., Cohen, S.M., Dellarco, V., McGregor, D., Meek, M.E., Vickers, C., Willcocks, D. & Farland, W. IPCS Framework for analyzing the Relevance of a Cancer Mode of Action for Humans. *Crit. Rev. in Toxicol.* (2006) 36:793-801.
5. Cohen, S.M., Meek, M.E., Klaunig, J.E., Patton, D.E., and Fenner-Crisp, P.A. (2003) The human relevance of information on carcinogenic Modes of Action: An Overview. *Crit. Rev. Toxicol.* 33:581-589
6. Holsapple, M.P., Pitot, H.C., Cohen, S.N., Boobis, A.R., Klaunig, J.E., Pastoor, T., Dellarco, V.L. & Dragan, Y.P. (2006) Mode of Action in Relevance of Rodent Liver Tumors to Human Cancer Risk. *Toxicol. Sci.* 89:51-56.
7. Meek, E.M., Bucher, J.R., Cohen, S.M., Dellarco, V., Hill, R.N., Lehman-McKemmon, L.D., Longfellow, D.G., Pastoor, T., Seed, J. & Patton, D.E. A Framework for Human Relevance analysis of Information on Carcinogenic Modes of Action. *Crit. Rev. Toxicol.* (2003). 33:591-653.