INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

DETECTION OF REPRODUCTIVE AND DEVELOPMENTAL TOXICITY FOR HUMAN PHARMACEUTICALS

S5(R3)

Final version
Adopted on 18 February 2020

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of ICH regions.

S5(R3)
Document History

Code	History	Date

Parent Guideline: Detection of Toxicity to Reproduction for Medicinal Products

S5A	Approval of the Guideline by the Steering Committee under *Step 2* and release for public consultation.	19 September 1992
S5A	Approval of the Guideline by the Steering Committee under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	24 June 1993

Addendum to the Parent Guideline: Toxicity to Male Fertility

S5B	Approval of the Addendum by the Steering Committee under *Step 2* and release for public consultation.	30 March 1995
S5B	Approval of the Addendum by the Steering Committee under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	29 November 1995
S5B(M)	Approval of minor revisions to the Addendum by the Steering Committee without further public consultation under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	9 November 2000
S5A and S5B(M)	The parent guideline is now renamed S5(R2) as the Addendum and its first revision have been added to the parent guideline.	November 2005

Current Step 4 Version

S5(R3)	Approval by the ICH Assembly under Step 2 and release for public consultation.	1 August 2017
S5(R3)	Adopted by the Regulatory Members of the ICH Assembly under *Step 4* (document dated 16 December 2019).	18 February 2020

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ICH Consensus Guideline

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LIST OF ABBREVIATIONS

AUC:	Area Under the Curve
C_{max}:	Maximum plasma concentration
C_{min}:	Minimum plasma concentration
DART:	Developmental and Reproductive Toxicity
DRF:	Dose Range Finding
EFD:	Embryo-Fetal Development
ePPND:	Enhanced Pre- and Postnatal Developmental
FEED:	Fertility and Early Embryonic Developmental
GD:	Gestation Day
GI:	Gastrointestinal
GLP:	Good Laboratory Practices
ICH:	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IV:	Intravenous
LOAEL:	Lowest Observed Adverse Effect Level
LLO:	Late Life Onset
MOA:	Mechanism of Action
MEFL:	Malformation or Embryo-Fetal Lethality
MFD:	Maximum Feasible Dose
MRHD:	Maximum Recommended Human Dose
NHP:	Non-Human Primate
NOAEL:	No Observed Adverse Effect Level
PD:	Pharmacodynamic
pEFD:	Preliminary Embryo-Fetal Development
PK:	Pharmacokinetic
PND:	Postnatal Day
PPND:	Pre- and Postnatal Developmental
SDLT:	Severely Debilitating or Life-Threatening
TK:	Toxicokinetic
WOCBP:	Women of Child Bearing Potential

1. INTRODUCTION & GENERAL PRINCIPLES

The purpose of this document is to recommend international standards for, and promote harmonization of, the assessment of nonclinical developmental and reproductive toxicity (DART) testing required to support human clinical trials and marketing authorization for pharmaceuticals. The guideline describes potential strategies and study designs to supplement available data to identify, assess, and convey risk. General concepts and recommendations are also provided that should be considered when interpreting study data.

This is a revision of the ICH guideline "S5 Detection of Toxicity to Reproduction for Medicinal Products" that was originally published in 1993. This revision brings the guideline into alignment with other ICH guidelines, elaborates on the use of exposure margins in dose level selection, incorporates a section on risk assessment, and expands the scope to include vaccines and biopharmaceuticals. It also describes qualification of alternative assays, potential scenarios of use, and provides options for deferral of developmental toxicity studies.

To assess a human pharmaceutical's effect on reproduction and development, there should generally be information available that addresses the potential impact of exposure to a pharmaceutical and, when appropriate, its metabolites (ICH M3 (1), ICH S6 (2)) on all stages of reproduction and development. No guideline can provide sufficient information to cover all possible cases, and flexibility in testing strategy is warranted.

1.1. Aim of Studies

The aim of DART studies is to reveal any effect of the pharmaceutical on mammalian reproduction relevant for human risk assessment. As appropriate, the set of studies conducted should encompass observations through one complete life cycle (i.e., from conception in one generation through conception in the following generation), and permit detection of immediate and latent adverse effects. The following stages of reproduction are generally assessed:

A) Premating to conception (adult male and female reproductive functions, development and maturation of gametes, mating behavior, fertilization).

B) Conception to implantation (adult female reproductive functions, preimplantation development, implantation).

C) Implantation to closure of the hard palate (adult female reproductive functions, embryonic development, major organ formation).

D) Closure of the hard palate to the end of pregnancy (adult female reproductive functions, fetal development and growth, organ development and growth).

E) Birth to weaning (parturition and lactation, neonate adaptation to extrauterine life, pre-weaning development and growth).

F) Weaning to sexual maturity (post-weaning development and growth, adaptation to independent life, onset of puberty and attainment of full sexual function, and effects on second generation).

The risks to all stages should be assessed, unless the stage is not relevant to the intended population. The stages covered in individual studies are left to the discretion of the Sponsor, although the timing of studies within the pharmaceutical development process is dependent on study populations and phase of pharmaceutical development (see ICH M3, ICH S6 and ICH S9 (3)).

2. SCOPE OF THE GUIDELINE

This guideline applies to all pharmaceuticals, including biopharmaceuticals, vaccines (and their novel constitutive ingredients) for infectious diseases, and novel excipients that are part of the final pharmaceutical product. For the purposes of this guideline, the term "pharmaceutical" is used to encompass all of these treatment modalities. This guideline does not apply to cellular therapies, gene therapies and tissue-engineered products. The methodological principles (e.g., study design, dose selection and species selection, etc.) outlined in this guideline apply to all compounds for which the conduct of reproductive and/or developmental toxicity studies is appropriate. This guideline should be read in conjunction with ICH M3, ICH S6, and ICH S9 regarding whether and when nonclinical DART studies are warranted.

3. GENERAL CONSIDERATIONS ON REPRODUCTIVE TOXICITY ASSESSMENT

The majority of pharmaceuticals being developed should be assessed for all stages of the reproductive cycle identified above, although there can be some exceptions which should be justified, as indicated below. To support clinical development, these stages have typically been evaluated using three *in vivo* study types: 1) a fertility and early embryonic development study (FEED - stages A and B), 2) embryo-fetal development studies in two species (EFD - stages C and D), and 3) a pre- and a postnatal development study (PPND – stages C through F). For each compound, the stages that are to be evaluated should be determined and the most appropriate studies to conduct should be identified. Key factors to consider when developing an overall integrated testing strategy to evaluate effects on reproduction and development include:

- The targeted patient population and conditions of use (especially in relation to reproductive potential and severity of disease);
- The formulation of the pharmaceutical and route(s) of administration intended for humans;
- Relevant data on toxicity (which can also include data from *in vitro*, *ex vivo* and non-mammalian studies, and structure-activity relationships), pharmacodynamics, pharmacokinetics, and pharmacological similarity to other pharmaceuticals;
- Aspects of the general biology of the pharmaceutical target, or known roles of the target in reproduction or development.

These concepts are discussed in more detail throughout the guideline.

To the extent that it does not diminish the overall risk assessment, the experimental strategy should minimize the use of animals. Approaches towards this goal can include the conduct of studies that combine typical study types (see Section 7), as well as appropriately

qualified alternative assays for risk assessment (see Annex 2). Since many clinical development programs are terminated prior to Phase 3, animal use can also be reduced by appropriately timing studies to support ongoing clinical development (e.g., embryo-fetal developmental toxicity data to support enrollment of women of childbearing potential) as per ICH M3.

DART studies should, in general, be conducted according to Good Laboratory Practice (GLP) regulations, as they will contribute to the risk assessment. However, if a relevant DART risk is identified in a non-GLP study, repetition of the study to confirm the finding(s) under GLP conditions is not necessarily warranted. A relevant risk is one that occurs at or near intended clinical exposures and is of a nature that is reasonably likely to translate to humans (see Section 9). It is recognized that GLP compliance is not expected for some study types, or aspects of some studies, employing specialized test systems or methods. However, high quality scientific standards should be applied with data collection records readily available. Areas of non-compliance should be identified within the study report and their impact on study results/data interpretation should be considered relative to the overall safety assessment.

3.1. Target Patient Population/ Therapeutic Indication Considerations

The intended patient population or therapeutic indication can influence the extent of DART testing. Studies evaluating all stages of reproduction and development are not warranted if the disease indicates that DART will have minimal impact on the risk of the pharmaceutical in the target population. For example, studies covering all stages are not necessarily appropriate for an exclusively post-menopausal female patient population, for use in the pediatric or juvenile pre-pubescent population, or for patient populations in hospitalized settings where pregnancy can be excluded.

3.2. Pharmacology Considerations

Before designing a testing strategy, it should be determined if the intended pharmacologic effects of a pharmaceutical are known to be incompatible with fertility, normal EFD, or assessment of particular endpoints (e.g., a general anesthetic and assessment of mating behavior). This assessment can be based on data with other pharmaceuticals with similar pharmacology, known effects of target engagement, or on knowledge of effects in humans with related genetic diseases. For example, it would be appropriate to modify the design of a PPND study for a pharmaceutical developed to prevent pre-term labor. If the intended pharmacologic effects are incompatible with the study endpoints, testing for a particular reproductive endpoint is not warranted, with justification.

3.3. Toxicity Considerations

Repeated–dose toxicity studies with sexually mature animals can provide important information on toxicity to reproductive organs that can affect the design of a DART study. The existing toxicology data for the compound should always be considered, taking into account the dose levels, toxicokinetic profile, and dosing duration. For example, the standard fertility study design can be modified to alter the duration of dosing, or the start of cohabitation, for a compound that affects testicular tissue.

3.4. Timing Considerations

General guidance on the timing for conduct of studies assessing reproductive and developmental endpoints is described in ICH M3, ICH S6, and ICH S9. The timing for when to conduct specific DART assessments should take into consideration the need for these data to support the safe use of the pharmaceutical in clinical trials or the intended patient population. Consequently, it can be appropriate to consider altering the timing of the assessment of specific reproductive stages. Additional options are discussed in Section 4.2.2 and 4.2.3.

3.5. Toxicokinetics (TK)

Exposure data can be generated in either reproductive (dose range finding (DRF) or pivotal) or repeated-dose toxicity studies. However, given the potential for meaningful changes in TK parameters induced by pregnancy, it is recommended to determine if pregnancy alters exposure. If dose selection is based on exposure ratio (see section 6.1.3), GLP-compliant TK data in pregnant animals is expected. Sampling day(s) should be justified.

When warranted, determination of the pharmaceutical's concentration in the embryo or fetus can facilitate interpretation of discordant or equivocal evidence of developmental hazard. This information can be collected in a separate study to determine the actual exposure. However, a direct comparison to the potential levels in the human conceptus is not appropriate.

Evidence of lactational excretion can be obtained, when warranted, by sampling milk or by demonstrating exposure in offspring during the pre-weaning period.

General concepts regarding TK data collection are discussed in ICH S3A (4).

4. DESIGN AND EVALUATION OF IN VIVO MAMMALIAN STUDIES

The strategy to evaluate the potential reproductive and developmental risk of a pharmaceutical generally includes one or more *in vivo* studies. The key factor is that, in total, they leave no gaps between stages and allow for evaluation of all stages of the reproductive process, although in some species (e.g., the non-human primate (NHP)) it is not possible to evaluate all stages. For most pharmaceuticals, the 3-study design will usually be appropriate, although various combinations of these study designs can be conducted to address specific product needs and to reduce animal use. Study details for the FEED, EFD, and PPND studies, and combinations thereof, can be found in Annex 1. The stages covered in individual studies are left to the discretion of the sponsor. All available pharmacological, toxicokinetic, and toxicological data for the pharmaceutical should be considered in determining which study design(s) should be used.

4.1. Strategy to Address Fertility and Early Embryonic Development (FEED)

The aim of the FEED study is to test for adverse effects resulting from treatment initiated prior to mating of males and/or females and continued through mating and implantation. This comprises evaluation of Stages A and B of the reproductive process. Results from repeated-dose toxicity studies of at least two weeks duration can often be used to design the fertility study without conducting further dose ranging studies, although studies of such short duration can be insufficient to reveal all adverse effects.

A mating phase is expected in most cases when a FEED study is warranted to support exposure of the target population. Such studies are typically performed in rodents. If no adverse effects on fertility are anticipated, both sexes can be treated and cohabited together in the same study. If effects on fertility are identified in the study, the affected sex should then be determined. In contrast, if adverse effects are anticipated based on mode of action or on the results of repeated-dose studies, each treated sex can be cohabited with untreated animals of the opposite sex. This can be achieved using separate treatment arms within a single study or by the conduct of two separate FEED studies. Reversibility of adverse effects on fertility and early embryonic development can have an important impact on risk assessment.

The FEED study design in female rodents (see Annex 1) allows for the detection of effects on the estrous cycle, tubal transport, implantation, and development of preimplantation stages of the embryo. When estrous/menstrual cycles are evaluated, it is important to obtain baseline cycle data (over 2 or 3 cycles minimum) to distinguish between treatment-related effects and inter/intra animal variability. The monitoring of estrous cyclicity should continue through the time of confirmation of mating.

The FEED study design for male rodents that includes 2 to 4 weeks of treatment prior to cohabitation allows for the detection of effects on spermatogenesis and epididymal transport. When data from repeated-dose studies suggest toxicity to the testis, it can be appropriate to extend the duration of pre-cohabitation treatment to 10 weeks; this permits assessment of effects on the full spermatogenic cycle as well as epididymal transport. The FEED study additionally permits detection of functional effects (e.g., on libido, epididymal sperm maturation, ejaculation) that cannot be detected by histological examinations of the male reproductive organs.

When there is cause for concern based on mode of action or data from previous studies, additional examinations can be included in repeated-dose toxicity and/or fertility studies (e.g., sperm collection for counts and morphology/motility assessments, measuring hormone levels, or monitoring of the estrous/menstrual cycle) to further characterize potential effects on fertility.

4.1.1. Considerations for Biopharmaceuticals

If the biopharmaceutical is pharmacologically active in rodents or rabbits, a FEED study in one of these species is recommended. Mating evaluations are not generally feasible in non-rodents such as dogs and NHPs. For example, if NHPs are the only pharmacologically relevant species (as for many monoclonal antibodies, see ICH S6), histopathological examinations of the reproductive tissues from the repeated-dose toxicity studies of at least three months duration can serve as a substitute for the fertility assessments. Such an approach should include a comprehensive histopathological examination of the reproductive organs from both male and female animals (Note 1). Unless the biopharmaceutical is intended to treat advanced cancer, in which case FEED studies are not warranted, animals should be sexually mature at study initiation in order for an adequate evaluation of the reproductive tissues to be made. These data would only provide information on the structure of the reproductive tissues, as no functional assessment of fertility can be made and predicting effects on fertility and early embryonic development is not always possible based solely on the results of histopathology assessments.

4.2. Strategies to Address Embryo-Fetal Development (EFD)

The aim of the EFD studies is to detect adverse effects on the pregnant female and development of the embryo and fetus following treatment (Stage C) of the pregnant female during organogenesis. EFD studies include evaluation of fetal development and survival (Stages C through D).

For most small molecules, effects on EFD are typically evaluated in two species (i.e., rodent and non-rodent (typically rabbit)). At least one of the test species should exhibit the desired pharmacodynamic response. If the pharmaceutical is not pharmacodynamically active in any routinely used species (Section 5.1) then non-routine species (Section 5.2), genetically modified animals, or use of a species-specific surrogate molecule (Section 5.3) (e.g., in the case of oligonucleotides) can be considered, provided there is sufficient characterization of the model to ensure pharmacologic relevance. Genetically modified animals and surrogate molecules are generally most useful for hazard identification, but have limitations when used for risk assessment. Even when there are no relevant models (e.g., the pharmacological target only exists in humans, either normally or in the diseased state), EFD studies should be conducted in two species to detect the adversity of off-target effects or secondary pharmacology.

Clearly positive results for the induction of malformations or embryo-fetal lethality (MEFL), in a single species, at exposures similar to that at the projected clinical exposure at the maximum recommended human dose (MRHD) can be sufficient for risk assessment.

Under limited circumstances, other approaches can be used in place of definitive EFD studies (see Annex 2). Alternatively, there can be adequate information to communicate risk without conducting EFD studies. Evidence suggesting an adverse effect of the intended pharmacological mechanism on EFD (e.g., mechanism of action, phenotypic data from genetically modified animals) can be sufficient to communicate risk.

4.2.1. Considerations for Biopharmaceuticals

The effect of biopharmaceuticals on EFD should typically be assessed in two species (one rodent and one non-rodent) if both are pharmacologically relevant. However, the rodent is often not pharmacologically relevant, in which case EFD assessment in a single pharmacologically relevant non-rodent species can be conducted. In cases where the NHP is the only relevant species, an enhanced pre-and postnatal development (ePPND) study can be conducted instead of an EFD study. Biopharmaceuticals intended for the treatment of advanced cancer typically need only be assessed in a single pharmacologically relevant species (ICH S9).

When no relevant species can be identified because the biopharmaceutical does not interact with the orthologous target in any species relevant to reproductive toxicity testing, use of surrogate molecules or transgenic models can be considered, as described in ICH S6. Calculating safety margins relative to human exposures with surrogate molecules is not appropriate. If there are no relevant species, genetically modified animals or surrogates available, *in vivo* reproductive toxicity testing is not meaningful. In this case, the approach used for risk assessment, or rationale for not conducting studies, should be justified.

4.2.2. Alternative Approaches for Addressing EFD Risk

4.2.2.1. Use of Alternative Assays

A number of alternative *in vitro*, *ex vivo*, and non-mammalian *in vivo* assays (alternative assays) have been developed to detect potential hazards to embryo-fetal development. They have been used as drug discovery screens for adverse effects on EFD and have assisted in the understanding of the mechanism of toxicity, which can be useful for translating nonclinical data to human risk (especially for human-specific targets).
The continued use of alternative assays for these purposes is encouraged.

If properly qualified, alternative assays have the potential to defer or replace (in certain circumstances) conventional *in vivo* studies. This has the added benefit of potentially reducing animal use. Concepts to consider when qualifying these assays, and examples when the use of such assays could be appropriate, appear in Annex 2. Approaches that incorporate alternative assays should provide a level of confidence for human safety assurance at least equivalent to that provided by the current testing paradigms described above. Based on the direction of scientific development as of the writing of this document, it is expected that for regulatory purposes multiple alternative assays will be used within a tiered or battery approach. These testing strategies will be qualified within a certain context of use, which is defined by the chemical applicability domain of the assay, and by characterization of the biological mechanisms covered by the assay.

4.2.3. Potential Approaches to Defer Definitive In Vivo Testing as Part of an Integrated Testing Strategy

The design of an appropriate testing strategy relies on a cumulative weight-of-evidence approach. ICH M3 allows preliminary embryo-fetal developmental (pEFD) toxicity data from two species to support the limited inclusion of women of childbearing potential (WOCBP) (up to 150 WOCBP for up to 3 months) before conducting definitive EFD studies. Based on these considerations, this guideline expands on ICH M3 by allowing two additional options to support inclusion of WOCBP prior to Phase 3 clinical trials:

1) Qualified alternative assays which predict the outcome in one species (see Annex 2), can be combined with a pEFD from a second species to enable the limited inclusion of WOCBP (up to 150 WOCBP for up to 3 months). The alternative assay and the second species should generally cover both a rodent and a non-rodent species.
2) Additional endpoints incorporated into at least one GLP pEFD study (specifically increasing the group size of evaluable litters with inclusion of skeletal examinations) performed in a pharmacologically relevant species, if available, combined with a pEFD in a 2nd species allows all regions to include an unlimited number of WOCBP in clinical trials through Phase 2.

4.3. Strategy to Address Effects on Pre- and Postnatal Development (PPND)

The aim of the PPND study is to detect adverse effects following exposure of the maternal animal from implantation through weaning to evaluate effects on the pregnant or lactating female and development of the offspring. Since manifestations of effects induced during this period can be delayed, development of the offspring is monitored through sexual

maturity (i.e., Stages C to F). The rodent is usually used to assess PPND; however, other species can be used as appropriate (See Annex 1).

In most cases, a preliminary (dose range finding) PPND study is not warranted, because the appropriate information is generally available from prior studies. However, a preliminary PPND study with termination of the pups before or at weaning can be used to select dose levels or inform study design and/or to provide pup exposure data.

If a modified PPND/ePPND study design is being considered to support pediatric development, see ICH S11 (5).

4.3.1. Considerations for Biopharmaceuticals

For pharmaceuticals that can only be tested in the NHP, the ePPND study can provide a limited assessment of postnatal effects, but it is not generally feasible to follow the offspring through maturity (See Annex 1 and ICH S6).

5. TEST SYSTEM SELECTION

5.1. Routine Test Species

Mammalian species should be used to detect DART. The use of the same species and strain as in already completed toxicity studies can eliminate the need to use additional animals or conduct additional studies to characterize pharmacokinetics and metabolism, and/or for dose range finding. The species used should be well-characterized and relevant for detecting effects on the endpoints in a particular study (e.g., with respect to health, fertility, fecundity, background rates of malformation and embryo-fetal death, etc.).

5.1.1. Selection of Species for DART Testing

The rat is generally appropriate for DART testing and is the most often used rodent species for reasons of practicality, general knowledge of pharmacology in this species, the extensive toxicology data usually available for interpretation of nonclinical observations and the large amount of historical background data. The mouse is also often used as the rodent species for many of the same reasons.

For assessment of EFD only, a second mammalian non-rodent species is typically evaluated, although there are exceptions (e.g., vaccines and biopharmaceuticals, see Sections 5.1.2 and 5.2, respectively). The rabbit has proven to be useful in identifying human teratogens that have not been detected in rodents and is routinely used as the non-rodent species based on the extensive historical background data, availability of animals, and practicality.

5.1.2. Species Selection for Preventative and Therapeutic Vaccines

The animal species selected for testing of vaccines (with or without adjuvants) should demonstrate an immune response to the vaccine. The type of developmental toxicity study conducted, and the choice of the animal model, should be justified based on the immune response observed and the ability to administer an appropriate dose. Typically, rabbits, rats, or mice are used in developmental toxicity studies for vaccines. Even though

quantitative and qualitative differences can exist in the responses (e.g., in humoral and cellular endpoints) between species, it is usually sufficient to conduct developmental toxicity studies in a single species. Although the degree and time course of transfer of maternal antibodies across the placenta varies between species, a developmental toxicity study in rabbits, rats, or mice can still provide important information regarding potential embryo-fetal toxicity of the vaccine components/formulation and safety of the product during pregnancy. NHP should be used only if no other relevant animal species demonstrates an immune response.

When there is a lack of an appropriate animal model (including NHP), an EFD toxicity study in rabbits, rats, or mice can still provide important information regarding potential embryo-fetal toxicity of the vaccine components/formulation and safety of the product during pregnancy.

5.2. Non-routine Test Species

Species other than the rat, mouse or rabbit can be used to evaluate the effects of pharmaceuticals on various reproductive stages. When considering the use of other species, their advantages and disadvantages (summarized in Table 1 of Annex 1) should be considered in relation to the pharmaceutical being tested, the study design and selected endpoints, and the ability to extrapolate results to the human situation.

NHPs should be considered a non-routine test species. They are most typically used for evaluating effects on embryo-fetal development and early postnatal development for biopharmaceuticals that are only pharmacologically active in primates, as described in ICH S6. However, there are additional considerations that limit the utility of studies in NHPs for assessing some endpoints for DART risk assessment (see Annex 1 and ICH S6).

5.3. Use of Disease Models, Genetically Modified Models, and Surrogate Molecules

Animal models of disease, genetically modified models, and surrogate molecules can be valuable for investigating the effect of the intended pharmacology on development and reproduction. Studies in disease models can be of value in cases where the data obtained from healthy animals could be misleading or otherwise not apply to the disease conditions in the clinical setting. The model should be pharmacologically relevant and appropriate for the development and reproductive endpoints being assessed. The pathophysiology of the disease course in the model should be characterized. Some differences from the human pathophysiology would not preclude its use if these are unlikely to confound data interpretation. Animal-to-animal variability should be characterized and appropriate within the context of the study. If historical control information is limited, reference data for the study endpoints should be available or should be generated during the study to aid data interpretation.

Genetically modified models can be used to provide information about on-target effects of a pharmaceutical on DART parameters through permanent or conditional alterations in target activity. Such models can inform on whether the biology of the target is closely linked to adverse effects on reproduction and development in routine test species.

When the pharmaceutical does not have adequate activity against the target in the routine test species, surrogate molecules can be used to assess potential adverse effects on reproduction and development.

6. DOSE LEVEL SELECTION, ROUTE OF ADMINISTRATION AND SCHEDULE

The choice of dose levels, schedule and route of administration are important study design considerations and should be based on all available information (e.g., pharmacology, repeated-dose toxicity, pharmacokinetics, and dose range finding studies). Guidance on the principles of dose selection for small molecules and biopharmaceuticals is given in ICH M3 and ICH S6, respectively. When sufficient information on tolerability in the test system is not available, dose range finding studies are advisable.

6.1. Dose Selection

There are a number of dose selection endpoints that can be used for DART studies. All endpoints discussed in this section are considered equally appropriate in terms of study design. The high dose in the definitive studies should be one that is predicted to comply with one or more of the concepts set forth in sections 6.1.1 to 6.1.5 below. The selected doses should take into account observations made in previous studies (e.g., repeated-dose, TK, DRF, etc.). There can be instances where fewer than three dose levels are sufficient to provide the necessary information for risk assessment.

Justification for high dose selection using endpoints other than those discussed below can be made on a case-by-case basis.

6.1.1. Toxicity–based Endpoint

This endpoint is based on inducing a minimal level of toxicity in the parental animals at the high dose. Factors limiting the high dose determined from previously conducted studies could include, but are not limited to:

- Alterations in body weight (gain or absolute; either reductions or increases). Minor, transient changes in body weight gain or body weight are not appropriate for dose selection. When assessing weight change effects, the entire dosing duration of the study should be considered.
- Exaggerated pharmacological responses (e.g., excessive sedation or hypoglycemia)
- Toxicological responses (e.g., convulsions, excessive embryo-fetal lethality, clinical pathology perturbations). Specific target organ toxicity that would interfere with the study endpoints within the duration of the planned DART study.

6.1.2. Saturation of Systemic Exposure Endpoint

High dose selection based on saturation of systemic exposure measured by systemic availability of pharmaceutical-related substances can be appropriate. There is little value in increasing the administered dose if it does not result in increased plasma concentration of parent or metabolites.

6.1.3. Exposure Margin Based Endpoint

It can be appropriate to select doses based on predicted exposure margins relative to the exposure at the MRHD. For small molecules, a systemic exposure representing a large multiple of the human AUC or C_{max} at the MRHD can be an appropriate endpoint for high dose selection. Doses providing an exposure in pregnant animals > 25-fold the exposure at the MRHD are generally considered appropriate as the maximum dose for DART studies (Note 2). The 25-fold exposure margin should be established in a GLP-compliant dose range finding/pEFD or definitive study. Usually this multiple should be determined based on parent drug levels; however, consideration should also be given to ensuring an adequate exposure margin to major human metabolites (see ICH M3 and ICH M3 Q&A). In the case of prodrugs, it can be more appropriate to establish the exposure multiple on the basis of the active metabolite, particularly if the test species has a lower ratio of active metabolite to prodrug, compared to humans. The basis for the moiety used for comparison (parent drug or metabolite) should be justified.

For pharmaceuticals that have demonstrated pharmacodynamic activity in the test species only at exposures > 25-fold that projected at the MRHD, higher doses can be warranted to assess adverse effects of exaggerated pharmacology. However, irrelevant off-target effects are more likely to be observed.

When exposure-based endpoints are used as the basis for selection of the dose levels for EFD studies, TK data from pregnant animals in a GLP-compliant study is expected. The choice for the use of total vs. fraction unbound pharmaceutical exposures should be justified and consistent with the entire nonclinical development program as outlined in ICH S3A.

6.1.3.1. Exposure-based Approach for Biopharmaceuticals

Exposure-based margins can be appropriate to select doses for biopharmaceuticals as per ICH S6. Generally, the dose should provide the maximum intended pharmacological effect in the preclinical species or provide an approximately 10-fold exposure multiple over the maximum exposure to be achieved in the clinic, whichever is higher. ICH S6 should be consulted with regard to dose adjustment for differences in target binding affinity and other relevant factors.

6.1.4. Maximum Feasible Dose (MFD) Endpoint

The MFD can be used for high dose selection when the physico-chemical properties of the pharmaceutical (or formulation) associated with the route/frequency of administration and the anatomical/physiological attributes of the test species limit the amount of the pharmaceutical that can be administered. Use of the MFD should maximize exposure in the test species, rather than maximize the administered dose, as per ICH M3 Q&A (1). Note that changes to the frequency of dose administration can be considered to increase the total feasible daily exposure (see Section 6.3).

6.1.5. Limit Dose Endpoint

A limit dose of 1 g/kg/day can generally be applied when other dose selection factors have not been attained with lower dose levels (see also ICH M3 for other considerations).

6.1.6. Selection of Lower Dose Levels

It is generally desirable to establish a no observed adverse effect level (NOAEL) for DART. The selection of lower dose levels should take into account exposure, pharmacology, and toxicity, such that the dose-response of findings can be established when appropriate. The low dose should generally provide a low multiple (e.g., 1 to 5-fold) of the human exposure at the MRHD. Dose levels that yield exposures that are sub-therapeutic in humans should be justified.

6.2. Route

In general, the route of administration should be the clinical route. If, however, sufficient exposure cannot be achieved using the clinical route or the clinical route is not feasible, a different route should be considered. When multiple routes of administration are being evaluated in humans, a single route in the test species can be adequate provided that sufficient systemic exposure is achieved compared to that of all clinical routes and that there is adequate coverage for the metabolites.

6.3. Schedule

Dosing schedules used in the toxicity studies determine the exposure profile, which can be important in the risk assessment. Although mimicking the clinical schedule is often sufficient, a more or a less frequent schedule can be appropriate. For example, twice daily dosing can be warranted with compounds that are quickly metabolized in the test species, although pragmatic factors (e.g., study logistics, stress on animals) should be considered when a more frequent schedule is contemplated. It can also be important to alter the dosing schedule to ensure that adequate exposure is obtained at all critical stages of reproduction and/or development being evaluated in a given study.

6.4. Dose Selection and Study Designs for Vaccines

This guideline covers vaccines (adjuvanted or not) used in both preventative and therapeutic indications against infectious diseases. While not within the scope of this guideline, the principles outlined can be applicable to the nonclinical testing of vaccines for other indications as well (e.g., cancer).

The types of reproductive and/or developmental toxicity studies used for preventative and therapeutic vaccines depend on the target population for the vaccine and the relevant reproductive risk. Generally, DART studies are not warranted for vaccinees being developed for neonates, pre-pubertal children, or geriatric populations.

For reproductive toxicity studies of vaccines, it is typically sufficient to assess a single dose level capable of eliciting an immune response in the animal model (Section 5.1.2), using the clinical route of administration. This single dose level should be the maximum human dose without correcting for body weight (i.e., 1 human dose = 1 animal dose). If it is not feasible to administer the maximum human dose to the animal because of a limitation in total volume that can be administered, or because of dose-limiting toxicity, whether local or systemic, a dose that exceeds the human dose on a mg/kg basis can be used. To use a reduced dose, justification as to why a full human dose cannot be used in an animal model should be provided.

The vaccination regimen should maximize maternal antibody titers and/or immune response throughout the embryonic, fetal, and early postnatal periods. Timing and number of doses will depend on the onset and duration of the immune response of the particular vaccine. When developing vaccines to be given during pregnancy, a justification should be provided for the specific study design, based upon its intended use (e.g., protecting the mother during pregnancy or protecting the child early postnatally).

Daily dosing regimens can lead to overexposure to the vaccine constituents. Episodic dosing of pregnant animals rather than daily dosing is recommended. Also, episodic dosing better approximates the proposed clinical immunization schedule for most preventive and therapeutic vaccines. Considering the short gestational period of routine animal species, it is generally recommended to administer a priming dose(s) to the animals several days or weeks prior to mating in order to elicit peak immune response during the critical phases of pregnancy (i.e., the period of organogenesis). The dosing regimen can be modified according to the intended vaccination schedule in humans.

At least one dose should be administered during early organogenesis to evaluate potential direct embryotoxic effects of the components of the vaccine formulation and to maintain a high antibody response throughout the remainder of gestation. If embryo-fetal toxicity is observed, this can be further assessed using subgroups of animals that are dosed at certain time points.

In cases where a vaccine includes a novel active constitutive ingredient (including novel adjuvants), consideration of additional testing strategies similar to those for non-vaccine products can be appropriate.

7. POSSIBLE COMBINATION STUDY DESIGNS IN RODENTS

Although three separate study designs, i.e., FEED (stages A and B), EFD (stages C through D) and PPND (stages C through F) have been employed to develop the majority of pharmaceuticals, various combinations of these study designs can be conducted to reduce animal use. The main advantage of combination designs is that all relevant stages of the reproductive process can be assessed using fewer animals. Combination studies can also better mimic the exposure duration in the clinic, especially for drugs with long half-lives. A common combination study design is a combined Fertility and EFD study (stages A through D) with a separate PPND study (stages C through F).

Designs and study details for FEED, EFD, and PPND studies, and combinations thereof, can be found in Annex 1.

In cases where no effects on male or female fertility are anticipated, or where extending the dosing period is appropriate due to observation of reproductive organ toxicity in a repeated-dose toxicity study, a combination design of repeated-dose and fertility studies can be considered. After a defined dosing period within the repeated-dose toxicity study, males can be paired with sexually mature females (whether untreated, or dosed for at least two weeks prior to mating). This combination study can reduce the number of animals used, but the number of mating pairs per group should be at least 16. Further, if treated, dosing of females can be extended until the end of organogenesis, thereby allowing evaluation of EFD endpoints (Annex 1).

8. DATA REPORTING AND STATISTICS

8.1. Data Reporting

Individual values should be tabulated in a clear concise manner to account for all animals in the study. The data tables should allow ready tracking of individual animals and their conceptuses, from study initiation through study conclusion.

Fetal morphologic abnormalities should be described using industry-harmonized terminology. All findings for each litter should be clearly listed by conceptus. Summary listings should be prepared by type of abnormality. The inclusion or exclusion of data from non-pregnant animals in summary tables should be clearly indicated.

Interpretation of study data relies primarily on comparison with the concurrent control group. Historical control/reference data can be used to assist data interpretation. Recent historical control data from the performing laboratory is preferable. Contemporary data typically from a five-year period is desirable and permits identification of genetic drift.

8.2. Statistics

Statistical testing to assess the significance of differences between the treated and control groups is expected in definitive studies. Many of the datasets from DART studies do not follow a normal distribution, necessitating the use of non-parametric statistical methods. Cesarean, fetal and postnatal data summary statistics should be calculated using the litter as the unit of analysis. Statistical significance need not convey a positive signal, nor lack of statistical significance impute absence of effect. Determination of biological plausibility, based on all available pharmacologic and toxicologic data, is often useful.

9. PRINCIPLES OF RISK ASSESSMENT

As described in the preceding sections of this guideline, all available data garnered from the pharmaceutical, related compounds, human genetics, and knowledge of the role of target biology in human reproduction should be used to address potential reproductive risks in humans under the conditions of use, both during clinical trials and after marketing authorization. Any limitations (e.g., test system relevance, achieved exposure), uncertainties and data gaps in the available nonclinical DART data package should be addressed and their impact assessed. Generally, the results from definitive *in vivo* studies in an appropriate species with adequate exposures carry more weight than those from alternative assays or preliminary studies. Risk assessment is a continuous process through product development as more information becomes available.

Not all findings reported in DART studies are adverse. When a finding is deemed adverse, several factors should be considered in a weight-of-evidence evaluation for risk assessment. These can include exposure margins, biological plausibility, evidence of a dose-response relationship, potential for reversibility, the potential for confounding parental toxicity, and evidence for cross-species concordance. For rare malformations, the absence of increased frequency with dose does not always alleviate concern.

Comparison of pharmaceutical exposure at the NOAEL in the test species to the exposure at the MRHD is an important component of the risk assessment. This comparison should be based on the most relevant metric (e.g., AUC, C_{max}, $C_{min,}$ body surface area-adjusted

dose). In general, there is increased concern when the NOAEL occurs at exposures less than 10-fold the human exposure at the MRHD; above this threshold, concern is reduced. Effects that are limited to occurrence at more than 25-fold the human exposure at the MRHD are usually of minor concern for the clinical use of the pharmaceutical. The most relevant margin is generally the exposure metric in the most sensitive species, unless appropriately justified otherwise. Biological plausibility is assessed by comparison of pharmacologic mechanism of action with the known role of the target in reproduction or development. A finding that can be interpreted as a consequence of pharmacology suggests that it will be of concern for humans. This relationship is further strengthened by evidence that the finding is dose-related, whether characterized as increasing incidence or severity. Absence of biological plausibility does not preclude off-target toxicity, particularly if this is characterized by a dose-response relationship.

Understanding the potential for reversibility will alter the risk assessment. Effects on male and female fertility that are reversible after cessation of treatment are of less concern. Conversely, critical irreversible developmental endpoints, such as death or malformation, are of increased concern. Other forms of developmental toxicity (e.g., growth retardation, functional deficits), may or may not be reversible. Generally, transient findings (e.g., skeletal variations, such as wavy ribs in rodents) are of less concern when they occur in isolation. Similarly, variations that are indicative of growth retardation in the presence of reduced fetal weight are of less concern. However, an overall increase in the incidence of variations (qualitatively similar or not) can suggest increased concern for dysmorphogenesis in the presence of an equivocal increase in malformations.

The role of parental toxicity should be considered in determination of the relevance of findings. Embryo-fetal toxicity observed in the presence of maternal toxicity should be considered carefully to determine the likelihood that the finding is relevant for humans. Specifically, evaluation of the concordance between individual litter findings and the severity of maternal toxicity in the dam could be helpful in this assessment. It should not be assumed that developmental toxicity is secondary to maternal toxicity, unless such a relationship is demonstrated de novo, or relevant published literature can be cited.

Also, consistency of findings reported among studies, or between species can strengthen the concern for an adverse effect. Increased fetal lethality seen in a rodent EFD study that is consistent with decreased live litter sizes in the PPND study is an example of cross-study concordance. Observations of increased post implantation loss in rats and rabbits is an example of cross-species concordance. Further knowledge of the mechanism of reproductive or developmental effects identified in animal studies can help to explain differences in responses between species and provide information on the human relevance of the effect (e.g., corticosteroid-induced cleft palate in mice).

A specific risk assessment conducted for breastfeeding would be predicated on hazards identified by the *in vivo* littering study (PPND or ePPND). These hazards can include adverse effects on offspring growth and development that are attributed to excretion of the pharmaceutical in the milk. Systemic exposure data in the pups from the littering study, if available, can also be compared with projected lactational exposures in the human infant. While interspecies differences in milk composition preclude a direct quantitative correlation of animal milk levels to human milk levels of a pharmaceutical, the presence of pharmaceutical in animal milk generally indicates the presence of pharmaceutical in human milk.

Lastly, available human data can influence the overall assessment of human reproductive risk.

10. ENDNOTES

Note 1: In particular, the testes and epididymides should be sampled and processed using methods which preserve the tissue architecture of the seminiferous epithelium. A detailed qualitative microscopic evaluation with awareness of the spermatogenic cycle is a sensitive means to detect effects on spermatogenesis. While generally not warranted, additional experimental endpoints (e.g., immunohistochemistry, homogenization resistant spermatid counts, flow cytometry, quantitative analysis of staging) can be incorporated into the study design to further characterize any identified effects. In females, a detailed qualitative microscopic examination of the ovary (including follicles, corpora lutea, stroma, interstitium, and vasculature), uterus and vagina should be conducted with awareness of the reproductive cycle and the presence of primordial and primary follicles.

Note 2: An analysis of 22 known human or presumed human teratogens showed that if MEFL was observed, exposure at the lowest observed adverse effect level (LOAEL) in at least one species was < 6-fold the exposure at the MRHD (Andrews et al. (6)). This indicates that using a > 25-fold exposure ratio for high-dose selection in the EFD toxicity studies would have been sufficient to detect the teratogenic hazard for all these pharmaceuticals. The analysis also showed that for human teratogens that were detected in animal species, the exposure at the NOAEL in at least one species was < 4-fold the exposure at the MRHD.

In addition, a survey was conducted on EFD toxicity studies by the IQ DruSafe Leadership Group (Andrews et al. (7)). This survey identified 153 and 128 definitive rat and rabbit EFD studies, respectively, that achieved ≥ 15-fold animal to human parent drug exposure ratios (using human exposure at the intended therapeutic dose) in the absence of confounding (i.e., dose-limiting) maternal toxicity. These data show that dosing animals to achieve exposures ≥ 25-fold human exposures when there is no maternal toxicity (that would otherwise limit the high dose), only infrequently detects MEFL. In all these cases, MEFL findings were not observed until exposures exceeded 50-fold and findings at such high exposures are not believed to be relevant to human risk assessment. In the absence of confounding maternal toxicity, the selection of a high dose for EFD and PPND studies that represents a > 25-fold exposure ratio to human plasma exposure of total parent compound at the intended maximal therapeutic dose is therefore considered pragmatic and reasonably sufficient for detecting outcomes relevant for human risk assessment.

11. GLOSSARY

Disclaimer: The definitions in this glossary are specific for their use within this guideline.

Alternative assay(s): *In vitro, ex vivo* or non-mammalian *in vivo* assay(s) intended to predict malformations or embryo-fetal lethality; see MEFL.

Applicability domain: refers to the definition of the physicochemical properties of the substances that can be reliably tested in the assay and the biological mechanisms of action covered by the assay.

Assay qualification (for regulatory use): Confirmation of the predictivity of an alternative assay(s) to identify MEFL, as observed *in vivo*.

Constitutive ingredients: Chemicals or biologic substances used as excipients, diluents, or adjuvants in a vaccine, including any diluent provided as an aid in the administration of the product and supplied separately.

Developmental toxicity: Any adverse effect induced prior to attainment of adult life. It includes effects induced or manifested from conception to postnatal life.

GD 0: The day on which positive evidence of mating is detected (e.g., sperm is found in the vaginal smear / vaginal plug in rodents, or observed mating in rabbits).

Malformation: Permanent structural deviation that generally is incompatible with or severely detrimental to normal development or survival.

Preliminary EFD (pEFD) toxicity study: An embryo-fetal developmental toxicity study that includes exposure over the period of organogenesis, has adequate dose levels, uses a minimum of 6 pregnant animals per group, and includes assessments of fetal survival, fetal weight, and external and soft tissue alterations (see ICH M3).

Surrogate molecule: A molecule showing similar pharmacologic activity in the test species as that shown by the human pharmaceutical in the human.

Vaccine: For the purpose of this guideline, this term refers to preventative or therapeutic vaccines for infectious diseases. Vaccine (inclusive of the term vaccine product) is defined as the complete formulation and includes antigen(s) (or immunogen(s)) and any additives such as adjuvants, excipients or preservatives. The vaccine is intended to stimulate the immune system and result in an immune response to the vaccine antigen(s). The primary pharmacological effect of the vaccine is the prevention and/or treatment of an infection or infectious disease.

Variation: Structural change that does not impact viability, development, or function (e.g., delays in ossification) which can be reversible, and are found in the normal population under investigation.

12. REFERENCES

1. International Council for Harmonisation M3: Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals together with ICH M3 Questions & Answers.

2. International Council for Harmonisation S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.

3. International Council for Harmonisation S9: Nonclinical Evaluation for Anticancer Pharmaceuticals.

4. International Council for Harmonisation S3A: Note for Guidance on Toxicokinetics: The Assessment of Systemic Toxicity in Toxicity Studies together with ICH S3A Questions and Answers.

5. International Council for Harmonisation S11: Nonclinical Safety Testing in Support of Development of Pediatric Medicines.

6. Andrews PA, Blanset D, Lemos Costa P, Green M, Green ML, Jacobs A, et al. Analysis of exposure margins in developmental toxicity studies for detection of human teratogens. Regul Toxicol Pharmacol. 2019a;105:62-8.

7. Andrews PA, McNerney ME, DeGeorge JJ. Reproductive and developmental toxicity testing: An IQ-DruSafe industry survey on current practices. Regul Toxicol Pharmacol. 2019b;107:104413.

ANNEX 1 *IN VIVO* STUDY DESIGNS

Outlined below are advantages and disadvantages to the use of various species utilized in DART studies.

Table 1: Principle Advantages and Disadvantages of Various Species for Developmental and Reproductive Toxicity Testing

Routine Species		
Species	**Advantages**	**Disadvantages**
Rat	• Well-understood biology • Widely used for pharmacodynamics and drug discovery • Robust reproductive capacity with short gestation • Large group sizes and litter size • Data available from repeated-dose toxicity study • Suitable for all stages of testing • Widespread laboratory experience and availability • Extensive historical data	• Different placentation to human (e.g., timing, inverted yolk sac) • Dependence on prolactin as the primary hormone for establishment and maintenance of early pregnancy, which makes them sensitive to some pharmaceuticals (e.g., dopamine agonists) • Highly sensitive to pharmaceuticals that disrupt parturition (e.g., nonsteroidal anti-inflammatory drugs in late pregnancy) • Less sensitive than humans to fertility perturbations • Limited application for foreign proteins o Limited or no pharmacologic activity o Potential impact of immunogenicity

Rabbit	• Similar advantages to rats • Non-rodent model • Suitable for serial semen sampling and mating studies • Placental transfer of antibodies more closely approximates primates than rodents, an advantage for DART testing of vaccines	• Limitations similar to rat for foreign proteins • Limited historical data for fertility and pre-/postnatal studies • Sensitive to gastrointestinal disturbances; (e.g., some antibiotics) • Prone to spontaneous abortion • General physical condition difficult to monitor using clinical signs • Should generate PD, toxicity, and TK data as not generally used for toxicology programs (except for vaccines)
Mouse	• Similar advantages to rats • Genetically modified models available or can be generated • Surrogate molecules are often available • Uses small amounts of test material	• Similar limitations to rats • Small fetus size and tissue volumes • Stress sensitivity • Malformation clusters are known to occur

Non-routine Species		
Species	**Advantages**	**Disadvantages**
Cynomolgus Monkey (NHP)	• Generally more phylogenetically and physiologically similar to humans than other species • More likely than rodents to show similar pharmacology to humans • Placentation similar to human • Data available from repeated-dose toxicity study • Transfer of antibodies across the placenta similar to humans	• Small group size, hence low statistical power and wide variability across groups • Low fecundity o Single offspring • High background pregnancy lossLimited availability of breeding animals • Long menstrual cycle (30 days) and gestation (165 days) • Impractical for fertility (mating) studies • F1 reproduction function not practical to evaluate due to late sexual maturity (around 3 to 6 years of age) • Sexual maturity cannot be determined by age and body weight

		• Ethical considerations • Less historical control data and laboratory experience/capability • Highly variable age, weight and pregnancy history at the start
Mini-pig	• Alternate non-rodent for general toxicity testing • Short period of organogenesis (GD 11-35) • Defined genetic background and specific-pathogen-free animals • Sexual maturity by 7 months • Larger litter size compared to NHP • Suitable for serial semen sampling and mating studies • Sufficient historical background data on reproductive endpoints	• Limited number of experienced laboratories • Long gestation (114 days) • Uses a large amount of test material • Minimal to no prenatal transfer of antibodies

Limited Use Species (primarily used for investigative purposes)		
Species	**Advantages**	**Disadvantages**
Hamster	• Alternate rodent model that can be pharmacologically relevant	• High postnatal loss due to cannibalization • Limited historical control data and laboratory experience • Limited availability of postnatal behavioral and functional tests • IV route difficult • Aggressive • Sensitive to GI disturbances • Should generate PD, toxicity, and TK data as not generally used for toxicology programs • Blood sampling is difficult

Dog	• Usually have repeated-dose toxicity data • Readily amenable to semen collection	• Long gestation (63 days) • Limited historical control data and laboratory experience • Limited availability of postnatal behavioral and functional tests • Uses a large amount of test material

Other mammalian species not listed here can also be used to evaluate the effects of pharmaceuticals on DART endpoints.

1.1 *In Vivo* Study Design Considerations

Generally, within and between reproductive studies animals should be of comparable age, weight and parity at the start. The easiest way to fulfil these factors is to use animals that are young, sexually mature adults at the time of the start of dosing. The number of animals per group specified in individual studies is a balance based on scientific judgment from many years of experience with these study designs, and ethical considerations on the appropriate use of animals. Smaller group sizes can be sufficient to demonstrate anticipated adverse effects on reproduction or development at clinically relevant exposures of the pharmaceutical.

Evaluation of 16 to 20 litters for rodents and rabbits provides a degree of consistency among studies. Below 16 litters inter-study results become inconsistent, and above 20 to 24 litters per group, consistency and precision is not greatly enhanced. These numbers refer to litters available for evaluation. If groups are subdivided for different evaluations the number of animals starting the study should be adjusted accordingly.

The suggested study designs below can be modified, particularly with respect to parameters, timings, and assessments and still meet the study objectives. Expert judgment should be used for adapting these framework designs for individual laboratories and purposes.

1.1.1 Fertility and Early Embryonic Development (FEED) Study

The FEED study is designed to assess the maturation of gametes, mating behavior, fertility, preimplantation development of the embryo, and implantation. For females, this includes effects on the estrous cycle and tubal transport. For males, it includes detection of functional effects (e.g., epididymal sperm maturation) that cannot be detected by histological examinations of the male reproductive organs.

A combined male/female FEED study, in which both sexes are administered test article, is commonly used (See Table 2). However separate male only or female only studies can be conducted by substituting the appropriate number of untreated females or males in the study designs.

Table 2: FEED Study Design: Rodents, combined male and female study

Parameter	
Group size	at least 16 of each sex
Number of dose groups	4 (including 1 control)
Administration period[a]	M: ≥ 2 weeks prior to cohabitation through at least confirmation of mating F: ≥ 2 weeks prior to cohabitation through implantation (GD6)
Mating ratio	1 male:1 female
Mating period[b]	≥ 2 weeks
Estrous cycle evaluation	Daily, commencing 2 weeks before cohabitation and until confirmation of mating
Clinical observations/mortality	At least once daily
Body weight	At least twice weekly
Food consumption	At least once weekly (except during mating)
Male necropsy[c]	Preserve testes and epididymides for possible histological examination; and evaluate on a case by case basis. Perform macroscopic examination and preserve organs with findings for possible histological evaluation; keep corresponding organs of sufficient controls for comparison.
Sperm analysis[d]	Optional
Female necropsy[e]	On a case by case basis, preserve ovaries and uteri for possible histological examination and evaluation. Perform macroscopic examination and preserve organs with findings for possible histological evaluation; keep corresponding organs of sufficient controls for comparison.
Scheduled cesarean section	Cesarean sections typically performed mid-gestation; corpora lutea counts, number of implantation sites, live and dead embryos
Uterine implantation data	

a: Available data from repeated-dose toxicity studies and genotoxicity studies should be used to justify dosing duration, especially for detecting effects on spermatogenesis. A premating treatment interval of 2 weeks for females and 2 weeks for males can be used provided no effects have been found in repeated-dose toxicity studies of at least 2 weeks duration that preclude this. Treatment of males should continue throughout confirmation of mating, although termination following confirmation of female fertility can be valuable. Treatment of females should continue through at least implantation. This will

permit evaluation of functional effects on fertility that cannot be detected by histopathological examination in repeated-dose toxicity studies and effects on mating behavior.

b: Most rats or mice will mate within the first 5 days of cohabitation (i.e., at the first available estrus), but in some cases females can become pseudopregnant. Leaving the female with the male for longer than 2 weeks can allow these females to restart estrous cycles and become pregnant.

c: It can be of value to delay euthanasia of the males until the outcome of mating is known. In the event of an effect on fertility, males could be mated with untreated females to ascertain any potential male-mediation of the effect. A more complete evaluation of toxicity to the male reproductive system can be achieved if dosing is continued beyond mating and euthanasia delayed so that the males are exposed for the total duration of a spermatogenic cycle (e.g., 10 weeks).

d: Sperm analysis (e.g., sperm counts, motility, and/or morphology) sometimes can be useful if issues arise to support risk assessment.

e: Termination of females around days 13-15 of pregnancy in general is adequate to assess effects on fertility and reproductive function (e.g., to differentiate between live implantations and resorption sites). There is an option to terminate females near the end of gestation.

1.1.2 Embryo-Fetal Developmental (EFD) Toxicity Study

The EFD toxicity study is designed to assess maternal toxicity relative to that in non-pregnant females, and to evaluate potential effects on embryo-fetal survival, intrauterine growth, and morphological development.

Suggested study designs for rodents, rabbits and cynomolgus monkeys are described below.

1.1.2.1. Dose Range Finding Embryo-Fetal Developmental (EFD) Toxicity Study

Dose range finding studies in mated females are most often used to select appropriate dose levels, or dose schedules, for the definitive rodent and rabbit EFD studies. Tolerability and TK data from existing repeated-dose toxicity studies can, however, be sufficient for this purpose.

1.1.2.2 Preliminary Embryo-Fetal Developmental (pEFD) Toxicity Study

The pEFD toxicity study (Table 3) is similar in design to the definitive EFD toxicity study. A typical pEFD toxicity study design includes dosing over the period of organogenesis, has adequate dose levels, evaluates a minimum of 6 pregnant females per group, and includes assessments of fetal survival, fetal weight, external fetal abnormalities and soft tissue abnormalities (see ICH M3).

1.1.2.3 Definitive Embryo-Fetal Developmental (EFD) Toxicity Study

The females are submitted to cesarean section near term. Assessments of fetal survival, fetal weight, external fetal abnormalities, soft tissue abnormalities and skeletal examinations are performed (Table 3). The timing given in Table 3 is for rodent, rabbit and cynomolgus monkeys; for other species appropriate timing should be used.

Table 3: EFD Toxicity Study Designs for Rodent, Rabbit and NHP

	pEFD	**EFD**		
Parameter	**Rodent/Rabbit**	**Rat (Mouse)**	**Rabbit**	**NHP[a]**
GLP Status	Optional[c]	Yes	Yes	Yes
Minimum number of pregnant females	6	16	16	16[b]
Number of dose groups	4 (including 1 control)	4 (including 1 control)	4 (including 1 control)	At least 2 (including 1 control)
Administration period[d]	Species appropriate	GD6/7-17 (6/7-15)	GD6/7-19	Approximately GD 20 - to at least GD 50
Antemortem endpoints				
Clinical observations/mortality	At least once daily	At least once daily	At least once daily	At least once daily
Body weight	At least twice weekly	At least twice weekly[e]	At least twice weekly[e]	At least once weekly
Food consumption	At least once weekly	At least once weekly	At least once weekly	Optional
Toxicokinetics	Optional[c]	Yes	Yes	Yes
Postmortem endpoints				
Cesarean section [f]	Species appropriate	GD20/21 (17/18)	GD28/29	GD100
Macroscopic examination	Yes	Yes	Yes	Optional
Gravid uterine weight	Optional	Optional	Optional	NA
Corpora lutea	Yes	Yes	Yes	NA
Implant sites	Yes	Yes	Yes	NA
Live and dead conceptuses	Yes	Yes	Yes	Yes
Early and late resorptions	Yes	Yes	Yes	NA
Gross evaluation of placenta	Yes	Yes	Yes	Yes
Weight of placenta	Optional	Optional	Optional	Optional
Fetal body weight	Yes	Yes	Yes	Yes
Fetal sex	Yes	Yes	Yes	Yes
Fetal external evaluations[g]	Yes	Yes	Yes	Yes

Fetal soft tissue evaluations[g]	Yes	Yes[g]	Yes	Yes
Fetal skeletal evaluations[h]	Optional[c]	Yes[g]	Yes	Yes

a: If a NHP other than the Cynomolgus monkey is used, the study design should be adapted.

b: Group sizes in EFD studies should yield a sufficient number of fetuses in order to assess potential adverse effects on morphological development.

c: If the pEFD is used to defer a definitive EFD study, then the pEFD should be done in accordance with GLP regulations, TK data in pregnant animals should be collected, and skeletal evaluations should be performed.

d: For rodents and rabbits, females are dosed with the test substance from implantation to closure of the hard palate (i.e., stage C of the reproductive process, see Section 1.1). For NHP, females are dosed from confirmation of pregnancy (approximately GD 20) to at least Day 50 (end of major organogenesis)

e: Daily weighing of pregnant females during treatment can provide useful information.

f: For rodents and rabbits, cesarean sections should be conducted approximately one day prior to expected parturition. Preserve organs with macroscopic findings for possible histological evaluation; keep corresponding organs of sufficient controls for comparison. For NHP, cesarean sections should be conducted on approximately GD 100.

g: All fetuses should be examined for viability and abnormalities. To permit subsequent assessment of the relationship between observations made by different techniques fetuses should be individually identified.

h: Although it is preferable to examine all rodent fetuses for both soft tissue and skeletal alterations (if methods allow), it is acceptable to submit 50% of fetuses in each litter to separate examinations.

1.1.3 Pre- and Postnatal Developmental (PPND) Toxicity Study

The PPND toxicity study is designed to assess enhanced toxicity relative to that in non-pregnant females, pre- and postnatal viability of offspring, altered growth and development, and functional deficits in offspring, including sexual maturation, reproductive capacity at maturity, sensory functions, motor activity, and learning and memory.

The females are permitted to deliver and rear their offspring to weaning at which time at least one male and one female offspring per litter are selected for rearing to adulthood and mating to assess reproductive competence (see Table 4).

Table 4: PPND Toxicity Study Design: Rats

Parameter	
Group size	At least 16 litters
Number of dose groups	4 (including 1 control)
Administration period	From implantation (GD 6/7) through weaning (postnatal day (PND) 20)
F0 Females	
Clinical observations/mortality	At least once daily
Body weight	At least twice weekly
Food consumption	At least once weekly until mid-lactation
Parturition observations	GD 21 until complete
Necropsy	PND 21 At necropsy, preserve and retain tissues with macroscopic findings and corresponding control tissues for possible histological evaluation, count uterine implantation sites
F1 Pre-weaning	
Clinical observations/mortality	Daily from PND 0
Pre-and postweaning survival	Daily from PND 0
Body weight and sex	PND 0/1 and then at least twice per week
Optional Standardization of litter size	≥ PND 4, to 4 or 5 pups per sex
Physical development[a]	Preweaning landmarks of development and reflex ontogeny (e.g. eye opening, pinna unfolding, surface righting, auditory startle, air righting, and response to light)
F1 Post-weaning	
Selection for post-weaning evaluation and group size[b]	PND 21, at least 1 male and 1 female/litter where possible to achieve 16 animals per group/sex
Clinical observations/mortality	Daily
Body weight	Weekly
Optional Food consumption	Weekly
Sexual maturation[c]	Females: vaginal opening Males: preputial separation
Other functional tests[d]	Assess sensory functions, motor activity, and learning and memory.
Reproductive performance	At least 10 weeks old, paired for mating (1M:1F) within the same group (not siblings)

a: The best indicator of physical development is bodyweight, however, measurement of bodyweight alone is not an acceptable substitute for the evaluation of other developmental parameters.

b: At least one animal per sex per litter should be retained to conduct behavioral and other functional tests, and to assess reproductive function. There can be circumstances where more animals per litter can be retained for independent functional assessments.

c: Body weight should be recorded at the time of attainment to determine whether any differences from control are specific or related to general growth.

d: Learning and memory should be evaluated in a complex learning task. Assessments of locomotor activity and startle reflex with prepulse inhibition (if conducted) should be evaluated over a sufficient period of time to demonstrate habituation.

1.1.3.1 Enhanced Pre- and Postnatal Developmental (ePPND) Toxicity Study in Non-Human Primate (NHP)

The ePPND toxicity study (Table 5) is a study in NHP that combines the endpoints from both the EFD and PPND studies. In this study dosing is extended throughout the gestation period to parturition (e.g., GD20 to parturition). See ICH S6 for information on timing and additional parameters to be evaluated.

Table 5: ePPND Toxicity Study Design: for Cynomolgus Monkey[a]

Parameter	
Group size[b]	Approximately 16 pregnant females
Number of dose groups	At least 2 (including 1 control)
Administration period	From confirmation of pregnancy (approximately GD 20) to parturition
F0 Females	
Clinical observations/mortality	At least once daily
Body weight	At least weekly
Parturition observations	Document day of completion
Placenta	Collect and preserve if possible
Necropsy and tissue evaluation	Only as warranted
Exposure Assessment	TK profiles and/or systemic drug levels should be measured, as appropriate
F1	
Clinical observations/mortality	Daily from PND 0
Body weights	Weekly
Morphometry/Physical and/or functionalassessment	At regular intervals, as appropriate
Neurobehavioural test battery .	At least 1 interval during the first 2 weeks postpartum

Grip strength	PND 28
Mother-infant interaction	Minimally in early postnatal period to confirm nursing; as appropriate thereafter
Exposure assessment	Systemic drug levels should be measured, as appropriate
External evaluation	At regular intervals
Skeletal evaluation	Approximately PND 28 or later
Visceral evaluation	At necropsy
Necropsy	At minimum 1 month, depends on aim of the evaluations Preserve and retain tissues for possible histological evaluation

a: If an NHP other than the Cynomolgus monkey is used, the study design should be adapted.

b: Group sizes in ePPND studies should yield a sufficient number of infants in order to assess potential adverse effects on pregnancy outcome, as well as dysmorphology and postnatal development, providing the opportunity for specialist evaluation if warranted (e.g., immune system). Most ePPND studies accrue pregnant animals over several months.

1.1.4 Combination Studies

The possibility also exists to combine study types to meet the goals of the development program. This is accomplished by incorporating appropriate endpoints measured in the separate studies summarized above into a single study. Concepts for various combination studies are provided below.

1.1.4.1 FEED and EFD

The aim of the combined FEED/EFD study is to test for toxic effects resulting from treatment from before mating (males/females) through mating, implantation and until the end of organogenesis. This comprises evaluation of stages A through D of the reproductive process (see Section 1.1). This study design is most often used with rodents, although it could be used with non-rodents.

A combined male/female FEED/EFD can be used, but a separate female only option is possible where male fertility is assessed in a separate study such as a repeated dose study of suitable duration. The study would then use untreated males for mating purposes only. For specific study design and observational parameters see Sections 1.1.1. and 1.1.2 of this Annex.

1.1.4.2 Male Fertility and Repeated-Dose Toxicology Study

It is also possible to evaluate male fertility during a rodent repeated-dose toxicity study. In this combination study, males that have been dosed for a defined number of weeks are paired with untreated females. Following cohabitation, the males continue to be dosed until the scheduled termination of the repeated-dose toxicity study. The untreated females are subjected to cesarean section approximately two weeks after evidence of mating. The study endpoints collected are identical to those outlined in Section 1.1.1 of this Annex. To adequately assess

effects, at least 16 males per group should be included in the study. Female fertility and other FEED endpoints will need to be evaluated in a separate study.

ANNEX 2 ALTERNATIVE ASSAYS

Data generated from qualified alternative assays (see glossary) conducted alone or in conjunction with one or more *in vivo* studies can be utilized to support hazard identification and risk assessment under limited circumstances.

Potential uses can include:

- circumstances where there is evidence suggesting an adverse effect on EFD (e.g., a mechanism of action affecting fundamental pathways in developmental biology, phenotypic data from genetically modified animals, class effects) (see Section 1.2.2 and Figure 1 of this Annex)
- toxicity in animal species precludes attaining systemic exposures relevant to the human exposures under conditions of use
- as support for a weight of evidence assessment when there are equivocal findings in animal studies
- as partial support for clinical trials including up to 150 WOCBP for up to 3 months duration (see Section 4.2.3 of Guidance)
- pharmaceuticals being developed for certain severely debilitating or life-threatening diseases or late-life onset diseases (see Sections 1.2.3, 1.2.4 and Figure 2 of this Annex).

When alternative assays are used to support risk assessment, incorporation of these assays into an integrated testing strategy should be justified. Assay(s) used for risk assessment should be conducted in accordance with GLP and qualified for context of use (i.e. applicability domain and regulatory conditions under which assay results are reliable). Strategies incorporating alternative assays should also assess the effects of drug metabolites when warranted (ICH M3). This annex does not recommend specific assays; instead, basic scientific principles are included to assist in assay qualification for regulatory use. Alternative assays used to explore mechanism of action, or otherwise not intended to substitute for *in vivo*-derived EFD endpoints, are not expected to be qualified in this rigorous manner.

1.1 Qualification of Alternative Assays for Prediction of MEFL

Test methods must be appropriate in order for test results to be of value. Accordingly, the endpoints measured should be scientifically justified with respect to assay objectives and predictions. The relationships among the assay's predictions, endpoint(s) assessed, and the applicability domain, should be supported empirically. To qualify[1] an alternative assay or a combination of assays for use in risk assessment for regulatory purposes, a comprehensive

[1] Qualified alternative assays within the context of this guideline have not been subject to formal validation as those can only be applied under certain specific circumstances.

description of the methodology and findings should be provided, including the following:

- A thorough description and justification of the predictive model, including which species (e.g., rat, rabbit and/or human) and endpoint(s) it is predicting. The currently available *in vitro* alternative assays used for evaluating potential hazards to development are designed to detect MEFL.
- An evaluation of the biological plausibility of the model including a description of the mechanisms of embryo-fetal development (e.g., cell migration, differentiation, vasculogenesis, neurulation, gastrulation) and subsequent developmental adverse effects studied with the model. In addition, any limitations of each of the individual assays should be discussed. The description should include a discussion and supporting data to show that the duration and timing of exposure supports the prediction of MEFL *in vivo*.
- An assessment of the accuracy and ability for the alternative assay to detect MEFL. The performance of the assay is compared to the data generated from *in vivo* studies with compounds that induce MEFL in the absence of confounding maternal toxicity. If the compound is not a marketed pharmaceutical, then *in vivo* data should be provided.
- A discussion determining whether an effect is negative or positive in the assay.
- Definition and justification of the threshold for molecular and metabolic markers predicting MEFL.
- The details of the algorithm employed for determining positive and negative outcomes *in vivo*. The predictive model should correlate concentrations tested in the alternative assay(s) to the *in vivo* exposure, preferably in pregnant animals, that results in an adverse outcome in the species being predicted.
- The list of compounds in each of the training sets (data used to discover potentially predictive relationships) and test sets (data used to assess the strength and utility of a predictive relationship) for qualification of the assay and the basis for selection of these compounds.
- Data sources (e.g., literature, study reports, regulatory reviews) for all *in vivo* exposure and MEFL data used for compounds in the qualification data set, if not obtained from the Reference Compound List (Section 1.3 of Annex 2).
- Data demonstrating the test method's performance covering an appropriate range of biological and chemical domains that are justified for the intended use of the alternative assay (context of use).
- Data demonstrating the sensitivity, specificity, positive and negative predictive values, and reproducibility of an assay or battery of assays to predict *in vivo* developmental outcomes. The performance of the training and test sets can be evaluated separately and/or together, provided the selected approach is justified.
- In cases when more than one assay is conducted, a separate description of the performance of each assay, in addition to the integrated assessment used for the predictive model. A clear description of how the results of individual assays are integrated into the final prediction.
- Historical data for assay development and use (e.g., viability, numbers and types of

malformations), including positive controls.

The sponsor should state to which health authorities (if any) the assay qualification has been previously been submitted. Note that acceptance of an assay by one regulatory authority does not bind other health authorities to accept the assay. Last, evaluation of human teratogens not detected *in vivo* by rat and/or rabbit is encouraged since some alternative assay(s) might predict MEFL that are not detectable by *in vivo* studies.

1.2 Examples of EFD Testing Strategies Utilizing Alternative Assays

This section provides illustrative examples of integrated testing strategies into which alternative assays are incorporated to test for adverse effects on EFD.

1.2.1 Potential Approach to Defer In Vivo Testing as Part of an Integrated Testing Strategy

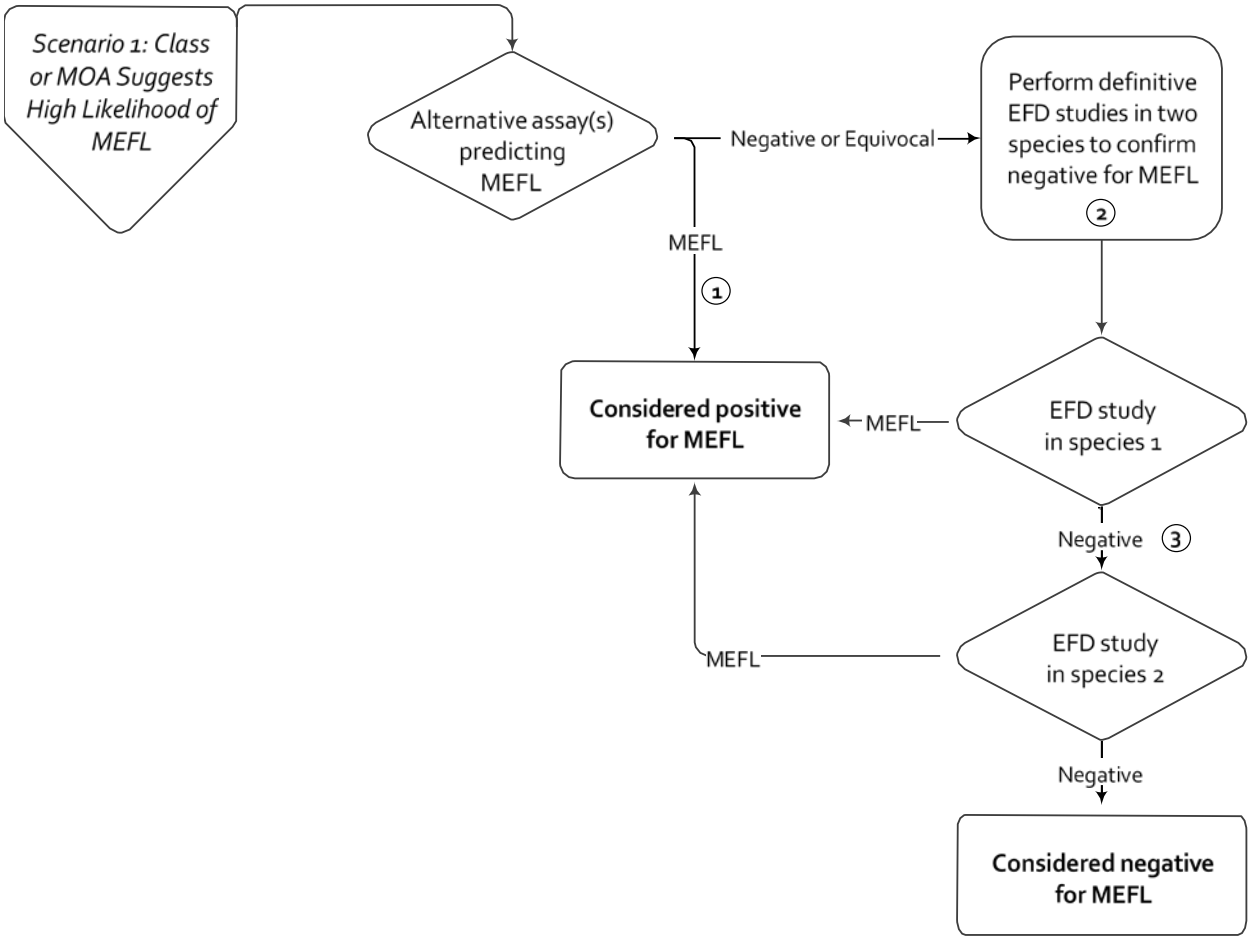
See Section 4.2.3 of the Guidance.

1.2.2 Pharmaceuticals Expected to Be Embryo-fetal Toxicants

For pharmaceuticals that are expected to adversely affect embryo-fetal development based on mechanism of action, pharmacologic class or target biology, it can be appropriate to confirm this activity in a qualified alternative assay(s) (see Figure 1 of this Annex).

When a qualified alternative assay clearly predicts MEFL at clinically relevant extrapolated exposures, this can be sufficient to identify the compound as an EFD risk, and further testing would generally not be warranted. If the alternative assay does not predict MEFL, this should be confirmed in definitive *in vivo* EFD studies in two species. Conducting the studies in series, as shown in Annex 2 Figure 1, can allow for reduction in animal use, as the second *in vivo* assay would not be warranted if the first one is positive. Under this scenario, since the pharmaceutical is expected to adversely affect embryo-fetal development, there is no merit in using *in vivo* EFD studies to attempt to negate a positive alternative assay response.

Figure 1: Use of Alternative Assays for Pharmaceuticals Expected to be EFD Toxicants



1) No additional assessment is warranted if unequivocal MEFL signal is observed at clinically relevant extrapolated exposures.
2) Alternatively, pEFD studies can be used; however, negative results should be confirmed by a definitive study in the relevant species
3) Conducting *in vivo* EFD studies in series, as shown, can permit reduction in animal use, as 2nd *in vivo* assay is not warranted if the first study is positive.

1.2.3 Pharmaceuticals Intended to Treat Severely Debilitating or Life-Threatening Diseases

Considering the risk/benefit for pharmaceuticals intended to treat severely debilitating or life-threatening conditions (compared to less severe chronic diseases) where the likelihood of pregnancy is low, the use of qualified alternative assay(s) can be considered an appropriate component of the EFD risk assessment (see Annex 2 Figure 2).

When a qualified alternative assay clearly predicts MEFL in the first species (e.g., rat) at clinically relevant extrapolated exposures, this can be considered, on a case-by-case basis, to sufficiently characterize the EFD risk. However, if the results are equivocal or thought to represent a false positive, definitive *in vivo* studies in one or two species should be conducted to assist human risk assessment. If no EFD signal is observed in the two definitive *in vivo* studies at appropriate exposure margins the results of the alternative assay could be considered of minimal concern for human risk. However, for alternative assays that have been qualified to predict human MEFL (i.e., not predicting only animal MEFL), additional data (e.g., mechanistic or genetic) should be provided to support a conclusion that the alternative assay results represent a false positive finding. If one or both of the *in vivo* studies are positive for EFD toxicity, the compound is considered to be positive for EFD risk. Conducting the studies in series, as shown in Annex 2 Figure 2, can allow for reduction in animal use, as the second *in vivo* assay would not be warranted if the first one is positive.

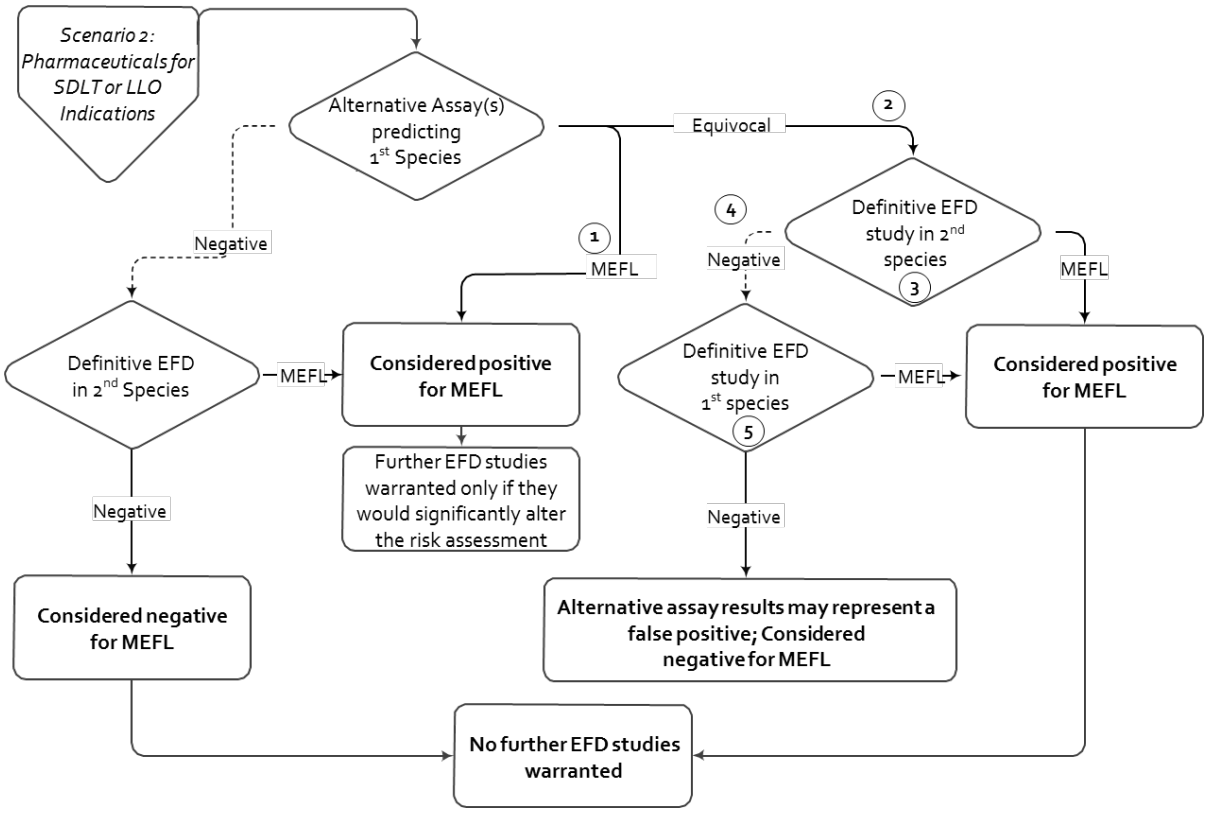
If the alternative assay for the first species predicts a negative outcome (i.e., no MEFL), a definitive *in vivo* EFD study in the second species should be conducted to confirm the assessment. If positive, the compound is considered positive for EFD risk. If negative, the compound is considered negative for EFD risk, and no further testing is generally warranted, unless it is judged that additional studies would significantly alter the risk assessment.

1.2.4 Pharmaceuticals Intended to Treat Late-life Onset Diseases

Some diseases are typically only diagnosed at a later age, but may nonetheless be diagnosed in reproductively capable women at a low incidence (e.g., bullous pemphigoid, which is typically diagnosed after age 60). Given the generally low rate of fertility in the female population with such late-life onset diseases, there is a diminished likelihood that a pharmaceutical used exclusively in this population will lead to an increase in the incidence of birth defects. Whether an EFD assessment is warranted under this scenario should be determined on a case-by-case basis. This scenario is not intended for situations where the treatment population is presumptively infertile (e.g., post-menopausal osteoporosis), for which no EFD assessment would typically be warranted.

The testing strategy under this scenario is similar to that depicted for severely debilitating or life-threatening diseases, with the exception that the first *in vivo* assessment in the second species can be conducted as a pEFD study.

Figure 2: Use of Alternative Assays for Severely Debilitating or Life-threatening or Late Life Onset Diseases



1) A clearly positive MEFL signal at clinically relevant extrapolated exposures can be sufficient to consider a pharmaceutical positive for EFD toxicity, without further assessment, on a case-by-case basis.
2) While pEFD studies can be used, negative results from definitive *in vivo* EFD studies in two species are warranted to establish that alternative assay results represent a false positive.
3) For late-life onset diseases, given low likelihood of pregnancy in this patient population a pEFD study in the 2nd species can generally be sufficient.
4) Conducting *in vivo* EFD studies in series, as shown, can permit reduction in animal use, as 2nd *in vivo* assay is not to be conducted if the first is positive.
5) Same species as the alternative assay is intended to predict.

1.3 Reference Compound List

The Reference Compound List contains 29 compounds that have been shown to induce MEFL in nonclinical studies (in the absence of overt maternal toxicity) and/or humans (Table 1 of this Annex).

Only findings of MEFL were recognized for NOAEL and LOAEL determinations. Doses associated with the induction of reversible or minor manifestations of developmental toxicity (e.g., changes in fetal weight, growth suppression, and skeletal variations) were not used for this assessment. (see Section 9, of the Guidance).

The general robustness of the studies (e.g., compliance with GLP regulations, the number of animals in the study, number of dose levels) was considered when determining which NOAEL and LOAEL values to use. When multiple sources were available, the data from a study designed in a manner consistent with the design recommended in the ICH S5(R2) guideline was accepted as the definitive data. When there were multiple robust sources of data that did not closely align, the highest NOAEL (to avoid bias towards claiming a low margin) and lowest LOAEL (as is routinely done in regulatory assessments) were generally used, even if the data were from different studies.

The compounds in this list as well as others can be used to support qualification of an alternative assay or battery of assays.

Compounds not causing MEFL (negative compounds) should also be used to assess assay specificity. Such compounds would lack MEFL regardless of additional effects on embryo/fetus such as fetal body weight changes, structural variations or delayed/reduced ossification. These compounds can be negative at all *in vivo* doses tested, or can be positive (MEFL observed) at higher doses/exposures provided the alternative assay within its context of use predicts the transition from negative to positive. That is, the alternative assay should predict a negative result at some extrapolated level under the conditions for which the *in vivo* study yielded a negative result (no MEFL). In the Reference Compound List, three compounds are provided as an example for negative controls (Cetirizine, Saxagliptin, Vildagliptin). These compounds did not induce MEFL in rat and rabbit at an exposure multiple (AUC and Cmax) of >25 fold at the MRHD.

Table 1: Reference Compound Positive Control Examples for Qualifying Alternative Assays

Positive Controls	**Human Teratogen**	**Rat MEFL**	**Rabbit MEFL**
Acitretin	X	X	X
Aspirin	X	X	
Bosentan		X	
Busulfan	X	X	X
Carbamazepine	X	X	X
Cisplatin		X	
Cyclophosphamide	X	X	X
Cytarabine	X	X	
Dabrafenib		X	
Dasatinib		X	

Positive Controls	**Human Teratogen**	**Rat MEFL**	**Rabbit MEFL**
Fluconazole	X	X	X
5-Fluorouracil	X	X	X
Hydroxyurea	X	X	X
Ibrutinib		X	X
Ibuprofen	X	X	
Imatinib		X	
Isotretinoin (13-*cis*-retinoic acid)	X	X	X
Methotrexate	X	X	X
Pazopanib		X	X
Phenytoin (Diphenylhydantoin)	X	X	X
Pomalidomide	presumed	X	X
Ribavirin		X	X
Tacrolimus		X	X
Thalidomide	X	X	X
Topiramate	X	X	X
Tretinoin (all-*trans*-retinoic acid)	X	X	X
Trimethadione	X	X	
Valproic acid	X	X	X
Vismodegib	presumed	X	

1.3.1 Positive Control Reference Compounds

ACITRETIN (ETRETIN)
CAS No.: 55079-83-9

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
7.5 mg/kg oral GD7-16 (Kistler) C_{max} = 1.5 µg/mL[a] AUC = 6.6 µg·h/mL[a]	15 mg/kg oral GD7-16 (Kistler) C_{max} = 3.0 µg/mL[a] AUC = 13.2 µg·h/mL[a]	15 mg/kg: malformed humeri, dilated renal pelvis 30 mg/kg: cleft palate; malformed humeri, radii and ulnae	0.2 mg/kg oral GD7-19 (Kistler) no PK data available	0.6 mg/kg oral GD7-19 (Kistler) no PK data available	0.6 mg/kg: cleft palate, open eyelid, skeletal 2 mg/kg: cleft palate, skull and tail malformations, ectrodactyly of the fore- and hindfeet and malformations of the long bones	50 mg (0.83 mg/kg, 29.4 mg/m^2) Exposure values at steady state: C_{max} = 0.79 µg/mL[b] $AUC_{(0\text{-}24h)}$: 3.6 µg·h/mL[b]	NOAEL: <u>rat</u> C_{max} = 1.9 (1.5/0.79) AUC = 1.8 (6.6/3.6) <u>rabbit[c]</u> C_{max} = 0.2 (0.2/0.83) AUC = 0.08 (2.4/29.4) LOAEL: <u>rat</u> C_{max} = 3.8 (3.0/0.79) AUC = 3.7 (13.2/3.6) <u>rabbit[c]</u>	Acitretin is the major metabolite (free acid) of etretinate (ethyl ester)

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
							C_{max} = 0.7 (0.6/0.83) AUC = 0.2 (7.2/29.4)	

a: Extrapolated from reported values at 5 mg/kg (Brouwer): C_{max} = ~1.0 µg/mL from visual inspection of graph, AUC = 4.4 µg·h/mL.

b: Steady state values after 21 daily doses administered with food (FDA, United States): C_{max} = 0.786 µg/mL, $AUC_{(0\text{-}24h)}$ = 3.569 µg·h/mL.

c: In the absence of rabbit PK data, C_{max} ratio was based on mg/kg dose ratio and AUC was based on mg/m^2 dose ratio.

References

Brouwer KR, McNamara PJ. Influence of pregnancy on the pharmacokinetic disposition of two aromatic retinoids (etretinate and acitretin) in the rat. II. Single and multiple oral dosing studies. Drug Metab Dispos. 1989;17:652-5.

FDA, United States. Approval package review of NDA 019821, part 01 (28 Oct 1996), page 86.

Kistler A, Hummler H. Teratogenesis and reproductive safety evaluation of the retinoid etretin (Ro 10-1670). Arch Toxicol. 1985;58:50-6.

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FDA, United States. Pharm/tox review of NDA 019821 (08 Jun 1988), page 13. [There were no details provided for study findings, study appears to be the same as reported by Kistler and Hummer.]

ACETYLSALICYLIC ACID (ASPIRIN)
CAS No.: 50-78-2

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
125 mg/kg oral GD6-17 (n=20 Sprague Dawley) [Gupta][a] aspirin C_{max} = ~25 µg/mL[b] AUC = 6.6 – 25.3 µg·h/mL[b] salicylate C_{max} = 132 µg/mL[c] AUC = 8333 µg·h/mL[d]	200 mg/kg oral GD7-17 (n=20 Sprague Dawley) [Nakatsuka][e] aspirin C_{max} = ~40 µg/mL[b] AUC = 10.5 – 40.5 µg·h/mL[b] salicylate C_{max} = 211 µg/mL[c] AUC = 13,333 µg·h/mL[d]	Nakatsuka (200 mg/kg): malformations including craniorachischisis, abdominal hernia, exencephaly, club foot, open eyelid, severe defects of vertebral and costal bones; increased resorptions Gupta (250 mg/kg): ablepharia, cranio-rachischisis, exencephaly, various low occurrence head malformations,	350 mg/kg oral GD7-19 (n=20 NZW) [Cappon][f] aspirin: aspirin PK data in rabbits is not available salicylate C_{max} = 490 µg/mL[g] AUC = 4865 µg·h/mL[g]	Not Applicable: no MEFL findings in rabbits up to a maternally toxic dose	None	650 mg (10.8 mg/kg) q4h 3900 mg daily oral (2294 mg/m^2 daily) aspirin C_{max}= 7.08 µg/mL[h] $AUC_{(0-24h)}$ = 48.3 µg·h/mL[h] salicylic acid C_{max} = 45.2 µg/mL[i] AUC = 1448 µg·h/mL[i]	**Aspirin** NOAEL: rat C_{max} = 3.5 (25/7.08) AUC = 0.1 – 0.5 (6.6/48.3 to 25.3/48.3) rabbit[j] C_{max} = 32.4 (350/10.8) AUC = 1.8 (4200/2294) LOAEL: rat C_{max} = 5.6 (40/7.08) AUC = 0.2 – 0.8 (10.5/48.3 to 40.5/48.3) rabbit LOAEL not identified	The aspirin metabolite, salicylate (salicylic acid) has much higher concentrations in comparison to the parent and is pharmacologically active. Since aspirin concentrations were often BLQ, salicylate exposure data are also reported. salicylic acid MW = 138.12 g/mol aspirin

		bent fore and hind paw, kinked tail, protruding tongue, gastroschisis, ectopic adrenal, various low occurrence cardio-vascular malformations, VSD, DH, hypoplastic kidney, hypoplastic testes; decreased implantations, increased resorptions and post implantation loss					**Salicylate** NOAEL: <u>rat</u> C_{max}: 2.9 (132/45.2) AUC: 5.8 (8333/1448) <u>rabbit</u> C_{max}: 10.8 (490/45.2) AUC: 3.4 (4865/1448) LOAEL: <u>rat</u> C_{max}: 4.7 (211/45.2) AUC: 9.2 (13,333/1448) <u>rabbit</u> LOAEL not identified	MW = 180.16 g/mol

a: Nakatsuka and Fujii reported a NOAEL of 100 mg/kg in Sprague Dawley rats; the highest NOAEL of the 2 studies is reported here.

b: Extrapolated or actual reported value at 200 mg/kg oral dose in Sprague Dawley rats (Wientjes): C_{max} = 40 µg/mL (visual inspection of Figure 1); AUC = 629 – 2430 µg·min/mL (recalculated as 10.5 – 40.5 µg·h/mL). C_{max} data for aspirin is also available in Wistar rats administered 200 mg/kg (Higgs).

c: Extrapolated from reported value at 200 mg/kg oral dose in Sprague Dawley rats (Wientjes): C_{max} = 211 µg/mL (Table 5); no AUC values were reported for salicylate. C_{max} data for salicylate is also available in Wistar rats administered 200 mg/kg (Higgs) and in Fischer rats administered 90 mg/kg (Kapetanovica).

d: Extrapolated from reported value at oral 90 mg/kg/day on D15 in Fischer rats (Kapetanovica): AUC = 6000 µg·h/mL. Note the AUC in Table 2 is reported as 6.0 µg·h/mL, but this is incompatible with the plot in Figure 1a. An AUC estimated from concentrations visually estimated from Figure 1a was 5319 µg·h/mL (personal calculation); thus it is assumed that the reported value should actually be 6000 µg·h/mL.

e: Gupta reported a LOAEL of 250 mg/kg in Sprague Dawley rats; the lowest LOAEL of the 2 studies is reported here.

f: Data from Cappon is reported since the study design complied with ICH S5 standards. Data are also available in which 200 mg/kg was reported as the NOAEL (McColl, Schardein), but these studies were pre-ICH S5. McColl reported small auricles in hearts (18% v 4.5% in controls) and increased presence of 13th rib (93% vs 56% in controls) at 200 mg/kg aspirin, but these are considered variations. Schardein reported marked reduction in litter size at 200 mg/kg/day, but this dose was maternally toxic.

g: Extrapolated from reported values on D3 after 50 mg/kg/day oral dose in NZW rabbits (Marangos): C_{max} = 70 µg/mL and AUC = 695 µg·h/mL. Note that the extrapolation is 7-fold and that there are no data available on the linearity of the pharmacokinetics in rabbits.

h: Extrapolated to 6 daily doses every 4 hours from reported values after a single 1000 mg dose (Schurer): C_{max} = 10.89 µg/mL, AUC = 12.38 µg·h/mL. The C_{max} after a single dose likely represents the C_{max} at steady state since the half life is short (approximately 0.5 hours) and no accumulation is expected using the equation: accumulation = $1/(1 - e^{-k \cdot tau})$, where k = 0.693/t½ with t½ = 0.5 hours and tau = 4 hours. For $AUC_{(0\text{-}24h)}$, the single dose AUC at 1000 mg was extrapolated to 650 mg and multiplied by 6 (the maximum recommended doses in 24 hours). Data are also available following administration of 500 mg (Nagelschmitz).

i: Extrapolated to 6 daily doses every 4 hours from reported values after a single 1000 mg dose (Schurer): C_{max} = 53.5 µg/mL, AUC = 371.32 µg·h/mL. For C_{max}, an accumulation factor of 1.3 was applied that was estimated from the equation: accumulation = $1/(1 - e^{-k \cdot tau})$, where k = 0.693/t½ with t½ = 2.0 hours and tau = 4 hours (i.e., $1/(1 - e^{-1.386}) = 1/(1 - 0.25) = 1/0.75 = 1.3$). For $AUC_{(0\text{-}24h)}$, the single dose AUC at 1000 mg was extrapolated to 650 mg and multiplied by 6 (the maximum recommended doses in 24 hours). Data are also available following administration of 500 mg (Nagelschmitz).

j: In the absence of PK data, C_{max} ratio was based on mg/kg dose ratio and AUC was based on mg/m^2 dose ratio.

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ALL-*TRANS*-RETINOIC ACID (ATRA), TRETINOIN
CAS No.: 302-79-4

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins[a]** **NOAEL/Human** **LOAEL/Human**	**Notes**
5 mg/kg oral GD6-15 (Wistar) [Seegmiller] C_{max} = 0.15 µg/mL[b] $AUC_{(0-8h)}$ = 0.25 µg·h/mL[b]	10 mg/kg oral GD6-15 (Wistar) [Seegmiller] C_{max} = 0.30 µg/mL[b] $AUC_{(0-8h)}$ = 0.50 µg·h/mL[b]	cleft palate, sporadic gross external and soft tissue malformations, skeletal alterations	2 mg/kg oral GD6-18 [Tzimas, 1994] C_{max} = 0.10 µg/mL[c] $AUC_{(0-24h)}$ = 0.207 µg·h/mL[c]	6 mg/kg oral GD6-18 [Tzimas, 1994] C_{max} = 0.30 µg/mL[c] $AUC_{(0-8h)}$ = 0.622 µg·h/mL[c]	fetal resorptions and a decrease in live fetuses; visceral ectopia, skin erosions, acaudia, torsion of hindlimbs, and omphalocele	45 mg/m^2/day in two divided doses C_{max} = 0.394 µg/mL[d] AUC = 0.537 µg·h/mL[d]	NOAEL: <u>rat</u> C_{max} = 0.4 (0.15/0.394) AUC = 0.5 (0.25/0.537) <u>rabbit</u> C_{max} = 0.3 (0.100/0.394) AUC = 0.4 (0.207/0.537) LOAEL: <u>rat</u> C_{max} = 0.8 (0.30/0.394) AUC = 0.9 (0.50/0.537) <u>rabbit</u> C_{max} = 0.8 (0.300/0.394)	tretinoin induces its own metabolism, so PK margins are highly dependent on day of assessment

							AUC = 1.2 (0.622/0.537)	

a: Since tretinoin induces its own metabolism, which causes a significant decrease in plasma exposures with repeated dosing, single dose PK data in animals and humans were used for calculating exposure margins.

b: Extrapolated or actual value after single 5 mg/kg oral dose on GD9 in Wistar rats (Tzimas 1997): C_{max} = 0.15 µg/mL, $AUC_{(0\text{-}8h)}$ = 0.25 µg·h/mL. Pharmacokinetic data are also available after a single dose of 6 mg/kg on GD12 (Collins, 1995): C_{max} = 0.320 µg/mL from visual inspection of graph, $AUC_{(0\text{-}8h)}$ = 0.820 µg·h/mL; as well as after 6 daily doses (Collins 1994, 1995): C_{max} = 0.046 or 0.052 µg/mL, and $AUC_{(0\text{-}24h)}$ = 0.098 µg·h/mL or $AUC_{(0\text{-}10h)}$ = 0.090 µg·h/mL, respectively.

c: Extrapolated or actual value after single 6 mg/kg oral dose on GD12 in Swiss hare rabbits (Collins 1995): C_{max} = 0.300 µg/mL from visual inspection of graph, $AUC_{(0\text{-}8h)}$ = 0.622 µg·h/mL. Pharmacokinetic data are also available following 6 daily doses in Swiss hare rabbits (Collins 1995): C_{max} = 0.110 µg/mL, $AUC_{(0\text{-}10h)}$ = 0.281 µg·h/mL; and from (Tzimas 1994): C_{max} = 0.105 µg/mL, $AUC_{(0\text{-}24h)}$ = 0.321 µg·h/mL.

d: PK data after first dose (US label).

References

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US label tretinoin.

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FDA, United States. Pharmtox review of NDA 021108/S000 (31 Aug 2000), page 16,26. [p. 16: same study as Seegmiller; p. 26: review mentions “only a modest increase in intrauterine death” at 2.5 mg/kg in an oral rat developmental toxicity study, but there are no study details to allow confirmation].

US label tretinoin. [fetal resorptions and a decrease in live fetuses were stated as findings in all species studied, but the dose at which these occurred was not mentioned]

Bosentan
CAS No.: 147536-97-8

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Notes**
60 mg/kg oral GD6-15 (FDA, United States, p. 39, 155) C_{max} = 4.5 µg/mL[a] AUC = 13.2 µg·h/mL[a]	300 mg/kg oral GD6-15 (FDA, United States, p. 39, 155) C_{max} = 16.25 µg/mL[b] AUC = 53.5 µg·h/mL[b]	<u>Cesarean sections</u> 300 mg/kg: agenesis of soft palate (1 litter) 1500 mg/kg: agenesis of soft palate (14 litters), shortened tongues, abnormal origin of the right subclavian artery (1 litter); abnormalities of the skull (shortened and misshapen mandibles, abnormally shaped palatine, abnormally shaped tympanic annulus and hyoid bone, fusion of the pterygoid process with the tympanic annulus, bent internal pterygoid process) <u>Spontaneous delivery fetuses (PPND groups) that died on study</u>:[c] 300 mg/kg: agenesis of the soft palate, anophthalmia, and microphthalmia	1500 mg/kg/day oral (750 mg/kg BID) GD7-18 (FDA, United States, p. 66) C_{max} = 1.435 µg/mL[d] AUC = 27.7 µg·h/mL[d]	LOAEL not identified	none	

a: Extrapolated from reported values in plasma after 10 doses of 200 mg/kg oral bosentan in pregnant rats (FDA, United States, p. 78): C_{max} = 15 µg/mL, AUC = 44 µg·h/mL.

b: Interpolated from reported values in plasma after 10 doses of 200 and 600 mg/kg oral bosentan in pregnant rats (FDA, United States, p. 78): at 200 mg/kg, C_{max} = 15 µg/mL, AUC = 44 µg·h/mL; at 600 mg/kg, C_{max} = 20 µg/mL, AUC = 82 µg·h/mL.

c: In a separate PPND study with higher levels of impurities and pup sacrifice on PND4, agenesis of the soft palate was also observed in 3 litters at 120 mg/kg (FDA, United States, p. 58)

d: Actual values in plasma after 12 doses of 1500 mg/kg/day oral bosentan administered as 2 divided doses (750 mg/kg each) 5 to 6 hours apart in pregnant Himalayan rabbits (FDA, United States, p. 78): C_{max} = 1.435 µg/mL, AUC = 27.70 µg·h/mL.

References

FDA, United States. Pharmacology Review NDA 021290 (30 Aug 2001).

Busulfan
CAS No.: 55-98-1

Rat NOAEL Dose C_{max} AUC	**Rat LOAEL Dose C_{max} AUC**	**Rat Findings**	**Rabbit NOAEL Dose C_{max} AUC**	**Rabbit LOAEL Dose C_{max} AUC**	**Rabbit Findings**	**Human Dose[a] C_{max} AUC**	**Margins NOAEL/Human LOAEL/Human**	**Notes**
NOAEL not identified	3 mg/kg oral single dose GD12 (18 mg/m^2) [Dodo] C_{max} = 0.84 µg/mL[b] AUC = 2.70 µg·h/mL[b]	3 mg/kg: fused carpal bones 10 mg/kg: low incidence of limb and rib malformations 30 mg/kg: high incidence of limb and rib malformations	1.3 mg/kg oral GD7–14 (15.6 mg/m^2) [Somers] no rabbit PK data found	3.6 mg/kg oral GD7-14 (43.2 mg/m^2) [Somers] no rabbit PK data found	increased resorptions and decreased live young, abnormalities in liver and gall bladder	4 – 8 mg daily oral (0.06 – 0.13 mg/kg, 2.4 – 4.7 mg/m^2) <u>for 8 mg dose</u> C_{max} = 0.128 µg/mL[c] AUC = 0.529 µg·h/mL[c]	NOAEL: <u>rat</u> NOAEL not identified <u>rabbit[d]</u> C_{max} = 10 (1.3/0.13) AUC = 3.3 (15.6/4.7) LOAEL: <u>rat</u> C_{max} = 6.6 (0.84/0.128) AUC = 5.1 (2.7/0.529) <u>rabbit[d]</u> C_{max} = 27.7 (3.6/0.13) AUC = 9.2 (43.2/4.7)	human dose is daily but MEFL NOAEL was single dose, margins likely even lower if rats dosed through organogenesis

a: Note that Busulfex is a concentrated busulfan intravenous formulation with dimethylformamide indicated for bone marrow ablation. Myleran is the original busulfan oral drug product indicated for treatment of chronic myelogenous leukemia. The doses used below are for remission induction in chronic myelogenous leukemia.

b: Extrapolated from reported values after 1 mg/kg busulfan oral dose to fasted rats (strain not specified) (FDA, United States): C_{max} = 0.28 µg/mL, AUC = 0.9 µg·h/mL.

c: Extrapolated from the average of dose-normalized (to 2 mg) values across the range 2 to 6 mg (C_{max} = 0.03 µg/mL, AUC = 0.130 µg·h/mL) and dose-normalized values (to 4 mg) from 4 and 8 mg in a separate study (C_{max} = 0.068 µg/mL, AUC = 0.269 µg·h/mL) (US label, Ehrsson).

d: In the absence of rabbit PK data, C_{max} ratio was based on mg/kg dose ratio and AUC was based on mg/m^2 dose ratio.

References

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CARBAMAZEPINE
CAS No.: 298-46-4

Rat NOAEL Dose C_{max} AUC	**Rat LOAEL Dose C_{max} AUC**	**Rat Findings**	**Rabbit NOAEL Dose C_{max} AUC**	**Rabbit LOAEL Dose C_{max} AUC**	**Rabbit Findings**	**Human Dose C_{max} AUC**	**Margins NOAEL/Human LOAEL/Human**	**Notes**
200 mg/kg oral GD7-18 [Vorhees][a] C_{max} = 33 µg/mL[b] $AUC_{(0-24\ h)}$ = 547 µg·h/mL[b]	400 mg/kg oral GD4-14 [FDA, United States 1967, Vorhees] C_{max} = 65 µg/mL[b] $AUC_{(0-24h)}$ = 1094 µg·h/mL[b]	<u>400 mg/kg GD4-14 [FDA, United States 1967]</u> abortions <u>600 mg/kg GD7-18 SD rats [Vorhees]</u> increased resorptions, increased kinked tails <u>650 mg/kg [US label]</u> offspring showed low incidence of cleft palate, talipes, or anophthalmos	NOAEL was not identified [FDA, United States 1967]	225 mg/kg GD5-12 [FDA, United States 1967] C_{max} = 29 µg/mL[c] $AUC_{(0-24h)}$ = 267 µg·h/mL[c]	No malformations up to 450 mg/kg GD5-12 Decreased numbers of fetuses, increased resorptions at 225 – 450 mg/kg	Up to 800 mg twice daily (1600 mg/day) C_{max} = 11.7 µg/mL[d] $AUC_{(0-24h)}$ = 232 µg·h/mL[d]	NOAEL: <u>Rat</u> C_{max} = 2.8 (33/11.7) AUC = 2.4 (547/232) <u>Rabbit</u> No NOAEL identified LOAEL: <u>Rat</u> C_{max} = 5.6 (65/11.7) AUC = 4.7 (1094/232) <u>Rabbit</u> C_{max} = 2.5 (29/11.7) AUC = 1.2 (267/232)	Human exposure is invariant, independent of dose.

a: Data from Vorhees was used for establishing the NOAEL because the data were much more detailed than provided in the FDA, United States review, which suggested a NOAEL of 300 mg/kg.

b: Extrapolated or actual data after 200 mg/kg oral single dose in Sprague Dawley male rats (Shi): C_{max} = 32.7 µg/mL, $AUC_{(0\text{-}24h)}$ = 32.8 mg·min/mL (547 µg·h/mL).

c: Extrapolated from reported value after 80 mg/kg oral single dose in Angora grey rabbits (Kourmaravelou): C_{max} = 10.4 µg/mL, $AUC_{(0\text{-}24h)}$ = 94.8 µg·h/mL. Data are also available from Abushammala at a dose of ~20.6 mg/kg. The data from Kourmaravelou were used because the dose was closer to the LOAEL, which provided a smaller extrapolation range (<3-fold).

d: From actual data for 1600 mg dose of conventional tablet carbamazepine (FDA, United States 1996). C_{max} = 11.66 µg/mL, AUC = 232.27 µg·h/mL.

References

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US Label Tegretol.

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CISPLATIN
CAS No.: 15663-27-1

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Notes**
0.3 mg/kg IP on GD6,8,11, or 14 in Wistar rats (Keller) C_{max} = 0.32 µg/mL[a] AUC = 0.25 µg·h/mL[a]	1 mg/kg IP on GD8 or 11 in Wistar rats (Keller) C_{max} = 1.08 µg/mL[a] AUC = 0.85 µg·h/mL[a]	increased fetal mortality, decreased live fetuses per dam	NOAEL not identified	LOAEL not identified	No data found	

a: Extrapolated from values in plasma (unbound) after an intraperitoneal 5 mg/kg cisplatin single dose in male Donryu rats (Tamura): C_{max} = 5.4 µg/mL, $AUC_{(0\text{-inf})}$ = 254 µg·min/mL (4.23 µg·h/mL).

References

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Toro-Cordova A, Flores-Cruz M, Santoyo-Salazar J, Carrillo-Nava E, Jurado R, Figueroa-Rodriguez PA, et al. Liposomes loaded with cisplatin and magnetic nanoparticles: physicochemical characterization, pharmacokinetics, and *in vitro* efficacy. Molecules. 2018;23(9). pii: E2272. doi: 10.3390/molecules23092272. [PK following 6 mg/kg intravenous cisplatin: C_{max} = 21.3 µg/mL, $AUC_{(0-t)}$ = 7.49 µg·h/mL·kg, which is 2.25 µg·h/mL in 300 g rats.]

Summary of Cisplatin PK data evaluated

Note: There was no obvious choice for the best PK data to use. Chen required a 15-fold extrapolation, Darwish was unclear whether the data were total Pt or unbound drug, and Tamura used a different strain of rat (Donryu) than used for the EFD toxicity study (Wistar). There are substantial differences in PK between the intravenous and intraperitoneal routes (Sekiya, et al., 1985), so intravenous data were not used.

Reference	**Route**	**Dose (mg/kg)**	**C_{max} (µg/mL)**		**AUC (µg·h/mL)**		**Notes**
			Reported	**Normalized to 1.0 mg/kg**	**Reported**	**Normalized to 1.0 mg/kg**	
Chen	IP	15	10.36	0.69	81.74 (0-inf)	5.45	unbound drug (DDTC-derivatized)
Darwish	IP	6	5.66	0.94	9.77	1.63	unclear whether unbound or total drug
Tamura[a]	IP	5	5.4	1.08	4.23	0.85	unbound drug (ultrafilterable)
Okada	IV	5	7.3	1.5	3.0 (0-2h)	0.6	unbound drug (DDTC-derivatized)
Toro-Cordova	IV	6	21.3	3.55	2.25[b] (0-t)	0.375	unbound (ultrafilterable, DDTC-derivatized)

All studies used male Wistar rats except for Tamura et al., which used male Donryu rats.

a: PK parameters were derived from Figure 4 using scanning software (CurveUnscan).

b: Reported as $AUC_{(0\text{-}t)}$ = 7.49 µg·h/mL·kg, which is 2.25 µg·h/mL in 300 g rats.

Cyclophosphamide
CAS No.: 50-18-0

Rat NOAEL Dose C_{max} AUC	**Rat LOAEL Dose C_{max} AUC**	**Rat Findings**	**Rabbit NOAEL Dose C_{max} AUC**	**Rabbit LOAEL Dose C_{max} AUC**	**Rabbit Findings**	**Human Dose C_{max} AUC**	**Margins NOAEL/Huma LOAEL/Human**	**Notes**
NOAEL not identified (<2.5 mg/kg) [Chaube]	2.5 mg/kg IP GD9 [Chaube] Cytoxan C_{max} = 4.1 µg/mL[a] AUC = 3.65 µg·h/mL[a] PM C_{max} = 0.55 µg/mL[b] $AUC_{(0-24h)}$ = 2.13 µg·h/mL[b]	2.5 mg/kg GD9 [Chaube] embryolethal 5 mg/kg GD11 [von Kreybig, Mirkes] encephalocele, exencephaly, microcephaly, limb defects (ie, syndactyly and ectrodactyly), defective facial development (cleft palate)	NOAEL not identified (<30 mg/kg)	30 mg/kg IV single doses on GD6-14 [Mirkes, Fritz] Cytoxan C_{max} = 151 µg/mL[c] $AUC_{(0-8h)}$ = 24.1 µg·h/mL[d] PM C_{max} = 0.07 µg/mL[e] $AUC_{(0-8h)}$ = 0.297 µg·h/mL[e]	embryo-fetal resportions, omphalocele, cleft lip/ palate, forelimb skeletal defects	1600 mg/m^2 (40 mg/kg) IV (highest dose, q 3 – 4 weeks)[f] Cytoxan C_{max} = 106 µg/mL[g] AUC = 798 µg·h/mL[g] PM C_{max} = 14.4 µg/mL[h] AUC = 352 µg·h/mL[h]	NOAEL: rat: NOAEL not identified, but LOAEL margins were <0.1 rabbit NOAEL not identified, but LOAEL margins were <1.5 LOAEL: rat C_{max}: 0.04 (4.1/106) AUC: 0.005 (3.65/798) rabbit C_{max} = 1.4 (151/106) AUC = 0.03 (24.1/798) PM margins	• MW CP = 261.086 • MW PM = 221.018 • Cytoxan is a prodrug, MEFL has been attributed to both phosphoramide mustard (PM) and acrolein metabolites

							rat C_{max} = 0.04 (0.55/14.4) AUC = 0.006 (2.13/352) rabbit C_{max} = 0.005 (0.07/14.4) AUC = 0.0008 (0.297/352)	

a: Extrapolated from reported value after 20 mg/kg intravenous single dose in Sprague Dawley rats (Hong): C_0 = 125.3 µM (32.7 µg/mL), AUC/D = 265.3 min/L (in rats with mean BW = 0.330 kg the administered dose = 6.6 mg/rat; thus AUC = 265.3 min/L x 6.6 mg = 1751 mg·min/L = 29.2 µg·h/mL).

b: Extrapolated from reported value after 20 mg/kg intravenous single dose in Sprague Dawley rats (Hong): C_{max} = 20 µM (4.4 µg/mL) from visual inspection of graph, $AUC_{(0\text{-}24h)}$ = 76.9 µM·h (17.0 µg·h/mL) from calculation based on concentration values estimated by visual inspection of graph.

c: Extrapolated from reported value after 45 mg/kg cytoxan intravenous single dose in 2 New Zealand White rabbits (Holm): C_{max} = 227 µg/mL from visual inspection of graphs (mean of 2 rabbits). Values for the *R* and *S* isomers were added together; parent cytoxan is a racemic mixture. Data are also available after a 20 mg/kg intravenous single dose in New Zealand White rabbits (Anthony), but the reported C_{max} value (2.2 µM [0.574 µg/mL] from visual inspection of graph) is inconsistent with the reported AUC and thus was not used.

d: Extrapolated from reported value after 20 mg/kg cytoxan intravenous single dose in New Zealand White rabbits (Anthony): $AUC_{(0\text{-}8h)}$ = 3683 µmol·min/L (16.0 µg·h/mL). Data are also available after a 45 mg/kg intravenous single dose in 2 New Zealand White rabbits (Holm), but the reported AUC values for total racemate (3189 and 1259 µg·min/mL [53.15 and 20.98 µg·h/mL]) in 2 rabbits differed by 2.5-fold and t_{last} was ≤90 minutes so these values were not used.

e: Extrapolated from reported value after 20 mg/kg cytoxan intravenous single dose in NZW rabbits (Anthony): C_{max} = 0.22 µM (0.049 µg/mL) from visual inspection of graph, $AUC_{(0\text{-}8h)}$ = 53.7 µmol·min/L (0.198 µg·h/mL).

f: From SmPC.

g: Extrapolated from reported value after 1000 mg/m^2 intravenous single dose cytoxan (Chan): C_0 = 254.4 µM (66.4 µg/mL), $AUC_{(0\text{-}inf)}$ = 1910 µM·h (499 µg·h/mL).

h: Extrapolated from reported value after 1000 mg/m^2 intravenous single dose cytoxan (Chan): C_0 = 40.5 µM (9.0 µg/mL), $AUC_{(0\text{-inf})}$ = 996.3 µM·h (220 µg·h/mL).

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US label cyclophosphamide.

Cytarabine
CAS No.: 147-94-4

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
10 mg/kg IP single dose GD10,11, or 12 [Chaube] C_{max} = ~5.8 µg/mL[a] $AUC_{(0\text{-}inf)}$ = ~15.9 µg·h/mL[a]	20 mg/kg IP single dose GD11 or 12 [Chaube] C_{max} = ~11.6 µg/mL[a] $AUC_{(0\text{-}inf)}$ = ~31.7 µg·h/mL[a]	≥20 mg/kg cleft palate, micrognathia, deformed rear appendages, paws and tail; skeletal defects including distortion and fusion of the bones of the skull and appendages, embryofetal mortality	no rabbit data found[b]	no rabbit data found[b]	no rabbit data found	100 mg/m^2 IV every 12 hours (days 1 to 7) many regimens are used including CIV C_{max} = ~2.8 µg/mL[c] AUC = 6.6 µg·h/mL[c]	NOAEL: rat C_{max}: 2.1 (5.8/2.8) AUC: 2.4 (15.9/6.6) LOAEL: rat C_{max}: 4.1 (11.6/2.8) AUC: 4.8 (31.7/6.6)	• half-life is short, rapidly deaminated to inactive uridine arabinoside by cytidine deaminase • active moiety is Ara-CTP which inhibits DNA polymerase • MW = 243.217

a: Extrapolated or reported value after 20 mg/kg intraperitoneal [^{14}C]cytarabine single dose in male Sprague Dawley rats (Parker): C_{max} = ~11.6 µg/mL from visual inspection of graph, $AUC_{(0\text{-}inf)}$ = ~31.7 µg·h/mL from calculation based on concentration values estimated by visual inspection of graph. Note that the reported plasma concentrations represent total radioactivity and that at 4 hours only 71% of the total plasma radioactivity was attributed to intact cytarabine (Parker). The AUC value used, and the calculated margins, thus represents an upper bound and the true AUC for intact cytarabine would certainly be lower. Also note that teratology was performed in Wistar rats. PK data are also available in male Sprague Dawley rats administered 5 mg/kg intravenous cytarabine single dose (Zhang), in male Sprague Dawley rats administered 2.64

µg/kg intravenous [^{3}H]cytarabine (Simard), and in Wistar rats administered 5.4 mg/kg intramuscular cytarabine in solution with chitosan-beta-glycerophosphate (Mulik).

b: Rabbit PK data are available in male New Zealand white rabbits administered single doses 50 mg/kg intravenous cytarabine (Zimmerman): C_{max} = 400 µM (97 µg/mL) from visual inspection of graph, AUC estimated from CL = 8.16 mL/(min·kg) and dose = 50 mg/kg, AUC = dose/CL = (50/8.16)(1 h/60 min) = 102 µg·h/mL.

c: Extrapolated to 100 mg/m^2 BID dose from reported value after single 100 mg intravenous dose (1.67 mg/kg, 60 mg/m^2) (Wan): C_{max} = ~7.0 µmol/L (1.7 µg/mL) from visual inspection of graph, AUC = dose/CL = 100 mg/845 mL/min = 1.97 µg·h/mL (which gives AUC = 3.29 µg·h/mL at 100 mg/m^2 and 6.6 µg·h/mL for 100 mg/m^2 BID).

Mouse NOAEL **Dose** **C_{max}** **AUC**	**Mouse LOAEL** **Dose** **C_{max}** **AUC**	**Mouse Findings**	**Margins** **NOAEL/Human** **LOAEL Human**	**Notes**
0.5 mg/kg IP GD6-15 Swiss mice [Ortega] C_{max} = ~0.50 µg/mL[d] AUC = ~0.46 µg·h/mL[d] C_{max} = ~0.41 µg/mL[e] AUC = 0.315 µg·h/mL[e]	2 mg/kg IP GD6-15 Swiss mice [Ortega] C_{max} = ~2 µg/mL[d] AUC= ~1.83 µg·h/mL[d] C_{max} = ~1.62 µg/mL[e] AUC = 1.26 µg·h/mL[e]	cleft palate, renoureteral alterations, polydactyly, oligodactyly	NOAEL: mice C_{max}: 0.16 (0.46/2.8)[f] AUC: 0.06 (0.39/6.6)[f] LOAEL: mice C_{max}: 0.65 (1.81/2.8)[f] AUC: 0.23 (1.55/6.6)[f]	this table is included because: a) it shows that with the mouse teratology data, which was included in the US label, exposure margins at the NOAEL were <1, b) rat exposure margins at the NOAEL were much higher, c) rabbit data are not available, so it provides data in a 2nd species

d: Extrapolated from reported value after administration of a 30 mg/kg intraperitoneal single dose cytarabine to Swiss mice (Dedrick): C_{max} = ~30 µg/mL from visual inspection of graph, $AUC_{(0\text{-}24h)}$ = ~27.5 µg·h/mL from calculation based on concentration values estimated by visual inspection of graph. Note large extrapolation range.

e: Extrapolated from reported value after administration of a 2.466 mmol/kg (600 mg/kg) intravenous single dose cytarabine to mice (Bayne): C_{max} = 2 µmol/mL (486 µg/mL) from visual inspection of graph, AUC = 1.553 µmol·h/mL (378 µg·h/mL). Note large extrapolation range.

f: Mouse values were taken as the average of the 2 sources, which gave similar values despite the 20-fold difference in administered dose, suggesting PK was linear.

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Percy DH. Teratogenic effects of the pyrimidine analogues 5-iododeoxyuridine and cytosine arabinoside in late fetal mice and rats. Teratology. 1975;11:103-17. [Rats were dosed subcutaneously on GD18-21 and offspring sacrificed on PND10 and 20. NOAEL was 12.5 mg/kg and LOAEL was 25 mg/kg.]

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DABRAFENIB
CAS No.: 1195765-45-7

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Notes**
20 mg/kg oral pcD1-17[a] (FDA, United States, p. 115) C_{max} = 1.17 µg/mL[b] $AUC_{(0-t)}$ = 4.10 µg·h/mL[b]	300 mg/kg oral pcD1-17[a] (FDA, United States, p. 115) C_{max} = 2.17 µg/mL[c] $AUC_{(0-t)}$ = 22.6 µg·h/mL[c]	cardiac interventricular septal defects; decrease in the number of corpora lutea, number of implants, and the number of live fetuses	No rabbit data found	No rabbit data found	None	

a: From a combined female fertility and embryofetal development toxicity study in which females were dosed from 2 weeks prior to mating to post-coitum D17. Cesarean sections were performed on post-coitum D21.

b: Actual values in plasma after 20 mg/kg oral dabrefenib for 24 days in rats (FDA, United States, p. 119): C_{max} = 1.17 µg/mL, $AUC_{(0-t)}$ = 4.10 µg·h/mL.

c: Actual values in plasma after 300 mg/kg oral dabrefenib for 24 days in rats (FDA, United States, p. 119: C_{max} = 2.17 µg/mL, $AUC_{(0-t)}$ = 22.6 µg·h/mL.

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Dasatinib
CAS No.: 302962-49-8

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Notes**
NOAEL not identified	2.5 mg/kg oral GD6-15 (FDA, United States, p. 225) C_{max} = 0.021 µg/mL[a] $AUC_{(0-8h)}$ = 0.105 µg·h/mL[a]	increased post-implantation loss and resorptions, decreased litter size; bent scapula or humerus	6 mg/kg oral GD7-19 (FDA, United States, p. 236) C_{max} = 0.227 µg/mL $AUC_{(0-inf)}$ = 0.834 µg·h/mL	LOAEL for MEFL not identified	None: findings in the definitive study were limited to an increase in skeletal variations (delays in ossifications); embryolethality observed in the DRF at 10 mg/kg was associated with severe maternal toxicity	

a: Actual values in plasma after 10 days (GD15) of 2.5 mg/kg oral dasatinib in pregnant Sprague Dawley rats (FDA, United States, p. 227): C_{max} = 0.021 µg/mL, $AUC_{(0-8h)}$ = 0.105 µg·h/mL.

b: Actual values in plasma after 13 days (GD19) of 6 mg/kg oral dasatinib in pregnant NZW rabbits (FDA, United States, p. 238): C_{max} = 0.227 µg/mL, AUC = 0.834 µg·h/mL.

References

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2013;939:73-9. [PK at 2.5 mg/kg was substantially different than reported in FDA, United States review: C_{max} = 0.459 µg/mL, AUC = 3.289 µg·h/mL]

Fluconazole
CAS No.: 86386-73-4

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit** **NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit** **LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit** **Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
50 mg/kg oral [US label] C_{max} = 33.8 µg/mL[a] $AUC_{(0\text{-}inf)}$ = 380 µg·h/mL[b]	80 mg/kg oral [US label] C_{max} = 54 µg/mL[a] $AUC_{(0\text{-}inf)}$ = 608 µg·h/mL[b]	at ≥80 mg/kg: embryolethality, cleft palate, abnormal craniofacial ossification adactylia, brachygnathia [US Label, FDA, United States 1990a].	25 mg/kg oral [US Label, FDA, United States 1990a] C_{max} = 27 µg/mL[c] AUC = 521 µg·h/mL[d]	75 mg/kg oral [US Label, FDA, United States1990a] C_{max} = 81 µg/mL[c] AUC = 1563 µg·h/mL[d]	abortions (at maternally toxic dose)	400 mg C_{max} = 9.07 µg/mL[e] $AUC_{(0\text{-}24h)}$= 134.8 µg·h/mL[e]	NOAEL: <u>rat</u> C_{max} = 3.7 (33.8/9.07) AUC = 2.8 (380/134.8) <u>rabbit</u> C_{max} = 3.0 (27/9.07) AUC = 3.9 (521/134.8) LOAEL: <u>rat</u> C_{max} = 6.0 (54/9.07) AUC = 4.5 (608/134.8) <u>rabbit</u> C_{max} = 8.9 (81/9.07)	

							AUC = 11.6 (1563/134.8)	

a: Extrapolated from reported value after 20 mg/kg fluconazole oral single dose in rats (FDA, United States 1990a, p. 7): C_{max} = 13.5 µg/mL.

b: Extrapolated from reported value after 20 mg/kg fluconazole oral single dose in rats (Humphrey): $AUC_{(0\text{-}inf)}$ = 152 µg·h/mL.

c: Extrapolated from reported value after 10 mg/kg fluconazole oral single dose in rabbits (FDA, United States 1990a, p. 7): C_{max} = 10.8 µg/mL.

d: Calculated using plasma clearance value for rabbits (0.8 mL/min·kg, FDA, United States 1990a, p 8): AUC = Dose/Cl = (25 mg/kg)/(0.8 mL/min·kg)(1 h/60 min) = 521 µg·h/mL.

e: Actual value after 400 mg/day fluconazole oral single dose (FDA, United States 1990b, p. 7, 50-52): C_{max} = 9.07 µg/mL, $AUC_{(0\text{-}24h)}$ = 134.8 µg·h/mL. Data are also available after 14 days of repeated administration, which shows significant drug accumulation. C_{max} = 18.89 µg/mL, $AUC_{(0\text{-}24h)}$ = 349.9 µg·h/mL. Since PK was not available for repeated administration in animals, the single-dose human PK data were used for margin calculations.

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US label Diflucan.

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5-FLUOROURACIL
CAS No.: 51-21-8

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Huma** **LOAEL/Human**	**Notes**
10 mg/kg single dose IP GD9 [Wilson] C_{max} = 2.6 µg/mL[a] AUC = 3.89 µg·h/mL[a]	15 mg/kg single dose IP GD9 [Wilson] C_{max} = 3.87 µg/mL[a] AUC = 5.83 µg·h/mL[a]	Wilson: 15 mg/kg: malformations, embryofetal lethality Kuwagata: ≥17 mg/kg: micro-/anophthalmos, craniofacial defect, hydrocephaly, brain hernia	NOAEL not identified [DeSesso]	40 mg/kg SC GD12 [DeSesso] C_{max} = 111 µg/mL[b] AUC = 11 µg·h/mL[b]	limb anomalies 85% of term fetuses	500 mg/m^2 (400 – 600 mg/m^2) in a variety of dosing regimens, including doses up to 3000 mg/m^2 CIV for 46 hours[c] C_{max} = 29 µg/mL[d] AUC = 11.5 µg·h/mL[d]	NOAEL: rat C_{max} = 0.09 (2.6/29) AUC = 0.3 (3.89/11.5) rabbit no NOAEL identified LOAEL: rat C_{max} = 0.1 (3.87/29) AUC = 0.5 (5.83/11.5) rabbit C_{max} = 3.8 (111/29) AUC = 1.0 (11/11.5)	• note: half-life is very short (most patients have undetectable 5-FU levels in plasma 90 min after IV) and PK is nonlinear • 5FU is a pro-drug: thymidylate synthetase inhibitor is 5FdUMP • MW = 130.077

a: Extrapolated from reported value after 30 mg/kg 5FU intraperitoneal single dose in Sprague Dawley rats (Zhang): C_{max} = 7.74 µg/mL, AUC = 11.66 µg·h/mL.

b: Extrapolated from reported value after 20 mg/kg 5FU intravenous single dose in rabbits (Kar): C_{max} = 0.427 µmol/mL (55.5 µg/mL), AUC = 2.535 µmol·min/mL (5.5 µg·h/mL).

c: The dose of 500 mg/m^2 IV bolus was used for comparison although higher doses (e.g., ~1500 mg/m^2/day CIV) are used. Very low margins were calculated and using higher human doses would make them even lower.

d: Extrapolated from reported value after 14.7 mg/kg (544 mg/m^2) 5FU oral single dose (Schaaf): C_{max} = 32 µg/mL from visual inspection of graph, AUC = 12.55 µg·h/mL. Data are also available after a 370 mg/m^2 dose (Bocci): C_{max} = 48.41 µg/mL, AUC = 13.61 µg·h/mL.

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Shuey DL, Buckalew AR, Wilke TS, Rogers JM, Abbott BD. Early events following maternal exposure to 5-fluorouracil lead to dysmorphology in cultured embryonic tissues. Teratology. 1994b;50:379-86. [10 – 40 mg/kg SC on GD14, all malformations studied in explants]

US Adrucil label. [confirms malformations in rats as detailed by Kuwagata]

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Hydroxyurea
CAS No.: 127-07-1

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**[a]	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
100 mg/kg IP GD9-12 [Wilson] C_{max} = 47.3 µg/mL[b] AUC not available	137 mg/kg IP GD9-12 [Wilson] C_{max} = 80.6 µg/mL[b] AUC not available	embryofetal lethality, ocular and cerebral malformations	NOAEL not identified PK not available	30 mg/kg [US label] PK not available	650 mg/kg SC GD12 [DeSesso 1990]: cleft lip, cleft palate, reduction deformities of limbs and tail 750 mg/kg SC GD12 [DeSesso 1977]: skull and facial anomalies as well as severe reduction deformities of all limbs	<u>oral for oncology indications:</u> 80 mg/kg Q3D, 20 – 30 mg/kg/day <u>oral for sickle cell anemia</u> 15 – 35 mg/kg/day (555 – 1295 mg/m^2) C_{max} = 52 µg/mL[c] $AUC_{(0\text{-}inf)}$ = 184 µg·h/mL[c]	NOAEL: <u>rat</u> C_{max} = 0.9 (47.3/52) C_{max} dose = 2.9 (100/35)[d] AUC = 0.5 (600/1295)[e] <u>rabbit</u> NOAEL not identified LOAEL: <u>rat</u> C_{max} = 1.6 (80.6/52) C_{max} dose = 3.9 (137/35)[d] AUC = 0.6 (822/1295)[e] <u>rabbit</u>[f] C_{max} = 0.9 (30/35)	• PK is nonlinear with short half-life (15 min in rats, 2 – 4 h • in humans) • MW = 76.05g/mol • PK after IP and IV is similar (Wilson) • bioavailability is 70 – 80% in rats and humans, respectively (Beckloff) • no robust data for adverse human pregnancy outcomes

							AUC = 0.3 (360/1295)	

a: US label states that "Hydroxyurea is embryotoxic and causes fetal malformations (partially ossified cranial bones, absence of eye sockets, hydrocephaly, bipartite sternebrae, missing lumbar vertebrae) at 180 mg/kg/day in rats and 30 mg/kg/day in rabbits", but it is not clear which effects are in which species. Thus, 30 mg/kg is accepted as the LOAEL, but the findings are listed from publications with rabbits with SC doses of 650 and 750 mg/kg.

b: Actual values after 100 and 137 mg/kg hydroxyurea IP doses in pregnant Wistar rats (Wilson): C_{max} = 47.3 at 100 mg/kg and 80.6 µg/mL at 137 mg/kg.

c: Extrapolated from reported value after 1000 mg (16.7 mg/kg) hydroxyurea oral single dose (MHRA): C_{max} = 24.6 µg/mL, $AUC_{(0\text{-inf})}$ = 87.79 µg·h/mL. The dose for margin calculations was chosen to be 35 mg/kg/day. Although higher intermittent doses are used for oncology indications, the dose for sickle cell anemia is believed to be more relevant for assessing risk of developmental toxicity. As summarized in the table below, other human PK data are also available.

d: Although rat C_{max} data are available, this was after IP administration whereas the human data is after oral administration. Thus, in the absence of more direct PK comparisons, the estimated ratio based on mg/kg dose is also provided.

e: In the absence of rat AUC data, AUC ratio was based on mg/m^2 dose ratio.

f: In the absence of rabbit PK data, C_{max} ratio was based on mg/kg dose ratio and AUC was based on mg/m^2 dose ratio.

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MHRA Public Assessment Report PL 10880/128-9, page 48.

US label Hydrea and Droxea.

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Human Pharmacokinetic Data

Reference	**Population**	**Dose**	**Route**	**C_{max} (µg/mL)**	**AUC (µg·h/ml)**	**Notes**
Charache	sickle cell anemia	25 mg/kg	oral	19	$AUC_{(0-6)}$ = 1216	AUC units were published as "µg mL/min", value seems wrong since (C_{max} □ 6 h = 114 µg·h/mL)
Villani	HIV	mean 7.6 mg/kg BID	oral	0.135 nmol/L = 0.135 µmol/mL = 10.3 µg/mL	$AUC_{(0-12h)}$ = 540 µmol·h/L = 41.1 µg·h/mL; $AUC_{(0-24h)}$ = 82.1 µg·h/mL	

MHRA review	not stated – BE study	1000 mg (16.6 mg/kg)	oral	24.6	$AUC_{(0\text{-}inf)}$ = 87.79 µg·h/mL	use these values
Beckloff	cancer	20 mg/kg	oral	20.7	—	
		80 mg/kg	oral	128.1	—	

IBRUTINIB
CAS No.: 936563-96-1

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL[a]** **Dose** **C_{max}** **AUC**	**Rabbit Findings[b]**	**Notes**
40 mg/kg oral GD6-17 (FDA, United States, p. 126) C_{max} = 1.31 µg/mL[c] $AUC_{(0-24h)}$ = 5.348 µg·h/mL[c]	80 mg/kg oral GD6-17 (FDA, United States, p. 126) C_{max} = 2.627 µg/mL[d] $AUC_{(0-24h)}$ = 13.729 µg·h/mL[d]	malformations including dextrocardia, retroesophageal aortic arch, persistent truncus arteriosus, right-sided aortic arch, and interrupted aortic arch; increased post-implantation loss (increased early resorptions), decreased viable fetuses	30 mg/kg oral GD7-19 (FDA, United States, p. 135) C_{max} = 0.311 µg/mL[e] AUC = 1.31 µg·h/mL[e]	100 mg/kg oral GD7-19 (FDA, United States, p. 135)[c] C_{max} = 1.83 µg/mL[f] AUC = 21.00 µg·h/mL[f]	increased pre- and post-implantation loss (increased early resorptions), decreased viable fetuses, abortions	

a: The LOAEL for MEFL was a maternally toxic dose as indicated by increased mortality and abortions, clinical signs, and reductions in body weight and food consumption.

b: This was a dose range finding study with limited numbers of animals (n=6) and fetal evaluations limited to external morphology. It is thus unknown if there were visceral or skeletal alterations.

c: Actual values in plasma after 11 doses of 40 mg/kg oral ibrutinib in pregnant rats (FDA, United States, p. 130): C_{max} = 1.31 µg/mL, $AUC_{(0-24h)}$ = 5.348 µg·h/mL.

d: Actual values in plasma after 11 doses of 100 mg/kg oral ibrutinib in pregnant rats (FDA, United States, p. 130): C_{max} = 2.627 µg/mL, $AUC_{(0-24h)}$ = 13.729 µg·h/mL.

e: Actual values in plasma after 13 doses of 30 mg/kg oral ibrutinib in pregnant rabbits (FDA, United States, p. 136): C_{max} = 0.311 µg/mL, AUC = 1.31 µg·h/mL.

f: Actual values in plasma after 13 doses of 100 mg/kg oral ibrutinib in pregnant rabbits (FDA, United States, p. 136): C_{max} = 1.83 µg/mL, AUC = 21.00 µg·h/mL.

References

FDA, United States. Pharmacology Review NDA 020552 (21 Aug 2013).

Ibuprofen
CAS No.: 15687-27-1

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL[c]** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
180 mg/kg oral GD1-20 [Adams] C_{max} = 205 µg/mL[a] AUC = 597 µg·h/mL[a]	oral GD1-20: No LOAEL idenitifed [Adams] oral GD9-10: 300 mg/kg [Cappon 2003][b] <u>at 300 mg/kg</u> C_{max} = 341 µg/mL[a] AUC = 995 µg·h/mL[a]	GD1-20: None GD9-10: ventricular septal defects	60 mg/kg oral GD1-29 [Adams] C_{max} = 26.6 µg/mL[d] $AUC_{(0\text{-}inf)}$ = 80.5 µg·h/mL[d] 500 mg/kg oral GD9-11 [Cappon 2003][b] C_{max} = 222 µg/mL[d] $AUC_{(0\text{-}inf)}$ = 671 µg·h/mL[d]	No LOAEL identified	None	Maximum dose is 800 mg QID, 3200 mg/day (13.3 mg/kg/dose, 53 mg/kg/day) [US label] C_{max} = 59 µg/mL[e] AUC = 839 µg·h/mL[e]	NOAEL: <u>rat</u> C_{max} = 3.4 (205/59.7) AUC = 0.7 (597/839) <u>rabbit[c]</u> <u>60 mg/kg NOAEL</u> C_{max} = 0.5 (26.6/59) AUC = 0.1 (80.5/839) <u>500 mg/kg NOAEL</u> C_{max} = 3.8 (222/59) AUC = 0.8 (671/839) LOAEL: <u>rat</u> C_{max} = 5.8 (341/59)	

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL[c]** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
							AUC = 1.2 (995/839) <u>rabbit</u> no LOAEL	

a: Extrapolated from reported value after 25 mg/kg ibuprofen (suspension) single oral dose in Sprague Dawley rats (You): C_{max} = 28.4 µg/mL, $AUC_{(0\text{-}inf)}$= 4971.3 µg·min/mL (82.9 µg·h/mL). Note that different data (5- to 7-fold lower values) are available from the same laboratory at 25 mg/kg where the only difference appears to be that ibuprofen was administered in hard gelatin capsules versus a suspension (Newa): C_{max} = 5.32 µg/mL, AUC = 12.41 µg·h/mL.

b: To enhance detection of VSD and midline defects (seen in humans and with other NSAIDS), exposure was limited to the sensitive period of cardiovascular development and midline closure (i.e., GD9-10 in rats and GD9-11 in rabbits). By limiting the exposure period, maternal GI toxicity was reduced, allowing for the administration of higher doses.

c: Two values are included for the rabbit NOAEL since neither study design was ideal for assessing the risk of developmental toxicity according to current conventions. The study by Adams dosed rabbits on GD1-29 instead of the conventional GD7-19, whereas the study by Cappon dosed rabbits only on GD9-11 to enhance detection of VSD and midline defects.

d: Extrapolated from reported value after 56 mg/kg ibuprofen single oral dose in male New Zealand White rabbits (Kondal): C_{max} = 24.85 µg/mL, $AUC_{(0\text{-}inf)}$ = 75.14 µg·h/mL.

e: Extrapolated from reported value after 14.8 mg/kg (mean) ibuprofen single oral dose (Konstan): C_{max} = 65.5 µg/mL, $AUC_{(0\text{-}inf)}$ = 14.0 mg·min/mL (233 µg·h/mL). Note the C_{max} was multiplied by 0.9 (13.3/14.8) to give the extrapolated C_{max}. The C_{max} after a single dose likely represents the C_{max} at steady state since the half life is short (approximately 1.8 to 2 hours [US label]) and little accumulation is expected using the equation: accumulation = $1/(1 - e^{-k \cdot tau})$, where k = 0.693/t½ with t½ = 2 hours and tau = 6 hours (yielding an accumulation fatcor of 1.1). The AUC was multiplied by 4 to get the daily AUC for QID dosing (at 59.2 mg/kg/day), and then by 0.9 to give the extrapolated AUC for 53 mg/kg/day.

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IMATINIB
CAS No.: 152459-95-5 (220127-57-1 as mesilate)

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Notes**
30 mg/kg oral GD6-15 (FDA, United States, p. 69) C_{max} = 3.57 µg/mL[a] AUC = 39.28 µg·h/mL[a]	100 mg/kg oral GD6-15 (FDA, United States, p. 69) C_{max} = 12.14 µg/mL[b] AUC = 142.55 µg·h/mL[b]	exencephaly and/or protruding tongue, encephalocele, absent frontal or parietal bones; increased post-implantation loss, decreased live fetuses	60 mg/kg oral GD7-19 (FDA, United States, p. 72) C_{max} = 53.06 µg/mL[c] AUC = 699.8 µg·h/mL[c]	LOAEL not identified	None	

a: Interpolated from reported values in plasma after 15 and 50 mg/kg imatinib oral single dose in female rats (FDA, United States, p. 24): at 15 mg/kg, C_{max} = 1.69 µg/mL, $AUC_{(0\text{-}24h)}$ = 15.40 µg·h/mL; at 50 mg/kg, C_{max} = 6.07 µg/mL, $AUC_{(0\text{-}24h)}$ = 71.276 µg·h/mL.

b: Extrapolated from reported value in plasma after 50 mg/kg imatinib oral single dose in female rats (FDA, United States, p. 24): C_{max} = 6.07 µg/mL, $AUC_{(0\text{-}24h)}$ = 71.276 µg·h/mL.

c: Reported value after 60 mg/kg oral imatinib single dose in rabbits species (FDA, United States, p. 26): C_{max} = 53.06 µg/mL, $AUC_{(0\text{-}24h)}$ = 699.8 µg·h/mL.

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FDA, United States. Pharmacology Review NDA 021335 (04 May 2001).

Isotretinoin (13-*cis*-retinoic acid)
CAS No.: 4759-48-2

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit** **NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit** **LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
50 mg/kg oral dose on GD10 [Tembe] C_{max} = 0.9 µg/mL[a] $AUC_{(0-10h)}$ = 4.8 µg·h/mL[a]	100 mg/kg oral dose on GD10 [Tembe] C_{max} = 1.8 µg/mL[a] $AUC_{(0-10h)}$ = 9.6 µg·h/mL[a]	LOAEL: microtia and talipes. Higher doses: microcephaly, anotia, exopthalmos, protruding tongue, cleft lip, mandibular hypoplasia, cleft palate, overdeveloped papillae, analatresia, spina bifide,deformed tail, and acaudate; increased resorptions	3 mg/kg oral GD8-11 [Eckhoff] C_{max} = 0.95 µg/mL[b] AUC = 12.2 µg·h/mL[b]	15 mg/kg oral GD8-11 [Eckhoff] C_{max} = 3.1 µg/mL[c] AUC = 49.1 µg·h/mL[c]	increased resorptions, malformations including eye defects, tail defects, cardiomegaly, skin tag on face	0.5 mg/kg BID (1 mg/kg/day) C_{max} = 0.32 µg/mL[d] AUC = 7.52 µg·h/mL[d]	NOAEL: <u>rat</u> C_{max} = 2.8 (0.9/0.32) AUC = 0.6 (4.8/7.52) <u>rabbit</u> C_{max} = 3.0 (0.95/0.32) AUC = 1.6 (12.2/7.52) LOAEL: <u>rat</u> C_{max} = 5.6 (1.8/0.32) AUC = 1.3 (9.6/7.52) <u>rabbit</u> C_{max} = 9.7 (3.1/0.32)	

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit** **NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit** **LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
							AUC = 6.5 (49.1/7.52)	

a: Extrapolated from reported value after 500 mg/kg isotretinoin oral single dose in Wistar rats (Tembe): C_{max} = 9.07 µg/mL, $AUC_{(0\text{-}10h)}$ = 47.9 µg·h/mL.

b: Actual values after 3 mg/kg isotretinoin oral single dose in New Zealand White rabbits (Eckhoff): C_{max} = 0.952 µg/mL, AUC = 12.2 µg·h/mL.

c: Actual values after 15 mg/kg isotretinoin oral single dose in New Zealand White rabbits (Eckhoff): C_{max} = 3.099 µg/mL, $AUC_{(0\text{-}10h)}$ = 49.1 µg·h/mL.

d: Extrapolated from reported value after 80 mg (1.33 mg/kg) isotretinoin oral single dose with food (US label): C_{max} = 0.86 µg/mL, $AUC_{(0\text{-}10h)}$ = 10.0 µg·h/mL. The C_{max} extrapolation was based on a 0.5 mg/kg dose, whereas the AUC extrapolation was based on the daily dose of 1 mg/kg/day. PK data are also available while fasting, but the higher values from the fed state were used for margin calculations: C_{max} = 0.3 µg/mL, AUC = 3.7 µg·h/mL.

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Tembe EA, Honeywell R, Buss NE, Renwick AG. All-trans-retinoic acid in maternal plasma and teratogenicity in rats and rabbits. Toxicol Appl Pharmacol. 1996;141:456-72.

US label isotretinoin.

METHOTREXATE
CAS No.: 59-05-2

Rat NOAEL Dose C_{max} AUC	**Rat LOAEL Dose C_{max} AUC**	**Rat Findings**	**Rabbit NOAEL Dose C_{max} AUC**	**Rabbit LOAEL (Dose C_{max} AUC**	**Rabbit Findings**	**Human Dose[c] C_{max} AUC**	**NOAEL Margins**	**Notes**
NOAEL not identified	0.1 mg/kg IP GD9 [Jordan, Woo] C_{max} = 0.21 µg/mL[a] AUC = 0.067 µg·h/mL[a]	resorbed litters, malformations	NOAEL not identified	0.3 mg/kg IV GD10 [Jordan] C_{max} = 1.58 µg/mL[b] AUC = 0.61 µg·h/mL[b]	hydrocephalus, microphthalmia, cleft lip and palate, micrognathia, dysplastic sacral and caudal vertebrate, phocomelia, hemimelia, syndactyly, and ectrodactyly; embryolethality, resorptions	psoriasis: 10 – 25 mg Q7D (5.9 – 14.7 mg/m^2) oral or IV[c] ALL: induction – 3.3 mg/m^2 daily; maintenance – 15 mg/m^2 oral twice/week choriocarcinoma: 15 – 30 mg oral QD□□ 5 (8.8 – 17.6 mg/m^2) lymphoma: 10 – 25 mg QD□□ 4-8 oral (5.9 – 14.7 mg/m^2); 0.625 – 2.5 mg/kg (23 – 92.5 mg/m^2) mycosis fungoides: 5 – 50 mg Q7D oral (2.9 – 29 mg/m^2) RA: 7.5 mg Q7D oral (4.4 mg/m^2) C_{max} = 2.14 µg/mL[d]	NOAEL: rat NOAEL not identified rabbit NOAEL not identified LOAEL: rat C_{max} = 0.1 (0.21/2.14) AUC = 0.02 (0.067/3.28) rabbit C_{max} = 0.7 (1.58/2.14) AUC = 0.2 (0.61/3.28)	Note: animal MEFL data is after single dose, so margins would likely be eve n lower if dosed throughout organogenesis

						AUC = 3.28 µg·h/mL[d]		

a: Extrapolated from reported value after 0.31 mg/kg methotrexate intravenous single dose in Wistar rats (Scheufler 1982): C_0 = 0.64 µg/mL, $AUC_{(0.1\text{-}4h)}$ = 0.207 µg·h/mL. Other PK data are also available as shown in the table below. The data from Scheufler 1982 were chosen for margin calculations because it required the least degree of extrapolation in the same strain as the teratology study.

b: Extrapolated from reported value after 1.33 mg/kg methotrexate intravenous single dose in male rabbits (Iven): C_{max} = 7 µg/mL, AUC = 2.72 µg·h/mL. Data are also available after a 10 mg/kg methotrexate intravenous single dose in female New Zealand White rabbits (Stagni): C_{max} = 74 µg/mL, AUC = 31.4 µg·h/mL. The data from Iven were chosen for margin calculations because it required the least degree of extrapolation to the dose in the teratology study.

c: As noted there is a wide variety of doses, schedules, and routes used in a variety of indications (US label). An intravenous dose of 25 mg (14.7 mg/m^2) in psoriasis was chosen for PK margin comparisons since this was the highest dose in a non-oncology indication and would also provide a higher exposure than a 50 mg (29 mg/m^2) oral dose (mycosis fungoides) since oral bioavailability is only ~40%.

d: Extrapolated to 14.7 mg/m^2 f rom reported value after 30 mg/m^2 methotrexate intravenous single dose (Campbell): C_{max} = 4.37 µg/mL from visual inspection of graph, $AUC_{(0\text{-}inf)}$ = 6.69 µg·h/mL. Oral data are also available (Campbell): C_{max} = 0.50 µg /mL from visual inspection of graph, $AUC_{(0\text{-}inf)}$ = 2.34µg·h/mL.

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US label methotrexate.

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Rat Pharmacokinetic Data						
Reference	**Dose (mg/kg)**	**Route**	**Strain**	**C_{max} (µg/mL)**	**AUC (µg·h/mL)**	**Notes**
Wilson	0.3	IV	Wistar	0.40	—	C_0 was estimated from graph since 1st timepoint was 0.25 hours
Scheufler 1981	31	IV	Wistar	177	$AUC_{(0\text{-}inf)}$ = 38.4	C_{max} is C_0
Scheufler 1982	0.31	IV	Wistar	0.64	$AUC_{(0.1\text{-}4h)}$ = 0.207	
Kim	4.0	IV	Sprague Dawley	40	$AUC_{(0\text{-}inf)}$ = 2.88	C_{max} was from visual inspection of graph, AUC was 173 µg·min/mL

Pazopanib
CAS No.: 444731-52-6

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Notes**
1 mg/kg oral GD6-17 (FDA, United States, p. 218) C_{max} = 3.47 µg/mL[a] AUC = 0.028 µg·h/mL[a]	3 mg/kg oral GD6-17 (FDA, United States, p.218) C_{max} = 10.4 µg/mL[a] AUC = 0.083 µg·h/mL[a]	malformations in the great vessels, missing innominate artery	3 mg/kg oral GD7-19 (FDA, United States, p. 225) C_{max} = 0.130 µg/mL[b] $AUC_{(0-t)}$ = 0.517 µg·h/mL[c]	10 mg/kg oral GD7-19 (FDA, United States, p. 225) C_{max} = 1.063 µg/mL[d] $AUC_{(0-t)}$ = 1.723 µg·h/mL[d]	increased post-implantation loss	

a: Extrapolated or actual reported value in plasma after 3 mg/kg oral pazopanib for 28 days to Sprague Dawley rats (FDA, United States, p. 249): C_{max} = 10.4 µg/mL, AUC = 83 µg·h/L (0.083 µg·h/mL).

b: Actual values in plasma after 3 mg/kg pazopanib in rabbits (FDA, United States, p. 227): C_{max} = 0.130 µg/mL.

c: Extrapolated from reported value after 10 mg/kg pazopanib to rabbits (FDA, United States, p. 227): $AUC_{(0-t)}$ = 1.723 µg·h/mL.

d: Actual values in plasma after 10 mg/kg pazopanib in rabbits (FDA, United States, p. 227): C_{max} = 1.063 µg/mL, $AUC_{(0-t)}$ = 1.723 µg·h/mL.

References

FDA, United States. Pharmacology Review NDA 022456 (18 Sep 2009).

Additional References Evaluated

US Label Votrient.

PHENYTOIN
CAS No.: 57-41-0

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit** **NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit** **LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit** **Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
150 mg/kg oral GD6-15 [Kim] C_{max} = 13.4 µg/mL[a] AUC = 205 µg·h/mL[a]	300 mg/kg oral GD6-15 [Kim] C_{max} = 26.8 µg/mL[a] AUC = 410 µg·h/mL[a]	external findings (protruding tongue, meningoencepha-locele, domed head, anasarca, and limb hyperflexion), skeletal malformation (short rib)	50 mg/kg oral GD7-18 [McClain] C_{max} = 27 µg/mL[b] $AUC_{(0-24h)}$ = 193 µg·h/mL[c]	75 mg/kg oral GD7-18 [McClain] C_{max} = 34 µg/mL[d] $AUC_{(0-24h)}$ = 290 µg·h/mL[c]	open eyes, cleft palate, and limb abnormalities that included shortened and curved long bones, pes caves, syndactyly	up to 625 mg/day oral solution[e] C_{max} = 14.5 µg/mL[f] AUC = 291 µg·h/mL[g]	NOAEL: <u>rat</u> C_{max} = 0.9 (13.4/14.5) AUC = 0.7 (205/291) <u>rabbit</u> C_{max} = 1.9 (27/14.5) AUC = 0.7 (193/291) LOAEL: <u>rat</u> C_{max} = 1.8 (26.8/14.5) AUC = 1.4 (410/291) <u>rabbit</u> C_{max} = 2.3 (34/14.5) AUC = 1.0 (290/291)	

a: Actual or extrapolated from reported value after 150 mg/kg phenytoin oral dose on GD8 in Sprague Dawley rats (Rowland): C_{max} = 13.4 µg/mL, $AUC_{(0\text{-inf})}$ = 205 µg·h/mL. PK data are also available on GD17: C_{max} = 30.2 µg/mL, $AUC_{(0\text{-inf})}$ = 906 µg·h/mL.

b: Actual value after 50 mg/kg phenytoin oral single dose in female New Zealand White rabbits (McClain): C_{max} = 27 µg/mL. PK data are also available after 30 mg/kg phenytoin oral dose in male New Zealand White rabbits (Medhi): C_{max} = 12.8 µg/mL. The value from McClain was used because it was from females, required no extrapolation, and was generated in conjunction with the developmental toxicity study.

c: Extrapolated from reported value after 30 mg/kg phenytoin oral dose in male New Zealand White rabbits (Medhi): AUC = 116 µg·h/mL, from calculation based on concentration values estimated by visual inspection of graph since published value was inconsistent with other data in the paper.

d: Interpolated from actual values after 50 or 100 mg/kg phenytoin oral single dose in female New Zealand White rabbits (McClain): C_{max} = 27 µg/mL and 41 µg/mL at 50 and 100 mg/kg, respectively.

e: Phenytoin is available as an oral solution with an MRHD of 625 mg/day (dosing interval not clear) and as extended release capsules with an MRHD up to 600 mg/day (in 3 divided doses). For exposure comparisons, a dose of 250 mg (10 mL) as a single dose was used for C_{max} and a dose of 625 mg/day oral solution was used for AUC since exposure was higher for the solution than for extended release capsules (FDA, United States 1986).

f: Extrapolated to a 250 mg dose from reported value after 125 mg phenytoin oral solution single dose (FDA, United States 2002): C_{max} = 2.268 µg/mL, $AUC_{(0\text{-inf})}$ = 58.2 µg·h/mL. PK data are also available for a 100 mg oral solution dose and for extended release capsules (FDA, United States 1986). For C_{max}, an accumulation factor of 3.2 was applied that was estimated from the equation: accumulation = $1/(1 - e^{-k \cdot tau})$, where k = 0.693/t½ with t½ = 14.924 hours and tau = 8 hours (i.e., $1/(1 - e^{-0.372}) = 1/(1 - 0.690) = 1/0.31 = 3.2$).

g: Extrapolated to 625 mg/day from reported value after 125 mg phenytoin oral solution single dose (FDA, United States 2002): $AUC_{(0\text{-inf})}$ = 58.2 µg·h/mL.

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US label Dilantin oral solution.

US label Dilantin extended release capsules.

Pomalidomide
CAS No.: 19171-19-8

Rat NOAEL Dose C_{max} AUC	**Rat LOAEL Dose C_{max} AUC**	**Rat Findings**	**Rabbit NOAEL Dose C_{max} AUC**	**Rabbit LOAEL Dose C_{max} AUC**	**Rabbit Findings**	**Human Dose C_{max} AUC**	**Margins NOAEL/Human LOAEL/Human**	**Notes**
NOAEL not identified	25 mg/kg oral GD6-17 [FDA, United States 2013a] C_{max} = 2.7 µg/mL[a] $AUC_{(0-24)}$ = 34.3 µg·h/mL[a]	absence of urinary bladder and thyroid gland, fusion and misalignment of lumbar and thoracic vertebral elements (vertebral,	NOAEL not identified	10 mg/kg GD7-19 [FDA, United States 2013a] C_{max} = 0.072 µg/mL[b] AUCτ = 0.418 µg·h/mL[b]	interventricular septal defects; misaligned, fused or small caudal vertebrae	4 mg per day □ 21 (2.4 mg/m^2/day) C_{max} = 0.079 µg/mL[c] $AUC_{(0-24h)}$ = 0.402 µg·h/mL[d]	NOAEL: rat NOAEL not identified rabbit NOAEL not identified LOAEL: rat C_{max} = 34 (2.7/0.079)	

		central and/or neural arches) resorptions; increased post-implantation loss, decreased viable fetuses					AUC = 85 (34.3/0.402) <u>rabbit</u> C_{max} = 0.9 (0.072/0.079) AUC = 1.0 (0.418/0.402)	

a: Actual value on GD17 after 25 mg/kg pomalidomide oral dose in pregnant Sprague Dawley rats (FDA, United States 2013a, p. 152): C_{max} = 2.729 µg/mL, $AUC_{(0\text{-}24h)}$ = 34.34 µg·h/mL.

b: Actual value on GD17 after 10 mg/kg pomalidomide oral dose in pregnant New Zealand White rabbits (FDA, United States 2013a, p. 163): C_{max} = 0.072 µg/mL, AUC_{τ} = 0.418 µg·h/mL.

c: Actual value after 4 mg pomalidomide oral dose for 8 days in multiple myeloma subjects (FDA, United States 2013b, p. 24): C_{max} = 0.079 µg/mL.

d: Actual value after 4 mg/kg mg pomalidomide oral dose for 4 weeks (FDA, United States 2013a, p. 180): $AUC_{(0\text{-}24h)}$ = 0.402 µg·h/mL.

References

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Hoffmann M, Kasserra C, Reyes J, Schafer P, Kosek J, Capone L, et al. Absorption, metabolism and excretion of [14C]pomalidomide in humans following oral administration. Cancer Chemother Pharmacol. 2013;71:489-501. [PK in healthy volunteers, used data for patients from FDA, United States reviews]

Ribavirin
CAS No.: 36791-04-5

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Notes**
0.3 mg/kg oral GD6-15 (FDA, United States, p. 64) C_{max} = 3.8 ng/mL[a] AUC = 8.28 ng·h/mL[a]	1.0 mg/kg oral GD6-15 (FDA, United States, p. 64) C_{max} = 12.7 ng/mL[a] AUC = 27.6 ng·h/mL[a]	hydrocephaly, retinal folds, diaphragmic hernia, displaced adrenal, displaced oesophagus, vascular defects; extra vertabra, scoliosis, fused ribs and vertebrae, split sternum, ectrodactyly, malrotated hind limbs; increased post-implantation loss	0.3 mg/kg oral GD6-18 (FDA, United States, p. 68) No rabbit PK data found	1.0 mg/kg oral GD6-18 (FDA, United States, p. 68) No rabbit PK data found	anomalous cervicothoracic arteries	Ribavirin undergoes significant 1st pass metabolism. As a prodrug, it is rapidly anabolized to ribavirin monophosphate and ribavirin triphosphate, which play a role in its antiviral activity (Dixit). It is also deribosylated to triazole carboxamide (Lin). The contribution of each of these metabolites to the developmental effects in rats is unknown.

a: Extrapolated from reported value in plasma after 10 mg/kg ribavirin oral single dose in female Sprague Dawley rats (FDA, United States, p. 76): C_{max} = 0.127 µg/mL, AUC = 0.276 µg·h/mL. Note ≥10-fold extrapolation.

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Liao S, Jin X, Li J, Zhang T, Zhang W, Shi W, et al. Effects of silymarin, glycyrrhizin, and oxymatrine on the pharmacokinetics of ribavirin and its major metabolite in rats. Phytother Res. 2016;30:618-26. [at 30 mg/kg in fasted male Sprague Dawley rats: C_{max} = 1.36 µg/mL, $AUC_{(0\text{-}inf)}$ = 14.7 µg·h/mL]

Lin CC, Yeh LT, Luu T, Lourenco D, Lau JY. Pharmacokinetics and metabolism of [^{14}C]ribavirin in rats and cynomolgus monkeys. Antimicrob Agents Chemother. 2003;47:1395-8. [at 30 mg/kg in fasted male Sprague Dawley rats: C_{max} = 0.433 µg/mL, $AUC_{(0\text{-}inf)}$ = 3.04 µg·h/mL]

TACROLIMUS
CAS No.: 104987-11-3

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Notes**
1.0 mg/kg oral GD7-17 (FDA, United States, p. 18) C_{max} = 2.9 ng/mL[a] $AUC_{(0\text{-}inf)}$ = 10.9 ng·h/mL[a]	3.2 mg/kg oral GD7-17 (FDA, United States, p. 18) C_{max} = 20 ng/mL[b] $AUC_{(0\text{-}inf)}$ = 68.9 ng·h/mL[b]	slight increase in post implantation loss (late resorptions)	0.32 mg/kg oral GD6-18 (FDA, United States, p. 19) C_{max} = 0.93 ng/mL[c] AUC = 17.6 µg·h/mL[c]	1.0 mg/kg oral GD6-18 (FDA, United States, p. 19) C_{max} = 2.9 ng/mL[c] AUC = 55 ng·h/mL[c]	ventricular hypoplasia, interventricular septal defect, bulbous aortic arch and stenosis of arch and ductus arteriosus, omphalocele, gallbladder agenesis, skeletal malformations; increased post-implantation loss, decreased litter size	• Maternal toxicity seen in both rats and rabbits at LOAEL • Ratio of blood:plasma is 4:1 • Metabolites are 3-fold parent • 99% protein bound

a: Actual values in plasma after 1.0 mg/kg tacrolimus oral single dose in male rats (FDA, United States, p. 25): C_{max} = 2.9 ng/mL, $AUC_{(0\text{-}inf)}$ = 10.9 ng·h/mL.

b: Actual values in plasma after 3.2 mg/kg tacrolimus oral single dose in male rats (FDA, United States, p. 25): C_{max} = 20 ng/mL, $AUC_{(0\text{-}inf)}$ = 68.9 ng·h/mL.

c: Extrapolated from reported value after 2 mg/kg tacrolimus oral single dose in NZW rabbits (Piekoszewski): C_{max} = 5.79 ng/mL, AUC = 110 ng·h/mL.

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Thalidomide
CAS No.: 50-35-1

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings[a]**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
10 mg/kg[b] [Janer] C_{max} = 0.97µg/mL[c] $AUC_{(0\text{-}24h)}$ = 10.75 µg·h/mL[c]	50 mg/kg[b] [Newman, Schardein] C_{max} = 4.87µg/mL[c] $AUC_{(0\text{-}24h)}$ = 53.75 µg·h/mL[c]	decreased implanta-tion sites	20 mg/kg oral GD7-19 [Christian] <u>at GD19</u> C_{max} = 0.82 µg/mL[d] $AUC_{(0\text{-}24h)}$ = 4.18 µg·h/mL[d]	60 mg/kg oral GD7-19 [Christian] <u>at GD19</u> C_{max} = 2.16 µg/mL[e] $AUC_{(0\text{-}24h)}$ = 14.4 µg.h/mL[e]	• resorptions • rotated or flexed limbs (4/38 fetuses at 60 mg/kg and 15/25 fetuses at 180 mg/kg) • hydrocephaly (n=2/38) • increased postimplantation loss, including dead fetuses, and numerous external and visceral malformations at 180 mg/kg	50 mg oral[f] C_{max} = 0.62 µg/mL[g] $AUC_{(0\text{-}inf)}$ = 4.9 µg·h/mL[g]	NOAEL: <u>rat</u> C_{max} = 1.6 (0.97/0.62) AUC = 2.2 (10.75/4.9) <u>rabbit</u> C_{max} = 1.3 (0.82/0.62) AUC = 0.9 (4.18/4.9) LOAEL: <u>rat</u> C_{max} = 7.9 (4.87/0.62) AUC = 11.0 (53.75/4.9) <u>rabbit</u> C_{max} = 3.5 (2.16/0.62) AUC = 2.9 (14.4/4.9)	

a: Numerous developmental toxicity studies in rats have been reported in the literature with a variety of divergent results in different strains (Newman, Neubert, Janer, Schardein). Many of these older studies do not meet today's standards for design. Although malformations cannot be reproducibly induced, embryolethality appears to be a common effect at doses ≥100 mg/kg (Newman).

b: Based on literature reviews by Newman and Schardein, a dose of 50 mg/kg was chosen as the LOAEL. Based on review by Janer, 10 mg/kg appeared to be the highest dose with no evidence of developmental toxicity.

c: Extrapolated or actual value after 50 mg/kg thalidomide oral dose for 8 days in female Fischer rats (FDA, United States p. 86): C_{max} = 4.87 µg/mL, $AUC_{(0\text{-}24h)}$ = 53.75 µg·h/mL. PK data are also available after 30 mg/k oral single dose in female Fischer rats (FDA, United States, p. 22, 91): C_{max} = 10.4 µg/mL, $AUC_{(0\text{-}18h)}$ = 63.99 µg·h/mL; and after a 100 mg/kg oral single dose in male Sprague Dawley rats (FDA, United States, p. 73): C_{max} = 21.60 µg/mL, $AUC_{(0\text{-}48h)}$ = 348.5 µg·h/mL.

d: Actual value after 20 mg/kg thalidomide oral doses in pregnant New Zealand White rabbits (Christian). GD7: C_{max} = 1.77 µg/mL, $AUC_{(0\text{-}24h)}$ = 13.4 µg·h/mL; GD19: C_{max} = 0.824 µg/mL, $AUC_{(0\text{-}24h)}$ = 4.18 µg·h/mL.

e: Actual value after 60 mg/kg thalidomide oral doses in pregnant New Zealand White rabbits (Christian). GD7: C_{max} = 6.39 µg/mL, $AUC_{(0\text{-}24h)}$ = 78.7 µg·h/mL; GD19: C_{max} = 2.16 µg/mL, $AUC_{(0\text{-}24h)}$ = 14.4 µg·h/mL.

f: Currently approved doses range from 100 to 400 mg/day. A dose of 50 mg was used for PK comparisons because that was the lowest dose used to treat insomnia when thalidomide was first developed. Also, one 50 mg tablet of thalidomide during the time-sensitive window is sufficient to cause birth defects in 50% of pregnancies (Vargesson).

g: Actual value after 50 mg single dose to healthy volunteers (Teo, US label): C_{max} = 0.62 g/mL, AUC = 4.90 µg·h/mL.

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US label Thalomid.

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TOPIRAMATE
CAS No.: 97240-79-4

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
100 mg/kg oral GD6-15 [US label, FDA, United States 1996a] C_{max} = 49 µg/mL[a] AUC = 893 µg·h/mL[b]	400 mg/kg oral GD6-15 [US label, FDA, United States 1996a] C_{max} = 168.6 µg/mL[c] AUC = 3573 µg·h/mL[b]	ectrodactyly, hydronephrosis	20 mg/kg oral GD6-18 [US label, FDA, United States 1996a] C_{max} = 13 µg/mL[d] AUC = 67 µg·h/mL[d]	35 mg/kg oral GD6-18 [US label, FDA, United States 1996a] C_{max} = 23 µg/mL[d] AUC = 117 µg·h/mL[d]	embryofetal mortality at ≥35 mg/kg	400 mg/day in two divided doses C_{max} = 13.5 µg/mL[e] AUC = 229 µg·h/mL[e]	NOAEL: <u>rat</u> C_{max} = 3.6 (49/13.5) AUC = 3.9 (893/229) <u>rabbit</u> C_{max} = 1.0 (13/13.5) AUC = 0.3 (67/229) LOAEL: <u>rat</u> C_{max} = 12.5 (169/13.5) AUC = 15.6 (3573/229) <u>rabbit</u> C_{max} = 1.7 (23/13.5)	• In rats: Although reduced fetal BW and increased incidence of structural variations were observed at 20 mg/kg, the NOAEL for MEFL is assumed to be 100 mg/kg • In rats: Clinical signs of maternal toxicity were seen at ≥400 mg/kg and maternal BW gain was reduced at ≥100 mg/kg • In rabbits: maternal toxicity (decreased BW gain, clinical signs, and/or

							AUC = 0.5 (117/229)	mortality) was seen at ≥35 mg/kg

a: Extrapolated from reported value after 200 mg/kg topiramate for GD12-15 (4 days) in pregnant female Sprague Dawley rats (FDA, United States, p. 48): $C_{1.5h}$ = 97.3 µg/mL.

b: Extrapolated from reported value after 30 mg/kg topiramate for 8 days in female Sprague Dawley rats (FDA, United States, p. 12): C_{max} = 22.2 µg/mL, AUC = 268.2 µg·h/mL.

c: Actual value after 400 mg/kg topiramate for GD12-15 (4 days) in pregnant female Sprague Dawley rats (FDA, United States, p. 48): $C_{1.5h}$ = 168.6 µg/mL.

d: Extrapolated from reported value after 60 mg/kg topiramate for 14 days in female New Zealand White rabbits (FDA, United States, p. 13): C_{max} =39.1 µg/mL, AUC = 201 µg·h/mL.

e: Extrapolated from reported value after 100 mg/kg topiramate BID oral for 14 days (FDA, United States 1996b): C_{max} = 6.76 µg/mL, $AUC_{(0-24h)}$ = 57.2 µg·h/mL. PK data at a number of other doses and schedules and in combination with other drugs are also available (FDA, United States 1995b, Bialer).

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TRIMETHADIONE
CAS No.: 127-48-0

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
60 mg/kg oral GD6-15 [Buttar 1976] Trimethadione C_{max} = 58.9 µg/mL[a] $AUC_{(0\text{-}inf)}$ = 203 µg·h/mL[a] Dimethadione C_{max} = 97.7µg/mL[b] $AUC_{(0\text{-}inf)}$ = 4872 µg·h/mL[b]	240 mg/kg oral GD6-18 [Buttar 1976] Trimethadione C_{max} = 235 µg/mL[a] $AUC_{(0\text{-}inf)}$ = 814 µg·h/mL[a] Dimethadione C_{max} = 391 µg/mL[b] $AUC_{(0\text{-}inf)}$ = 19,488 µg·h/mL[b]	240 mg/kg GD6-15 [Buttar]: "adverse fetal effects on survival and litter size" 250 mg/kg GD7-18 [Vorhees]: embryolethality, malformations (primarily cardiac, with a lower incidence of esophageal and kidney defects)	No rabbit data found Trimethadione AUC = 10.78 µg·h/mL[c]	No rabbit data found	No rabbit data found	600 mg QID (10 mg/kg □ 4) [highest dose, US label] Trimethadione C_{max} = 42.75 µg/mL[d] $AUC_{(0\text{-}inf)}$ = 1000 µg·h/mL[d] Dimethadione C_{max} = 1251 µg/mL[e] $AUC_{(0\text{-}inf)}$ = 36,670 µg·h/mL[e]	Trimethadione NOAEL: rat C_{max} = 1.4 (58.9/42.75) AUC = 0.2 (203/1000) rabbit NOAEL not identified LOAEL: rat C_{max} = 5.5 (235/42.75) AUC = 0.8 (814/1000) rabbit LOAEL not identified Dimethadione NOAEL: rat C_{max} = 0.1 (97.7/1251)	Dimethadione is the only metabolite, has much higher exposures than trimethadione, and is a confirmed teratogen (Buttar 1978). Thus, margins for dimethadione are also listed.

							AUC = 0.1 (4872/36670) LOAEL: <u>rat</u> C_{max} = 0.3 (391/1251) AUC = 0.5 (19488/36670)	

a: Extrapolated from reported value after 100 mg/kg trimethadione oral single dose in male Wistar rats (Tanaka 1981): C_{max} = 98.1 µg/mL, $AUC_{(0\text{-}inf)}$ = 339 µg·h/mL.

b: Extrapolated from reported value after 100 mg/kg trimethadione oral single dose in male Wistar rats (Tanaka 1981): dimethadione C_{max} = 162.8 µg/mL, $AUC_{(0\text{-}inf)}$ = 8120 µg·h/mL.

c: Actual value after 4 mg/kg trimethadione intravenous single dose in Japanese White rabbits (Tanaka 1999): $AUC_{(0\text{-}inf)}$ = 10.78 µg·h/mL calculated from Cl = 0.371 L/(kg·h).

d: Extrapolated from reported value after 4 mg/kg trimethadione oral single dose (Kobayashi): C_{max} = 6.0 µg/mL, $AUC_{(0\text{-}inf)}$ = 100.1 µg·h/mL. For C_{max}, an accumulation factor of 2.85 was applied that was estimated from the equation: accumulation = $1/(1 - e^{-k \cdot tau})$, where k = 0.693/t½ with t½ = 9.6 hours and tau = 6 hours (i.e., $1/(1 - e^{-0433}) = 1/(1 - 0.649) = 1/0.351 = 2.85$).

e: Extrapolated from reported value after 4 mg/kg trimethadione oral single dose (Kobayashi): dimethadione C_{max} = 12.83 µg/mL, $AUC_{(0\text{-}inf)}$ = 3667 µg·h/mL. For C_{max}, an accumulation factor of 39 was applied that was estimated from the equation: accumulation = $1/(1 - e^{-k \cdot tau})$, where k = 0.693/t½ with t½ = 160 hours and tau = 6 hours (i.e., $1/(1 - e^{-0.026}) = 1/(1 - 0.974) = 1/0.026 = 39$).

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US label trimethadione.

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Tanaka E, Yoshida T, Kuroiwa Y. Dose-independent pharmacokinetics of trimethadione and its metabolite in rats. J Pharm Sci. 1985;74:340-1. [PK values after 4 mg/kg trimethadione oral single dose in male Wistar rats: trimethadione C_{max} = 3.0 µg/mL, $AUC_{(0\text{-}inf)}$ = 8.21 µg·h/mL, and dimethadione C_{max} = 10.2 µg/mL, $AUC_{(0\text{-}inf)}$ = 465.8 µg·h/mL. The values after 100 mg/kg (Tanaka 1981) were used instead].

Taylor JD, Bertcher EL. The determination and distribution of trimethadione (tridione) in animal tissues. J Pharmacol Exp Ther. 1952;106:277-85. [levels in rabbit brain after 1000 mg/kg IP]

Valproic Acid
CAS No.: 99-66-1 (sodium valproate: 1069-66-5)

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
65 mg/kg oral GD6-15, SD rats [FDA, United States, 1995] C_{max} = 73.8 µg/mL[a] AUC = 230 µg·h/mL[a]	200 mg/kg oral, SD rats, GD7-18 [Voorhees], GD8-17 [Binkerd]; [US Depacon label] C_{max} = 227 µg/mL[a] AUC = 707 µg·h/mL[a]	hydronephrosis, cardiovascular defects	150 mg/kg oral GD6-18 [FDA, United States, 1977] C_{max} = 410 µg/mL[b] AUC = 690 µg·h/mL[b]	350 mg/kg oral GD6-18 [FDA, United States, 1977] C_{max} = 957 µg/mL[b] AUC = 1610 µg·h/mL[b]	resorptions; external abnormalities (cleft palate, umbilical hernia, bilateral talipes, exencephaly, hypoplastic ears, gastrochisis, bilateral talipes); visceral malformations (intraventricular septal defects, misshapen ventricle, renal agenesis); skeletal malformations (supernumerary ribs, fused ribs)	60 mg/kg/day oral in 2 divided doses (30 mg/kg/dose) [highest approved dose, US Depakote and Depakene labels] C_{max} = 205 µg/mL[c] $AUC_{(0\text{-}inf)}$ = 4180 µg·h/mL[d]	NOAEL: <u>rat</u> C_{max} = 0.4 (73.8/205) AUC = 0.06 (230/4180) <u>rabbit</u> C_{max} = 2.0 (410/205) AUC = 0.2 (690/4180) LOAEL: <u>rat</u> C_{max} = 1.1 (227/205) AUC = 0.2 (707/4180) <u>rabbit</u> C_{max} = 4.7 (957/205) AUC = 0.4 (1610/4180)	

a: Extrapolated or actual value after 200 mg/kg valproic acid oral dose on GD17 in pregnant Sprague Dawley rats (Binkerd): C_{max} = 227 µg/mL, AUC = 707 µg·h/mL. PK data are also available on GD8: C_{max} = 341µg/mL, AUC = 1019 µg·h/mL

b: Extrapolated from reported value after 70 mg/kg valproic acid oral single dose in male New Zealand White rabbits (Bourin): C_{max} = 191.3 µg/mL, $AUC_{(0\text{-}inf)}$ = 322 µg·h/mL. Rabbit PK data are also available after 50 mg/kg oral (FDA, United States), 20 mg/kg oral (van Jaarsveld), 43 mg/kg intravenous (Nakashima), and 75 mg/kg intravenous (Yokogawa).

c: Extrapolated from reported value after 1000 mg valproic acid oral BID for 5 days (Nitsche): C_{max} = 114 µg/mL.

d: Extrapolated from reported value after 1000 mg valproic acid oral single dose (Nitsche): $AUC_{(0\text{-}inf)}$ = 1161 µg·h/mL.

References

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Bourin M, Guenzet J, Thomare P, Kergueris MF, Ortega A, Larousse C. Effects of administration route on valproate pharmacokinetics in the rabbit. Fundam Clin Pharmacol. 1991;5:331-9.

FDA, United States Approval Package, NDA 018081 (S-001, S-025) and 018082 (S-008) (1995), Part 2. p. 7-8,10,12,28.

FDA, United States Pharmtox reviews IND 011152 (March 1977), p. 31-32, 34.

Nitsche V, Mascher H. The pharmacokinetics of valproic acid after oral and parenteral administration in healthy volunteers. Epilepsia. 1982;23:153-62

Ong LL, Schardein JL, Petrere JA, Sakowski R, Jordan H, Humphrey RR, et al. Teratogenesis of calcium valproate in rats. Fundam Appl Toxicol. 198;3:121-6.

Vorhees CV. Teratogenicity and developmental toxicity of valproic acid in rats. Teratology. 1987;35(2):195-202.

US Depacon (valproate injection) label.

US Depakene (valproate capsule) label.

US Depakote (valproex tablets) label.

Additional References Evaluated

FDA, United States Pharmtox reviews IND 011152 (1977), p. 48. [after 50 mg/kg [^{14}C]valproic acid oral single dose in rabbits (FDA, United States): C_{max} = 86 µg/mL].

Katayama H, Mizukami K, Yasuda M, Hatae T. Effects of carnitine on valproic acid pharmacokinetics in rats. J Pharm Sci. 2016;105:3199-3204. [PK data in male Wistar rats after 32 mg/kg oral: C_{max} = 40.7 µg/mL, $AUC_{(0\text{-}inf)}$ = 3458 µg·min/mL (57.6 µg·h/mL)]

Nakashima M, Takeuchi N, Hamada M, Matsuyama K, Ichikawa M, Goto S. *In vivo* microdialysis for pharmacokinetic investigations: a plasma protein binding study of valproate in rabbits. Biol Pharm Bull. 1994;17:1630-4. [PK after 43 mg/kg intravenous valproic acid in anesthetized male Japanese Albino rabbits: C_0 = 157 µg/mL, $AUC_{(0\text{-}inf)}$ = 308 µg·h/mL]

Rha JH, Jang IJ, Lee KH, Chong WS, Shin SG, Lee N, Myung HJ. Pharmacokinetic comparison of two valproic acid formulations--a plain and a controlled release enteric-coated tablets. J Korean Med Sci. 1993 Aug;8(4):251-6.

van Jaarsveld MF, Walubo A, du Plessis JB. Interaction between valproic acid and acyclovir after intravenous and oral administration in a rabbit model. Basic Clin Pharmacol Toxicol. 2007;101:434-40. [PK after 20 mg/kg valproic acid oral single dose in New Zealand White rabbts: C_{max} = 64.2 µg/mL, $AUC_{(0\text{-}inf)}$ = 227 µg·h/mL].

Yokogawa K, Iwashita S, Kubota A, Sasaki Y, Ishizaki J, Kawahara M, Matsushita R, Kimura K, Ichimura F, Miyamoto K. Effect of meropenem on disposition kinetics of valproate and its metabolites in rabbits. Pharm Res. 2001;18:1320-6. [PK after 75 mg/kg intravenous dose in male albino rabbits: C_{max} = 238 µg/mL, $AUC_{(0\text{-}6h)}$ = 17.5 mg·min/L (292 µg·h/mL)]

Zaccara G, Messori A, Moroni F. Clinical pharmacokinetics of valproic acid--1988. Clin Pharmacokinet. 1988;15:367-89.

VISMODEGIB
CAS No.: 879085-55-9

Rat NOAEL Dose C_{max} AUC	**Rat LOAEL Dose C_{max} AUC**	**Rat Findings**	**Rabbit NOAEL Dose C_{max} AUC**	**Rabbit LOAEL Dose C_{max} AUC**	**Rabbit Findings**	**Human Dose C_{max} AUC**	**Margins NOAEL/Human LOAEL/Human**	**Notes**
NOAEL not identified	10 mg/kg GD6-17 oral [FDA, United States, 2011] C_{max} = 7.22 µg/mL[a] $AUC_{(0\text{-}24h)}$ = 50.5 µg·h/mL[a]	malformations included absent and/or fused digits on the hind limb, open perineum, multiple craniofacial anomalies	no rabbit data found	no rabbit data found	no rabbit data found	150 mg oral C_{max} = 13.0 µg/mL[b] $AUC_{(0\text{-}24h)}$ = 306 µg·h/mL[b]	NOAEL: <u>rat:</u> NOAEL not identified <u>rabbit:</u> no data found LOAEL: <u>rat</u> C_{max} = 0.6 (7.22/13) AUC = 0.2 (50.5/306) <u>rabbit</u> no data found	MW = 421.3

a: Reported value after 10 daily oral doses of 10 mg/kg vismodegib in female pregnant Wistar rats (FDA, United States, 2011): C_{max} = 7.22 µg/mL, $AUC_{(0\text{-}24h)}$ = 50.5 µg·h/mL

b: Reported value after 14 daily oral doses of 150 mg vismodegib (FDA, United States, 2012): C_{max} = 30.9 µM (13.0 µg/mL), $AUC_{(0\text{-}24h)}$ = 727 µmol·h/L (306 µg·h/mL).

References

FDA, United States. Pharmacology Review NDA 203388 (08 Sep 2011), p. 66-9.

FDA, United States. Clinical Pharmacology Review NDA 203388 (13 Jan 2012), p. 48.

1.3.2 Negative Control Reference Compounds

CETIRIZINE
CAS No.: 83881-51-0

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
NOAEL (MEFL) 75 mg/kg oral GD6-15 (FDA, United States 1989) C_{max} = 45 µg/mL[a] AUC = 301 µg•h/mL[b] Exposure data at lower doses 8 mg/kg oral GD6-15 (FDA, United States 1989) C_{max} = 4.6 µg/mL[a] AUC = 32 µg•h/mL[b] 25 mg/kg oral GD6-15 (FDA, United States 1989) C_{max} = 12 µg/mL[a]	225 mg/kg oral GD6-15 (FDA, United States 1989) C_{max} = 128 µg/mL[a] AUC = 1010 µg•h/mL[b]	225 mg/kg: pre- and post-implantation loss in presence of maternal toxicity (death, clinical signs)	NOAEL (MEFL) 135 mg/kg oral GD6-18 (FDA, United States 1989) C_{max} = 137 µg/mL[c] AUC = 642 µg•h/mL[c] Exposure data at lower doses 15 mg/kg oral GD6-18 (FDA, United States 1989) C_{max} = 15 µg/mL[c] AUC = 71 µg•h/mL[c] 45 mg/kg oral GD6-18 (FDA, United States 1989)	Not established	No MEFL observed	10 mg MRHD Exposure values after single dose: C_{max} = 0.33 µg/mL[d] $AUC_{(0\text{-}24h)}$: 3.0 µg•hr/mL[d]	NOAEL: rat (75 mg/kg/day) C_{max}: 136 (45/0.33) AUC: 111 (334/3.02) Rabbit (135 mg/kg/day) C_{max}: 415 (137/0.33) AUC: 213 (642/3.02) LOAEL: Rat (225 mg/kg/day) C_{max}: 388 (128/0.33) AUC: 334 (1010/3.02) rabbit Not applicable	None

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
AUC = 41 µg•h/mL[b]			C_{max} = 51 µg/mL[c] AUC = 116 µg•h/mL[c]					

a: From reported Cmax values in a 4-week repeated-dose toxicity study in rats at steady state (day 23) at doses of 25, 75 and 225 mg/kg/day. Cmax for 8 mg/kg/day was linearly extrapolated from these data. (FDA, United States 1993, page 4).

b: From reported AUC values in a 4-week repeated-dose toxicity study in rats at steady state (day 23) at doses of 25 mg/kg/day and 225 mg/kg/day. AUC for 8 and 75 mg/kg/day were linearly extrapolated from these data (FDA, United States 1993, page 4).

c: From reported Cmax and AUC values in pregnant rabbits exposed from GD6-18 at steady state (GD18) at doses of 25, 45 and 90 mg/kg/day. Cmax and AUC for 15 and 135 mg/kg/day were linearly extrapolated from these data. (FDA, United States 1993, page 5).

d: Single administration of 10 mg cetirizine with water (FDA, United States, 2003).

References

FDA, United States. Pharmacology review of NDA 019835 (11 Apr 1989) part 01, pages 10-11 (rat and rabbit EFD overview).

FDA, United States. Pharmacology review of NDA 019835 (11 Apr 1989) part 02, pages 10-30 (rat and rabbit EFD summary).

FDA, United States. Pharmacology review of NDA 019835 (18 Oct 1993), pages 4 (rat PK data) and 5 (rabbit PK data).

FDA, United States. Clinical Pharmacology and Biopharmaceutics Review 021621/S-000 (31 Oct 2003) (Clinical AUC, single dose pg 11)

US Label Zyrtec.

EU SmPC Zyrtec.

SAXAGLIPTIN
CAS No.: 361442-04-8

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
NOAEL (MEFL) 900 mg/kg oral GD6-15 (FDA, United States 2009) *Saxagliptin* C_{max} = 249 µg/mL[a] AUC_{0-24} = 647 µg•h/mL[a] *BMS-510849* C_{max} = 21.1 µg/mL[b] AUC_{0-24} = 144 µg•h/mL[a] Exposure data at lower doses 64 mg/kg oral GD6-15 *Saxagliptin* C_{max} = 17.7 µg/mL[a] AUC_{0-24} = 23.6 µg•h/mL[a]	Not established	No MEFL observed	NOAEL (MEFL) 200 mg/kg oral GD7-19 (FDA, United States 2009) *Saxagliptin* C_{max} = 43 µg/mL[c] AUC_{0-24} = 111 µg•h/mL[a] *BMS-510849* C_{max} = 125 µg/mL[c] AUC_{0-24} = 434 µg•h/mL[a] Exposure data at lower doses 8 mg/kg oral GD7-19 *Saxagliptin* C_{max} = 2 µg/mL[c] AUC_{0-24} = 2.5 µg•h/mL[a]	Not established	No MEFL observed	5 mg MRHD Exposure values after single dose: *Saxagliptin* C_{max} = 0.024 µg/mL[d] $AUC_{(0-24h)}$: 0.078 µg•hr/mL[d] *BMS-510849* C_{max} = 0.047 µg/mL[d] $AUC_{(0-24h)}$: 0.214 µg•hr/mL[d]	NOAEL: rat (900 mg/kg/day) *Saxagliptin* C_{max}: 10,375 (249/0.024) AUC: 8,294 (647/0.078) *BMS-510849* C_{max}: 449 (21.1/0.047) AUC: 673 (144/0.214) Rabbit (200 mg/kg/day) *Saxagliptin* C_{max}: 1,792 (43/0.024) AUC: 1,423 (111/0.078) *BMS-510849* C_{max}: 2,659 (125/0.047) AUC: 2,028 (434/0.214)	BMS-510849 is a major active metabolite of saxagliptin. (US Label and EU EPAR Onglyza)

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
BMS-510849 C_{max} = 1.5 µg/mL[b] AUC_{0-24} = 6.3 µg•h/mL[a] 240 mg/kg oral GD6-15 *Saxagliptin* C_{max} = 66.3 µg/mL[a] AUC_{0-24} = 121 µg•h/mL[a] *BMS-510849* C_{max} = 5.6 µg/mL[b] AUC_{0-24} = 28.9 µg•h/mL[a]			*BMS-510849* C_{max} = 5 µg/mL[c] AUC_{0-24} = 7.4 µg•h/mL[a] 40 mg/kg oral GD7-19 *Saxagliptin* C_{max} = 9 µg/mL[c] AUC_{0-24} = 12.3 µg•h/mL[a] *BMS-510849* C_{max} = 25 µg/mL[c] AUC_{0-24} = 47.9 µg•h/mL[a]				LOAEL: <u>rat</u> Not applicable <u>rabbit</u> Not applicable	

a: From reported AUC values in pregnant rats (GD15) and pregnant rabbits (GD19) at steady state at doses of 64, 240 and 900 mg/kg/day saxagliptin for rat and 8, 40 and 200 mg/kg/day saxagliptin for rabbit (FDA, United States, 2009, part 02, page 84)

b: From reported Cmax values in a 4-week repeated-dose toxicity study in female rats at steady state (day 28) at doses of 150, 300 and 225 mg/kg/day, corresponding to 50, 78 and 139 ug/mL for saxagliptin and 4.6, 7.9 and 11 ug/mL for the active metabolite.. Saxagliptin Cmax values were linearly extrapolated from these data. (FDA, United States, 2009, part 04, page 56)

c: From reported Cmax values in a rabbit EFD study at steady state (GD19) at 40 mg/kg/day saxagliptin (Cmax 8.5 µg/mL). Saxagliptin Cmax values were linearly extrapolated from these data.

d: Single administration of 5 mg saxagliptin (US Label Onglyza, page 12).

References

FDA, United States. Pharmacology Review 022350/S-000 (3 March 2009) Part 02, page 84 (rat and rabbit AUC data Saxagliptin and active metabolite)

FDA, United States. Pharmacology Review 022350/S-000 (3 March 2009) Part 03, pages 57-59 (rat and rabbit EFD studies).

FDA, United States. Pharmacology Review 022350/S-000 (3 March 2009) Part 04, page 56 (rat Cmax data Saxagliptin and active metabolite)

FDA, United States. Pharmacology Review 200678Orig1s000 (10 January 2010) for Saxagliptin + metformin, page 44 table 30 (rabbit Cmax data Saxagliptin and active metabolite)

US Label Onglyza.

EU EPAR Onglyza.

VILDAGLIPTIN
CAS No.: 274901-16-5

Rat NOAEL **Dose** **C_{max}** **AUC**	**Rat LOAEL** **Dose** **C_{max}** **AUC**	**Rat Findings**	**Rabbit NOAEL** **Dose** **C_{max}** **AUC**	**Rabbit LOAEL** **Dose** **C_{max}** **AUC**	**Rabbit Findings**	**Human** **Dose** **C_{max}** **AUC**	**Margins** **NOAEL/Human** **LOAEL/Human**	**Notes**
NOAEL (MEFL) 750 mg/kg oral GD6-17 (TGA, Australia 2010) AUC_{0-24} = 241 µg•h/mL[a] Exposure data at lower doses 75 mg/kg oral GD6-17 AUC_{0-24} = 23 µg•h/mL[a] 225 mg/kg oral GD6-17 AUC_{0-24} = 68 µg•h/mL[a]	Not established	No MEFL observed	NOAEL (MEFL) 150 mg/kg oral GD7-20 (TGA, Australia 2010) AUC_{0-24} = 80 µg•h/mL[a] Exposure data at lower doses 15 mg/kg oral GD7-20 AUC_{0-24} = 6 µg•h/mL[a] 50 mg/kg oral GD7-20 AUC_{0-24} = 19 µg•h/mL[a]	Not established	No MEFL observed	50 mg b.i.d. MRHD (100 mg/day) Exposure values after 50 mg b.i.d.: $AUC_{(0-24h)}$: 2.06 µg•hr/mL[b]	NOAEL: rat (750 mg/kg/day) AUC: 117 (241/2.06) Rabbit (150 mg/kg/day) AUC: 39 (80/2.06) LOAEL: Not applicable	

a: Calculated from exposure ratios compared to human exposure at MRHD (2.06 µg•hr/mL at 50 mg BID) of AUC data provided within the rat and rabbit EFD studies (TGA, Australia, 2010, page 19)
b: Human exposure data at 50 mg BID (TGA, Australia, 2010, page 14)

References

TGA, Australia. Australian Public Assessment Report for Vildagliptin (April 2010) pages 19 (EFD studies), 14, 24 (exposure data) and 72 (pregnancy).

EU EPAR Galvus

EU SmPC. Galvus