

1 **The Scientific Rationale**
2 **for**
3 **Deriving Database and Toxicodynamic**
4 **Uncertainty Factors**
5 **for**
6 **Reproductive or Developmental**
7 **Toxicants**

8
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74 **Goal of the Peer Consultation**

75

76 In light of the recent focus on children’s potential risk from chemical exposures, exemplified for
77 example by the use of a 10-fold safety factor as part of the 1996 U.S. Food Quality Protection
78 Act (FQPA), the existing rationale for uncertainty factors used to estimate various “safe” doses
79 has been investigated. Many scientific publications, opinions and thought pieces have been
80 developed around this topic since then. In addition, since 1994 the International Programme on
81 Chemical Safety (IPCS) has been developing a framework for using specific data in lieu of
82 default uncertainty factors as a basis of safe dose assessment. The development of this
83 framework has many scientific publications, presentations and thought pieces as well.

84

85 The goal of this peer consultation is to obtain expert input on the scientific rationale for choosing
86 two specific uncertainty factors to address developmental, neonatal, child, or reproductive
87 toxicity when deriving safe doses. Two distinct compilations are presented. First, we compile
88 toxicity data from experimental animals for classes of chemicals with diverse mechanisms of
89 action. This compilation can be used to explore the procedure for deriving safe doses when no
90 adequate human or animal reproductive or developmental studies are available, and can be used
91 to discuss the database uncertainty factor developed by U.S. EPA in the late 1980s based on
92 pesticide data.

93

94 Second, we compile information to evaluate the scientific rationale for making animal-to-human
95 extrapolations of developmental, neonatal, or reproductive endpoints that are reported as critical
96 effects in experimental animal studies. This second compilation can be used to explore
97 approaches for applying the IPCS framework on its Chemical Specific Adjustment Factors
98 (CSAFs) for toxicodynamics based on known similarities and differences in physiology and
99 anatomy.

100

101 Both efforts have usefulness for uncertainty factor selection when deriving safe doses that are
102 protective of children. Chapter 1 will provide some background on development and use of the
103 database uncertainty factor and the CSAF approach. Specific and more detailed compilations of
104 each area are found in Chapters 2 and 3.

105

106 **1. Background**

107

108 **1.1. Introduction**

109

110 The development of “safe” doses by health groups around the world focuses on the judgment of
111 critical effect and sensitive population. In developing these safe doses, the potential for effects
112 from chemical exposures on fetal and childhood development and on reproductive toxicity are
113 regularly explored. See, for example, the safe doses for various chemicals from several health
114 organizations on the International Toxicity Estimates for Risk (*ITER*, 2005). Figure 1 illustrates
115 a typical process of deriving such safe dose estimates, depending on the richness of the data set
116 and individual study results.

117

118 When adequate human studies on reproductive or developmental toxicity or other toxicity in
119 children are available to identify the critical effect, a “safe” dose can be developed directly as
120 shown by Column A (Human Child) in Figure 1. Several examples exist where this has been
121 done, such as the methyl mercury Reference Dose (RfD) on U.S. EPA’s Integrated Risk
122 Information System (U.S. EPA, 2005). An uncertainty factor for human variability in
123 toxicokinetics (HK) and toxicodynamics (HD) may or may not be needed with such data [see
124 methyl mercury or nitrate on *ITER* (2005) for examples of either from different organizations].
125 When only adult human studies are available and these studies identify the critical effect, a safe
126 dose can be developed following Column B (Human Adult). As before, an uncertainty factor for
127 intraspecies variability in HK or HD may or may not be needed with such data (see U.S. EPA,
128 2005 or *ITER*, 2005, for numerous examples).

129

130 When no human data are available, as is often the case, the approach outlined in Figure 1 under
131 Column C (Effect in Animals) would be used, with three alternate approaches depending on the
132 available data:

133

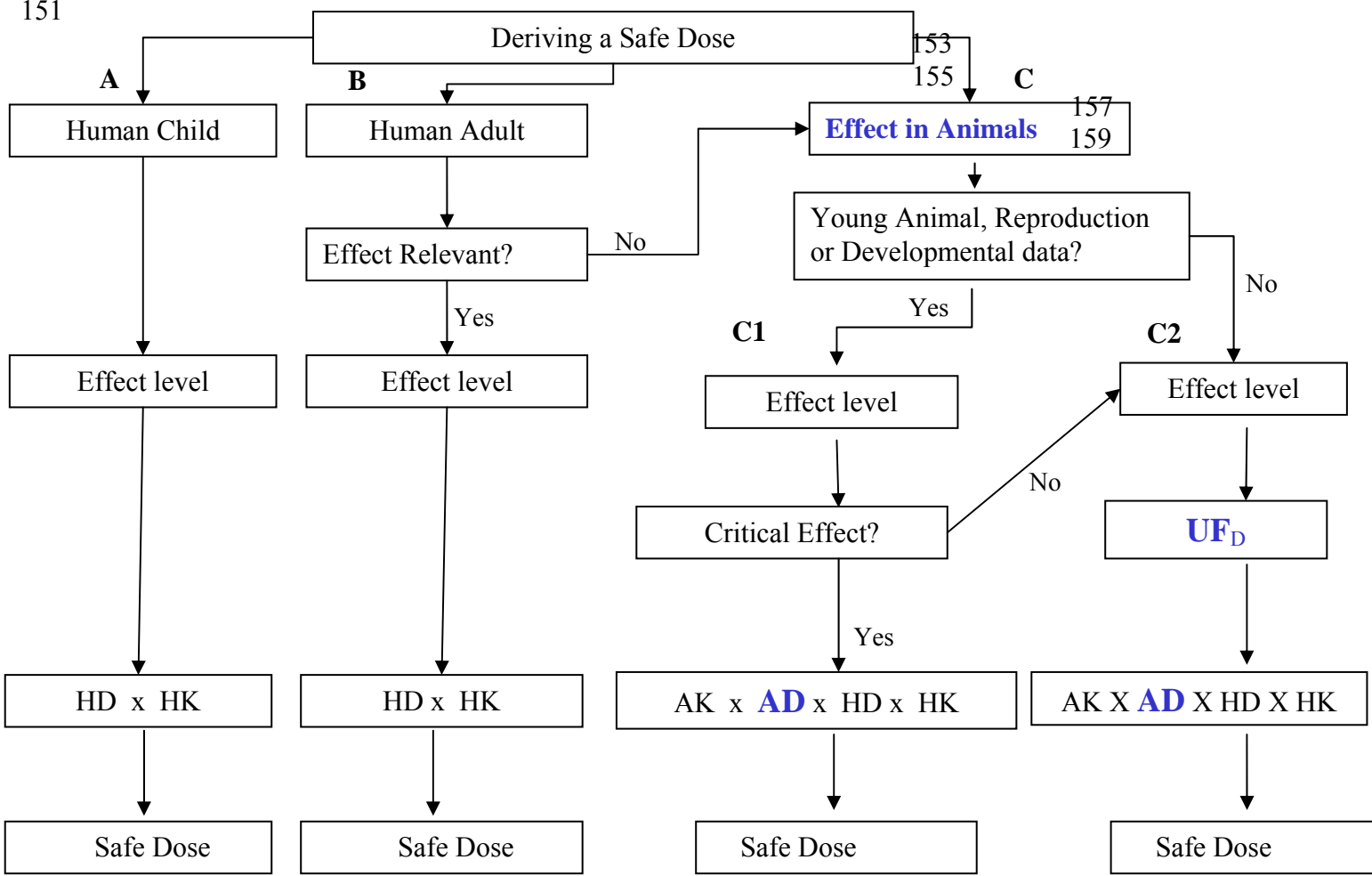
134 **Approach 1** is used when adequate young animal, reproductive, or developmental data exist
135 to suggest these endpoints are more sensitive than other systemic endpoints. In these cases,
136 the critical effect levels for young animal, reproductive, or developmental effects should be
137 adjusted to the extent possible with chemical specific data on toxicokinetics (i.e., AK =
138 experimental animal to human kinetic extrapolation) and toxicodynamics (i.e., AD =
139 experimental animal to human dynamic extrapolation). With these data, an uncertainty factor
140 for database (UF_D) is not needed.¹ (See Figure 1, Column C1.)

141

142 **Approach 2** is used when there are inadequate data to assess young animal, reproductive, or
143 developmental effects, and a systemic toxicity effect level is used. This systemic toxicity
144 level may be adjusted with a UF_D to account for the absence of data on reproductive or
145 developmental effects, as well as for other deficiencies in the database (see Figure 1, Column

¹Note that U.S. EPA often considers it appropriate to apply a database uncertainty factor (UF_D) based on limitations in the database that are not related to reproductive or developmental endpoints. For example, a lack of systemic effects data in a second species may result in use of a UF_D . This consideration would apply to Approaches 2 and 3 as well.

146 **Figure 1. General Outline for Deriving Safe Doses from the Viewpoint of Young Animal,**
 147 **Reproduction, and Developmental Data**
 148
 149
 151



HD = Human Dynamic variability
 HK = Human Kinetic variability
 AK = Experimental Animal Kinetic to human kinetic extrapolation
 AD = Experimental Animal Dynamic to human dynamic extrapolation

160 C2). As in Approach 1, to the extent possible, chemical specific data on toxicokinetics and
161 toxicodynamics should be used in lieu of the default uncertainty factor.
162

163 **Approach 3** is used when there are adequate young animal, reproductive and developmental
164 data that suggest that these endpoints are less sensitive than systemic endpoints and the data
165 do not raise a specific concern regarding young animal, reproduction or development. In this
166 case, the RfD is developed from experimental animals without the use of UF_D to account for
167 reproductive or developmental effects (see Figure 1, Columns C1 and C2). As before, to the
168 extent possible, chemical specific data on toxicokinetics and toxicodynamics should be used
169 in lieu of the default uncertainty factor.
170

171 The underlying bases for risk assessment methods to address the first two approaches above are
172 the focus of the compilations described in Chapters 2 and 3. Together, both updating the basis
173 for the database UF and improving guidance for chemical specific adjustment factors (CSAFs),
174 described by Meek et al. (2002) and the International Programme on Chemical Safety (IPCS
175 2001), will improve the evaluation of risks for developmental, neonatal, child, or reproductive
176 endpoints.
177

178 **1.2. Updating the Scientific Basis for the Database Uncertainty Factor**

179

180 Risk assessors prefer to estimate a “safe” dose using a complete dataset, which is often defined
181 as a reasonably small selection of toxicity studies that covers all life stages. Unfortunately, a
182 complete data set is often unavailable, which leads to a question of whether data from another
183 species, or data from different types of bioassays (such as reproductive or developmental
184 toxicity), would yield a lower critical effect level than what has already been identified in the
185 less-than-complete dataset. A common way to address this uncertainty is by applying an
186 additional uncertainty factor (e.g., the database uncertainty factor, or UF_D).
187

188 For example, U.S. EPA uses the UF_D in the development of a Reference Dose (RfD) to account
189 for one or more missing bioassays out of a complement of five types of studies (i.e., an example
190 of a complete data set). Three of these study types involve the direct testing of younger animals.
191 The initial support for this uncertainty factor is from a study by Dourson et al. (1992) on
192 pesticides. These investigators analyzed ratios of No Observed Adverse Effect Level (NOAEL)
193 or Lowest Observed Adverse Effect Level (LOAEL) among chronic dog, mouse, and rat studies,
194 and reproductive and developmental toxicity studies in rats to identify the potential impact of
195 missing study types. They concluded that several types of bioassays are needed in order to
196 develop a high confidence estimate of an RfD, and that, if one or more bioassay types are
197 missing, such as a developmental toxicity study in younger experimental animals, then an
198 additional uncertainty factor should be used to address this scientific uncertainty.
199

200 Although Dourson et al. (1992) concluded that the quantification on the use of such an
201 uncertainty factor needed additional work, U.S. EPA used these results to support the use of a 3-
202 or 10-fold uncertainty factor when only a few studies from the complement of five types of
203 studies were missing (the factor depends on which of these studies were available), and a 10-fold

204 uncertainty factor when only one study was available². The Dourson et al. analysis evaluated
205 only oral pesticide studies; additional classes of chemicals and other relevant (dermal and
206 inhalation) routes of exposure have not been evaluated.

207
208 In Chapter 2, we expand upon the earlier work that forms the basis for the UF_D. We identified
209 critical adverse effect NOAELs from chronic, reproductive, and developmental studies for more
210 than 150 diverse chemicals from recent authoritative reviews. The distributions of various no
211 effect level ratios (e.g., chronic toxicity rat NOAEL divided by the reproductive toxicity rat
212 NOAEL) are then presented and used to calculate the probability that current default UF_D values
213 of 3- and 10-fold adequately account for missing reproduction or developmental toxicity studies.
214

215 **1.3. Compound Specific Adjustment Factors for Toxicodynamics for Child, Reproduction** 216 **or Development Toxicity**

217
218 In the absence of evidence to the contrary, it is often assumed that effects observed in long-term
219 animal studies, or in reproductive and developmental animal studies are applicable to humans.
220 However, significant anatomical and physiological differences exist among species. The
221 *quantitative* implications of these differences and how to account for them have not yet been
222 translated to applied risk assessment guidance for child, reproductive, and developmental effects.
223 There are difficulties to quantitative extrapolation of dose-response data for these endpoints
224 across species. For example, animal studies often measure different endpoints than are observed
225 in epidemiology studies. For example, the incidence of spontaneous abortion or inability to
226 become pregnant is easily measured in experimental animals but less so in humans. The analysis
227 of *in vitro* studies may be inadequate for child, reproductive or developmental endpoints, which
228 are often dependent on complex endocrine and cellular interactions *in vivo*. These difficulties
229 often leave the risk assessor with the observation that child, reproductive, or developmental
230 endpoints are of concern, but with no adequate approach for estimating quantitatively the
231 toxicodynamic differences in susceptibility among species or the degree of variability within the
232 human population.

233
234 U.S. EPA (1996, 1991) has published hazard identification guidance for reproductive as well as
235 developmental toxicity. These guidelines aid in the interpretation of study results regarding
236 appropriate study designs, relevance to humans of various endpoints, and the adversity of various
237 effects. However, these guidelines do not identify the scientific basis for selecting uncertainty
238 factor values to derive a safe dose. Several independent retrospective analyses have shown that,
239 in general, the default uncertainty factors of 10 each for interspecies differences and human
240 variability used in various “safe” dose methods are adequate (e.g., Kroes, et al., 1993; Dourson et
241 al., 1996; Kalberlah and Schneider, 1998). However, whether or not these default values are
242 adequately protective for reproductive or developmental endpoints has not been systematically
243 investigated.

244

² Note that in the development of an RfD, it is U.S. EPA practice not to derive an RfD if the only study available is a developmental toxicity or a reproductive study that does not include evaluation of systemic toxicity endpoints. This is because, in the experience of U.S. EPA, standard toxicity bioassays often show critical effects at much lower doses than the developmental or reproductive endpoints.

245 The IPCS has developed guidance for deriving chemical specific adjustment factors (CSAFs)
246 when data are available to go beyond the default uncertainty factors (Meek et al., 2002; IPCS,
247 2001). The guidance provides a systematic approach for investigating quantitative differences in
248 toxicokinetics and toxicodynamics. The approach has been used to evaluate toxicokinetic
249 differences with several chemicals with developmental toxicity concerns (e.g., the boron RfD,
250 U.S. EPA, 2005; and Zhao et al., 1999). However, to our knowledge, this CSAF approach has
251 only been applied to toxicodynamic differences between species in one case study, specifically
252 for relative sensitivity to male reproductive effects among species (Mangelsdorf and Buschmann,
253 2002). We are not aware of similar research documentation for female reproductive effects or
254 for developmental toxicity endpoints.

255
256 Therefore, in Chapter 3, we show information that may allow the development of supplemental
257 guidance to the IPCS (2001) CSAF methods for evaluating toxicodynamic uncertainty for
258 reproductive and developmental endpoints. To accomplish this, we present Figures 6 and 7 to
259 address three types of endpoints: fertility, fetal development, and childhood development. These
260 figures guide the risk assessor to potentially comparable animal and human endpoints for use in
261 deriving CSAFs accounting for toxicodynamic differences. The figures contain typical
262 endpoints assessed for reproduction or development in the context of human and animal studies,
263 as well as comparative *in vitro* assays. Each endpoint is rated based on its usefulness in the
264 CSAF process. Limitations of using each of these endpoints for data-derived UF development
265 are also discussed.

266 **1.4. Data Compilation Approach and Meeting Materials for Peer Consultation**

267
268 This report presents two preliminary data collection and analysis efforts to evaluate the basis for
269 the current database uncertainty factor and to develop data for developing CSAFs for
270 experimental animal to human toxicodynamic extrapolation. Both efforts have usefulness for
271 uncertainty factor selection when deriving RfDs that are protective of children.
272
273
274

275 2. Updating the Scientific Basis for the Database Uncertainty Factor

276

277 2.1 Introduction

278

279 As described more fully by Health Canada, IPCS, U.S. EPA, and others (e.g., Meek et al., 1994;
280 IPCS, 1994, 2001; U.S. EPA, 1994; 2002a; Jarabek 1995a,b), uncertainty factors are applied in
281 the development of tolerable intakes or RfD/RfCs when the database is deficient in some aspect.
282 Health Canada and IPCS considers database deficiencies as the lack of data on critical endpoints,
283 lack of chronic data and/or lack of a study that defines a NOAEL (Meek, et al., 1994; IPCS,
284 1994). Others, such as U.S. EPA separate these areas into specific uncertainty factors. For
285 example, EPA applies a specific uncertainty factor for database (UF_D) that is intended to account
286 for the inability of any single study to adequately address all potential endpoints at various life
287 stages. As a rough guide, U.S. EPA uses the following choices of the database uncertainty factor
288 with databases of different confidence:

- 289
- 290 • With a database of high confidence, a 1-fold factor is generally used
 - 291 • With a database of low confidence, a 10-fold factor is generally used
 - 292 • With a database that falls in between high and low confidence, a 3-fold factor is generally used

293 Numerous examples of the database uncertainty factor can be found on the Integrated Risk
294 Information System (IRIS) (U.S. EPA, 2005). As more fully discussed later, research by U.S.
295 EPA and others gives some quantitative support to these choices (Dourson et al., 1992; Evans
296 and Baird, 1998).

297

298 The rationale for the minimum database for either high or low confidence RfDs/RfCs is also
299 provided in a number of texts (e.g., U.S. EPA, 1994; Haber et al., 2001). U.S. EPA (2002a)
300 describes this rationale from the viewpoint of minimum and robust data sets. In the absence of
301 adequate human data, U.S. EPA and others generally considers a "complete" database,³ that is,
302 complete for the purpose of calculating a chronic RfD/RfC for noncancer health effects, to be
303 composed as follows:

- 304
- 305 • two adequate⁴ mammalian subchronic or chronic toxicity studies by the appropriate route
in different species,

³ Generally, the presence of a "complete" database indicates that the acquisition of additional toxicity data is unlikely to result in a change to the RfD or RfC. Scientists at U.S. EPA typically consider such RfDs and RfCs to be "high confidence", reflecting the likely stability of the value to additional data. U.S. EPA considers a single, well-conducted, subchronic mammalian bioassay by the appropriate route as a minimum database for estimating an RfD or RfC. However, for such a limited database, the likelihood that additional toxicity data may change the value of the RfD or RfC is higher, and the associated confidence in the RfD/RfC is lower. Due to the conservatism inherent in the uncertainty factor approach, the acquisition of additional data often results in higher RfDs and RfCs (i.e., results in the conclusion that higher exposures are "safe"). For more details please see U.S. EPA (1994, 2002a) or Dourson (1994). Examples of confidence statements for RfDs and RfCs can be found in U.S. EPA's online IRIS database (www.epa.gov/iris).

⁴As determined by professional judgment. Typically, studies should have been adequately conducted and published in refereed journals, or be unpublished reports that adhered to Good Laboratory Practice (GLP) guidelines and have undergone final QA/QC (U.S. EPA, 1994). U.S. EPA (1998) and others have published guidelines in this area.

- 306 • one adequate mammalian multi-generation reproductive toxicity study by an appropriate
307 route, and
- 308 • two adequate mammalian developmental toxicity studies by an appropriate route in
309 different species.

310 This choice of studies reflects the fact that all life stages are covered by these tests. However,
311 gaps in the “complete database” may still exist because systemic effects are not fully evaluated
312 in old animals in subchronic studies or in weanling to young adult animals in a standard two-
313 generation study. Life stages typically covered by various studies are shown in Figure 2. This
314 choice of studies is also indirectly supported by work of Clegg (1978), FDA (Shibko, 1981),
315 Heywood (1981, 1983), Olson et al. (2000) and U.S. EPA (2002b). However, as more fully
316 discussed later this database may not be judged complete, if the observation of certain types of
317 toxicity (e.g., neurotoxicity) suggests the need for specialized tests. In contrast, this selection of
318 studies may not be fully needed if sufficient information on the critical effect in a sensitive
319 subgroup of humans is available.

320
321 For an oral safe dose, the chronic toxicity studies would generally be via the oral route, although
322 extrapolation from the inhalation route may occur. For an inhalation safe concentration, the
323 chronic toxicity studies should be via the inhalation route, with adequate evaluation of portal of
324 entry (respiratory) effects. Route-to-route extrapolation from oral data may be conducted, but
325 the conditions on route to route extrapolation described by U.S. EPA (1994) should be satisfied.
326 Data from other environmentally relevant routes can sometimes be used to satisfy the
327 requirements for developmental and multigenerational reproductive toxicity studies. For
328 example, if the critical effect for an RfC occurs outside the respiratory tract (i.e., is systemic),
329 and oral data show that developmental effects occur at doses much higher than the oral critical
330 effect, the oral developmental data can be used to satisfy the need for inhalation developmental
331 studies.

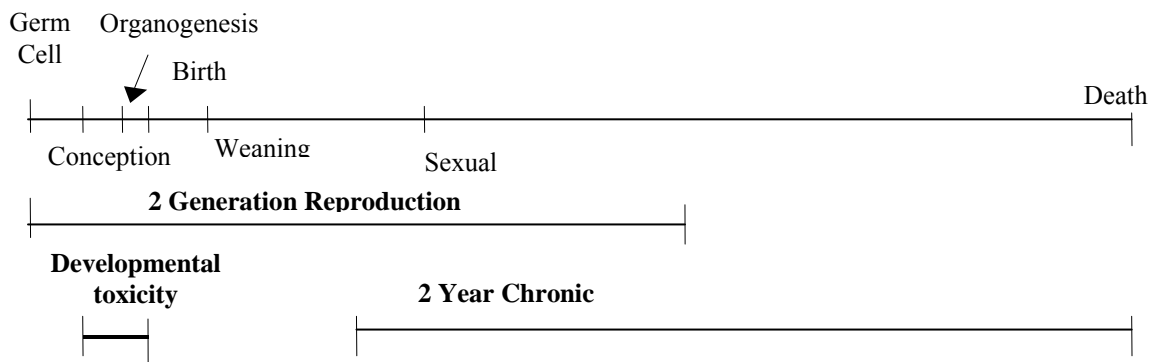
332 333 **2.2 Basis for the Current Practice⁵** 334

335 The specific number and types of toxicity tests used for safety assessment vary considerably
336 across regulatory programs. The registration requirements of different countries for substances
337 developed for specific biological activity, such as food use pesticides, are most stringent, and can
338 include many distinct mammalian toxicity tests. Similar requirements apply to pharmaceutical
339 agents. Other assessment approaches, such as the Organization for Economic Cooperation and
340 Development (OECD, 1997) Screening Information Data Set (SIDS) process, follow a tiered
341 approach, in which a base set of toxicity studies is evaluated initially and, depending upon the
342 results, the need for additional studies is determined. Similarly, the U.S. EPA’s approach for
343 evaluating “inert” ingredients in pesticide formulations consists of a base set of tiered toxicity
344 studies and guidelines for interpretation of results that lead to the triggering of more extensive
345 toxicity studies.

346
347 In the determination of “safe” doses from animal studies, few investigators have discussed or agreed
348 upon what comprises the necessary types of data (see, for example, early work of Clegg, 1978).
349 However, U.S. EPA has used an uncertainty factor, based in part on earlier work of FDA (Shibko,

⁵ Please note that much of this text for this section is taken from the publication of Dourson et al. (2002).

Figure 2. Animal lifespan in relationship to the timeline of existing toxicity tests (time frames are not to scale)

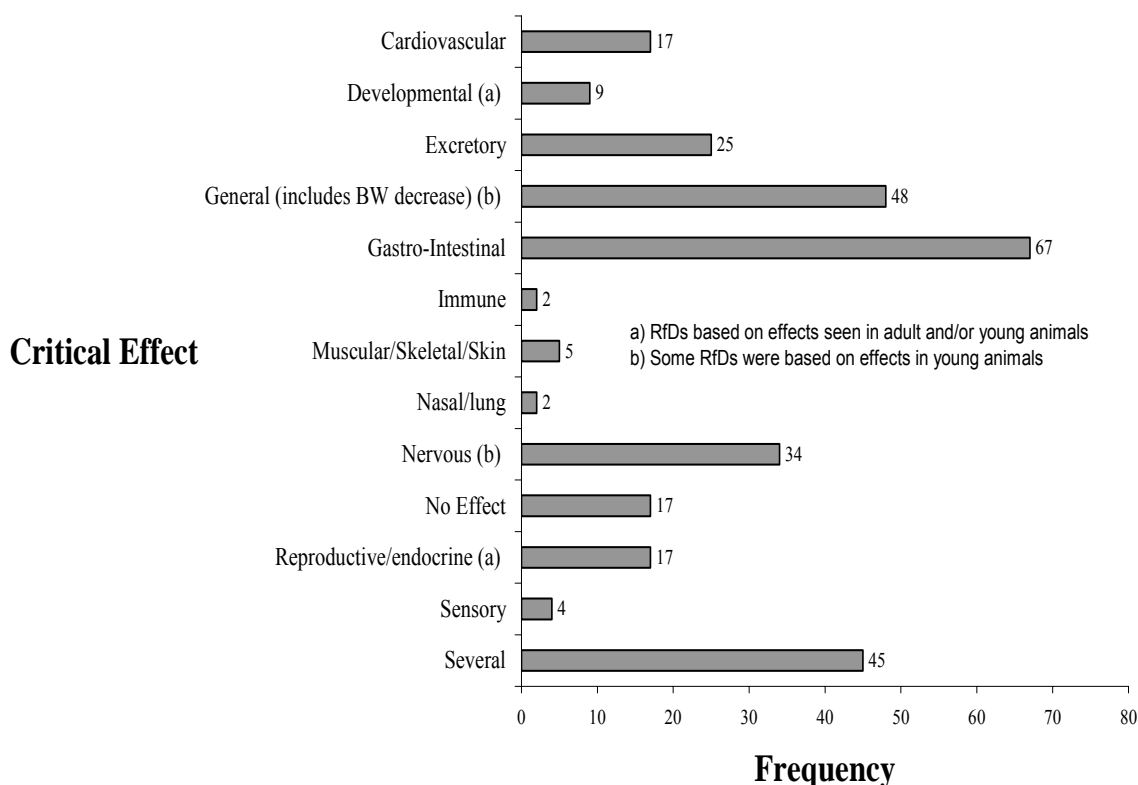


350
 351
 352 1981), to estimate safe exposure levels in the absence of adequate data from multiple toxicity studies
 353 (Barnes and Dourson, 1988). This factor is referred to as UF_D (Dourson, 1994; U.S. EPA, 2002a).
 354 U.S. EPA considers this factor necessary because of the inability of any one study to adequately test
 355 different species or different life stages of the same species. Other groups, such as Health Canada
 356 (Meek et al., 1994) and IPCS (1994), also consider deficiencies in this area as relevant to the
 357 establishment of a “safe” dose although the designation of a specific factor for this area is generally
 358 not done. All groups have found that the receipt of missing studies often yields a different critical
 359 effect and a lower NOAEL, and the use of this factor by all groups is based on the assumption that
 360 the critical effect can be discovered in a reasonably small selection of toxicity studies. In the context
 361 of setting safe exposure limits that protect children, evaluating the adequacy of and need for UF_D has
 362 become important because of concerns that incomplete toxicity testing will fail to identify effects
 363 relevant to children’s health.

364
 365 Initial attempts to understand how different toxicity studies identified the critical effect for safe
 366 exposure limits naturally focused on the frequency of different critical effects in the
 367 determination of such limits (see, for example, Figure 3). Such evaluations included systemic
 368 toxicity in laboratory animals through acute, short-term, subchronic, and chronic studies;
 369 specialized testing, such as evaluations of developmental toxicity, reproductive toxicity,
 370 immunotoxicity, and neurotoxicity; and toxicokinetic and toxicodynamic evaluations. If available,
 371 all of these studies are used to characterize a chemical's spectrum of potential human toxicity by
 372 identifying target organs and the dose ranges associated with adverse effects in laboratory animals
 373 of different life stages.⁶

⁶ *In vitro* data can be used to elucidate potential mechanisms of biological activity, to evaluate the relevance to humans of the endpoint observed in laboratory animals, to improve extrapolation from laboratory animals to humans, and to characterize intrahuman variability. Assessment of laboratory animal data should include an evaluation of the reliability of the experimental design and toxicological interpretation of the results. Moreover, once a critical effect and likely mode of action have been identified, results from the various studies should be

Figure 3. Distribution of Critical Effects for 292 RfD's on EPA's IRIS as of 7/1/95



374
375

376 Unfortunately, a problem with these initial evaluations is quickly evident. Quite simply, the
377 databases for many chemicals lack a sufficient number of studies that evaluate different
378 endpoints and life stages. Thus, the results in Figure 3, which show that ~9% of all U.S. EPA
379 RfDs are based on reproductive or developmental toxicity studies, do not give much assurance
380 that this percentage represents an accurate estimate of the number of times these effects might
381 serve as the basis of exposure limits if complete chemical-specific databases were more widely
382 available. Moreover, not every database includes functional neurological and immunological
383 bioassays, although systemic toxicity studies nearly always monitor these endpoints at a
384 histological level.

385

386 U.S. EPA conducted further work on the impact of missing data in developing RfDs, including data
387 for different life stages, and this research directly relates to evaluations of the need for and magnitude
388 of UF_D (Dourson et al., 1992). For example, data for 69 pesticides were analyzed and frequency
389 histograms of log₁₀ NOAEL ratios were developed for chronic dog, mouse, and rat toxicity studies

examined collectively to determine if a causal relationship is likely to exist between a chemical exposure and the hypothetical human effect. Species-specific differences in sensitivity to a chemical due to differing metabolism, physiology, or anatomy, also should be considered.

390 and for rat reproductive and developmental toxicity studies (see Figure 4).⁷ These pesticides were
391 selected because of the availability of many different types of toxicity studies on both adult and
392 young animals, including the full set of studies required for a high confidence RfD. On average,
393 chronic rat and dog studies, generally conducted on young adult to older animals, yielded similar
394 NOAELs. Reproductive and developmental toxicity studies, conducted on both adult and young
395 animals, were less likely to produce the lowest NOAELs when compared to the chronic rat and dog
396 studies. Chronic mouse studies, generally conducted on young adult to adult animals, were least
397 likely to yield the lowest NOAEL when compared to the chronic rat and dog studies, and thus only
398 occasionally resulted in the determination of a critical effect. The authors concluded that several
399 bioassays are needed in order to develop a high confidence estimate for an RfD and, if one or more
400 bioassays is missing (which is often the case when developing RfDs and other “safe” doses), then a
401 factor such as UF_D could be supported quantitatively. Specifically, when chronic rat and dog studies
402 are available but rat reproductive and rat developmental toxicity studies are missing, a UF_D of 3
403 applied to the lower of the chronic rat or dog NOAEL accounts for ~92% of the possible occurrences
404 of lower NOAELs being identified by the missing bioassays that include younger animals. A UF_D of
405 10 accounts for 98% of such occurrences.⁸ Therefore, the routine use of UF_D by U.S. EPA as shown
406 earlier to compensate for the lack of certain bioassays already addresses, in large part, the uncertainty
407 associated with the absence of specific studies, including studies that test younger animals.
408

409 Evans and Baird (1998) presented two approaches for estimating the quantitative value of UF_D
410 using a subset of studies on pesticides identified by Dourson et al. (1992), discussed above. One
411 method, based on regression analysis, provided a point estimate of UF_D. The other method,
412 based on non-parametric analysis, provided a distributional estimate of UF_D. In both cases, the
413 choice of UF_D depended on the definition of a complete database (see U.S. EPA’s definition
414 described above), the number of missing bioassays, and the specific bioassay missing.
415

416 Brand et al. investigated the usefulness of ratios of benchmark doses for performing
417 extrapolations. This approach does not account for several sources of error, including finite
418 sampling error. The authors concluded that the distributions of the ratios can potentially include
419 large errors, particularly in the estimated spread of the distribution, due to small sample sizes,
420 poorly spaced dose levels and imperfect dose centering, and other contextual factors (e.g., dose-
421 response shape or feeding protocol). They recommend these errors be accounted for in the
422 modeling where possible, and application of boot-strap techniques to estimate the precision of
423 the estimated ratios.
424

425 Based in part on the analysis of Dourson et al. (1992) of pesticides and published criteria for causal
426 significance of Hill (1965), U.S. EPA routinely uses UF_D to determine RfDs in cases where certain
427 bioassays are missing, including when studies that test younger animals are missing. This use allows
428 U.S. EPA to confidently develop RfDs for many compounds without the full complement of toxicity
429 tests. As described previously, U.S. EPA generally considers a “complete” database to be comprised

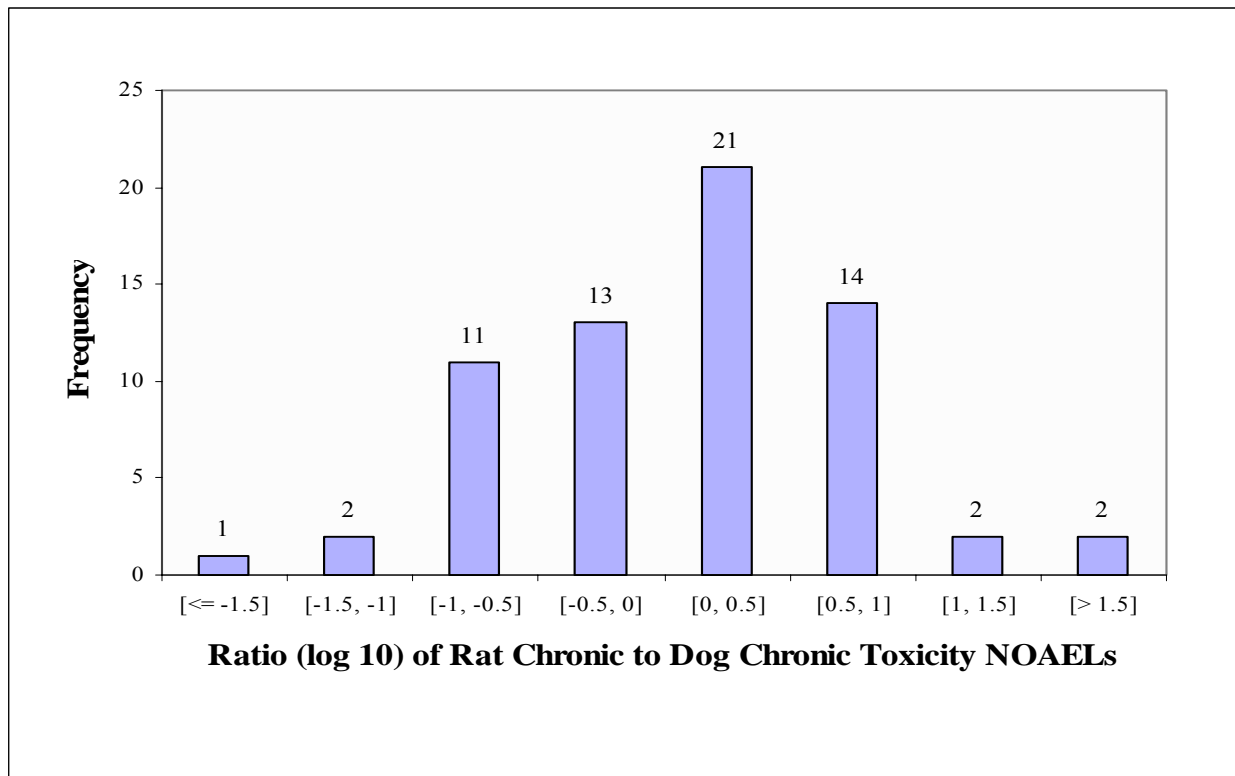
⁷ Log₁₀ ratios were used for ease of plotting the resulting large range of values.

⁸ The specific comparison made is found in Dourson et al. (1992), Table 6, line 18. The values of 0.08 at 10^{0.5} and 0.02 at 10^{1.0} are the probabilities that either the rat reproductive or rat developmental toxicity study NOAELs are lower than the corresponding NOAELs for either the chronic dog or rat bioassays. Thus, the chronic bioassay NOAELs, when divided by an uncertainty factor of 3 (10^{0.5}) or 10 (10^{1.0}), protect against either 92% or 98% of the potentially lower NOAELs that could be identified by bioassays that include younger animals, respectively.

430 **Figure 4. Frequency Histograms of the Logs (base 10) of the Ratios of NOAELs**

431

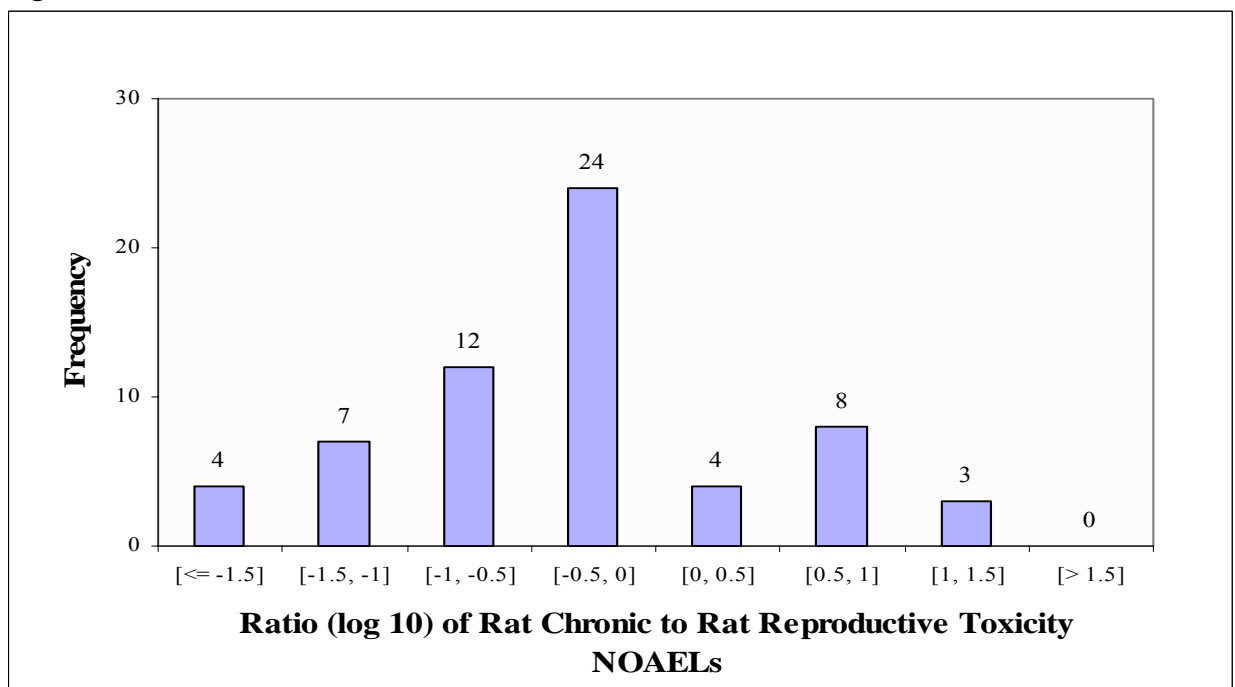
432 **Figure 4A**



433

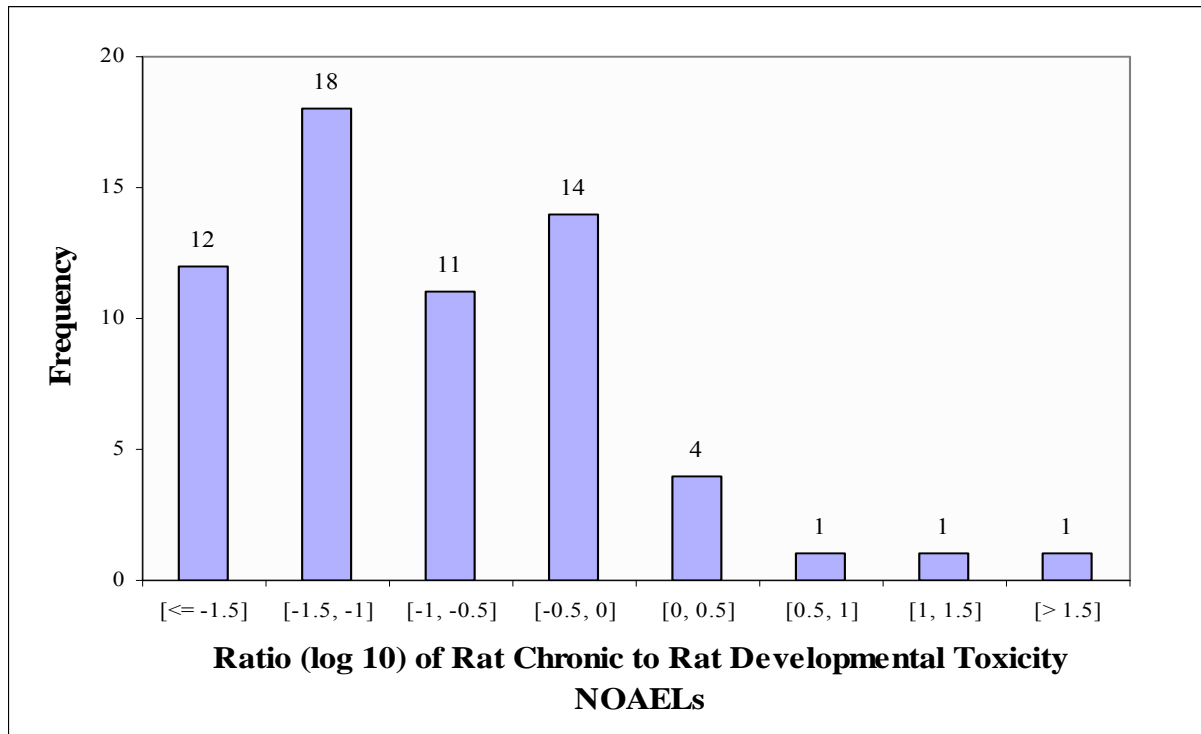
434

435 **Figure 4B**



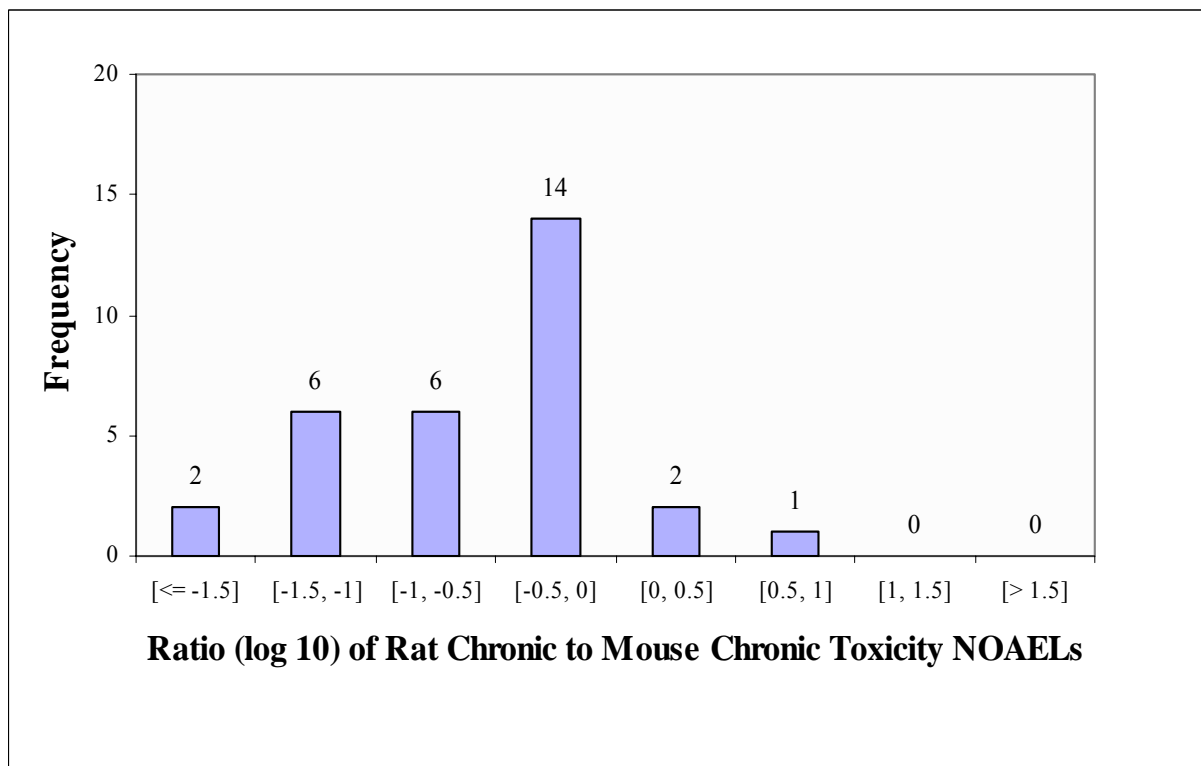
436

437 Figure 4C



438
439
440
441

Figure 4D



442

443 of two adequate mammalian chronic toxicity studies, one adequate mammalian multi-generation
444 reproductive toxicity study and two adequate mammalian developmental toxicity studies by the
445 appropriate route of exposure in different species.

446
447 This series of tests is considered complete because most of the animals' life stages will have
448 been investigated (again, please see Figure 2). The judgment of a "complete" database is
449 somewhat chemical-specific, however; the observation of certain types of toxicity in short-term
450 tests may suggest the need for specialized tests not included in the general definition of a
451 complete database.

452
453 For example, Makris et al. (1998) investigated the usefulness of developmental neurotoxicity
454 (DNT) tests as part of this database by comparing DNT NOAELs to NOAELs derived from
455 other types of toxicity tests. They found that for 9 of the pesticides investigated, 8 DNT
456 NOAELs were lower than developmental toxicity NOAELs, 6 were lower than reproductive
457 toxicity NOAELs, and 6 were lower than or approximately equal to neurotoxicity NOAELs.
458 However, DNT NOAELs were between 1.3 and 93-fold higher than the NOAELs used as the
459 basis of the lifetime RfDs for 7 of these same pesticides. For the remaining two pesticides, DNT
460 NOAELs were 70 and 90 percent of the chronic NOAELs. The mean ratio between DNT and
461 chronic NOAELs was 25-fold, suggesting that DNT NOAELs are generally much less sensitive
462 than chronic NOAELs. U.S. EPA scientists are currently analyzing additional data in a
463 continuation of this investigation (Makris, 2005).

464
465 A peer review of Makris et al. (1998) concluded, however, that either maternal toxicity or
466 developmental toxicity generally occur at comparable or lower dose levels than developmental
467 neurotoxicity (SAP, 1999). This peer review showed that for 10 of the 12 substances evaluated,
468 either the maternal toxicity NOAEL or the developmental toxicity NOAEL was the same as or
469 less than the DNT NOAEL. In only one case was the DNT NOAEL less than either the maternal
470 toxicity NOAEL or the developmental toxicity NOAEL, and in this case the effect reported for
471 the DNT NOAEL was questioned by the peer review. With respect to the applicability and
472 sensitivity of the DNT study, the majority of the peer review panel strongly indicated that the
473 DNT study was not more sensitive than either the developmental study or the reproductive study.

474
475 A recent study by Middaugh et al. (2003) lends some support to the critique of Makris et al.
476 (1998) by the SAP (1999). Middaugh et al. (2003) described the results of a survey of studies on
477 174 compounds, primarily pharmaceuticals, to evaluate the contribution of F1 neurobehavioral
478 testing to hazard identification. Although such testing had less of an effect than general
479 toxicology parameters, it contributed solely to defining the NOEL of the critical effect
480 approximately 3% of the time. It was the co-critical effect 15% of the time.

481
482 Thus, while the DNT study may (or may not) be more sensitive in some cases than other
483 specialized studies, its overall contribution to the determination of a lifetime RfD is likely to be
484 minimal, because it is generally not as sensitive as chronic bioassays. Its use in the development
485 of acute or other less than lifetime RfDs is perhaps more likely, because in these situations,
486 lifetime studies are seldom used. U.S. EPA is continuing its investigation of the DNT studies
487 and their usefulness for risk assessment determinations.

488

489 Based on available information to date then, an uncertainty factor of 3 or 10 commonly used by
490 U.S. EPA for varying degrees of data base incompleteness seems appropriate and more than
491 adequate when there is inadequate information for developmental effects, reproductive effects, or
492 developmental neurotoxicity.⁹ This conclusion is based on a fair number of pesticides, but could
493 be enhanced with a review of the data on other types of chemicals.

494
495 The purpose of the following compilation is to conduct this enhancement.

496 497 **2.3 Criteria Used in Selecting Chemicals for Analysis**

498
499 The toxicity data for 154 chemicals (comprising pesticides, metals, volatile organic compounds,
500 etc.) were obtained from the U.S. EPA's Integrated Information System (IRIS) (U.S. EPA, 2005)
501 and the Agency for Toxic Substances and Disease Registry (ATSDR, 2004) as shown in
502 Appendix A. Chemicals were selected because of availability of many different studies on both
503 adult and young animals. In order to be included in the analysis, the database for a particular
504 chemical had to include chronic toxicity studies in at least one species (rat, dog, or mouse), a
505 reproductive toxicity study in the rat, and a developmental toxicity study in at least one species
506 (rat or rabbit). The chronic toxicity studies were conducted for a significant period of the life
507 span (1- to 2-year rat, ½- to 2-year dog, or 1- to 2-year mouse). The reproductive studies were
508 single or multiple generation studies that evaluated effects in the parents and offspring. Only
509 studies rated at least as "core grade minimum," but not "core grade supplementary,"¹⁰ in the IRIS
510 summary for the chemical were included. Furthermore, studies that reported
511 acetylcholinesterase activity inhibition as critical effect were excluded since this inhibition is
512 often regarded only as an indicator of adverse effects. For reproductive and developmental
513 studies, the young animal NOAELs were assigned based only on reproductive and
514 developmental endpoints – not systemic effects or maternal endpoints. No subchronic toxicity
515 studies were included in this analysis because the NOAELs from these studies are generally
516 higher than those found in the chronic bioassays. While several of these studies identified both a
517 NOAEL and a LOAEL, our compilation mainly focused on the availability of the NOAELs. A
518 majority of the studies were based on the oral route of exposure (with dose levels expressed as
519 mg/kg-day), with a few based on inhalation exposure (expressed in terms of mg/m³ or ppm).

520 521 **2.4 Data Compilation**

522
523 Chronic toxicity data were compared to reproductive and/or developmental toxicity data to
524 determine the relative sensitivity of the bioassays. The comparison was made through the use of
525 log₁₀ ratios of the available NOAELs from rat, mouse, and dog chronic bioassays, and rat
526 reproductive, rat developmental, and rabbit developmental bioassays. Ratios were calculated, for
527 example, for rat chronic to rat reproductive, rat chronic to rat developmental, rat chronic to rabbit

⁹ In the case of specific information on these endpoints, the choice of NOAEL or LOAEL of the critical effect becomes more definitive. For example, when such endpoints are the critical effect, then the lifetime RfD is based on their NOAEL, even though the study is of shorter duration.

¹⁰ Core grade is the system U.S. EPA formerly used to indicate the extent to which a study conformed to published test guidelines. A "core grade minimum study" was considered sufficient for risk assessment and indicated that the study came close to, or met, test guideline requirements. A "core grade supplementary" was considered to provide useful supplementary information, but not suitable for risk assessment on its own.

528 developmental, and rat chronic to rat and rabbit developmental. Similar ratios were also
529 calculated for combinations of multiple NOAELs.

530
531 Calculations were then made directly from these data to determine the probabilities that the \log_{10}
532 ratios were greater than a particular value, such as $10^{0.5}$ and $10^{1.0}$. These values are directly
533 comparable to possible values of the database uncertainty factor, UF_D . For example, a value of
534 $10^{0.5}$ would correspond to an UF of 3. These probabilities were then used to estimate the percent
535 of the time that one or more NOAELs are greater than another or multiple NOAELs by a given
536 factor. These estimates are useful in determining the relative sensitivity of the bioassays.
537 Specifically, the frequency with which a given bioassay might provide a lower NOAEL value
538 (and thus be more sensitive) can be estimated. Moreover, these estimates can be used to
539 determine the probabilities that different values of UF_D account for missing reproductive and/or
540 developmental studies in the estimation of RfDs within the framework of U.S. EPA's current
541 procedure.

542
543 Based on a complete data set as defined by the U.S. EPA [i.e., one comprising two chronic
544 mammalian bioassays (e.g., rat and mouse), a mammalian reproductive study (e.g., rat), and
545 developmental toxicity studies in two mammalian species (e.g., rat and rabbit)], it is possible to
546 estimate the probability that missing a reproductive or developmental toxicity study would
547 provide a lower NOAEL if only a rat chronic bioassay, for example, is available. This analysis,
548 therefore, provides an estimate of how often the most sensitive endpoint would be missed in the
549 absence of reproductive or developmental studies, and directly relates to the value of UF_D .

550 551 **2.5 Results**

552
553 Figure 5 shows frequency histograms of the logs (base 10) of the ratios of NOAELs calculated
554 from chronic rat to rat reproductive (Figure 5A), chronic rat to rat developmental (Figure 5B),
555 chronic rat to the lower of rat reproductive and rat developmental (Figure 5C), chronic rat to
556 lower of rat reproductive and rabbit developmental (Figure 5D), and chronic rat to the lowest of
557 rat reproductive and rat or rabbit developmental bioassays (Figure 5E). The development of
558 other figures is certainly possible. All raw data used in these analyzes are found in Appendix A.
559 Other figures are shown in Appendix B.

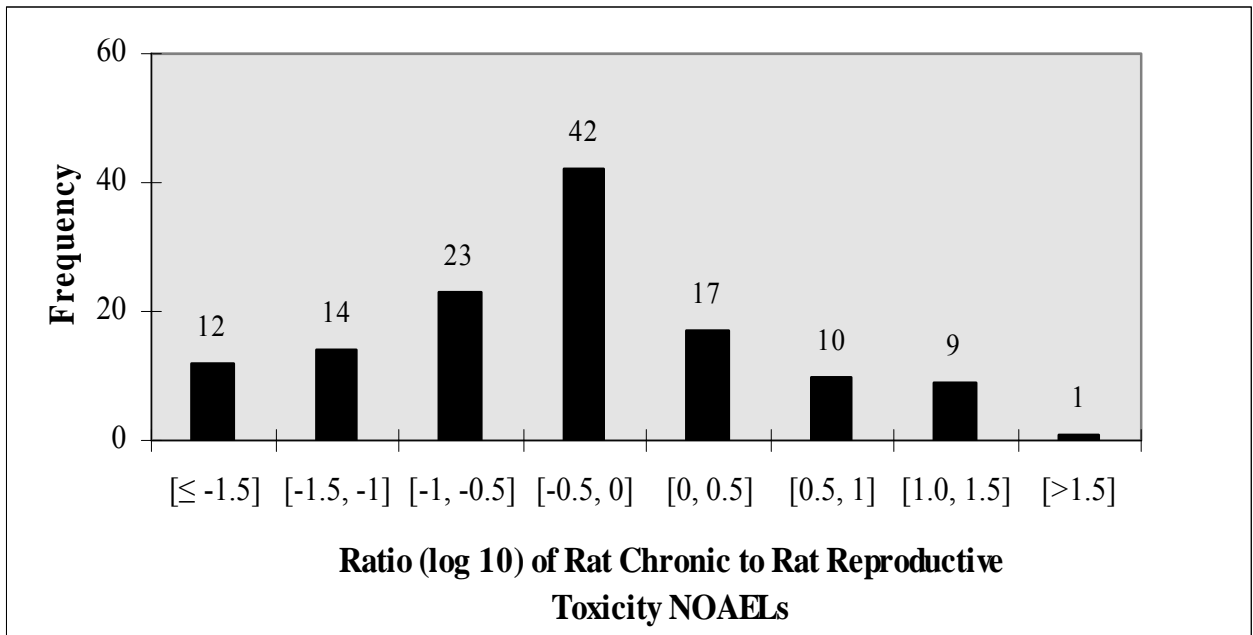
560
561 Note that the values on the horizontal axis of Figure 5 correspond to the values of k , which
562 directly relate to the value of UF_D . For example, Figure 5A shows that for 128 chemicals for
563 which rat chronic and rat reproductive toxicity NOAELs were available, the probability that the
564 rat chronic NOAEL is greater than the rat reproductive NOAEL by a factor of $10^{1.0}$ or greater is
565 $10/128$ (~0.08). This probability is calculated by taking the frequency of the data points to the
566 right of 10^1 . The probability that the rat chronic NOAEL is equal to or less than the rat
567 reproductive NOAEL is $91/128$ (0.71). This probability is calculated by taking the frequency of
568 the data points at or to the left of 10^0 . These results suggest that rat chronic NOAELs are lower
569 than reproductive NOAELs, in general, indicating that chronic bioassays are more sensitive than
570 the reproductive bioassays. Of course, other probabilities are possible from this table. The two
571 given here are only for illustration.

572

573 **Figure 5. Frequency Histograms of the Logs (base 10) of the Ratios of NOAELs**

574

575 Figure 5A

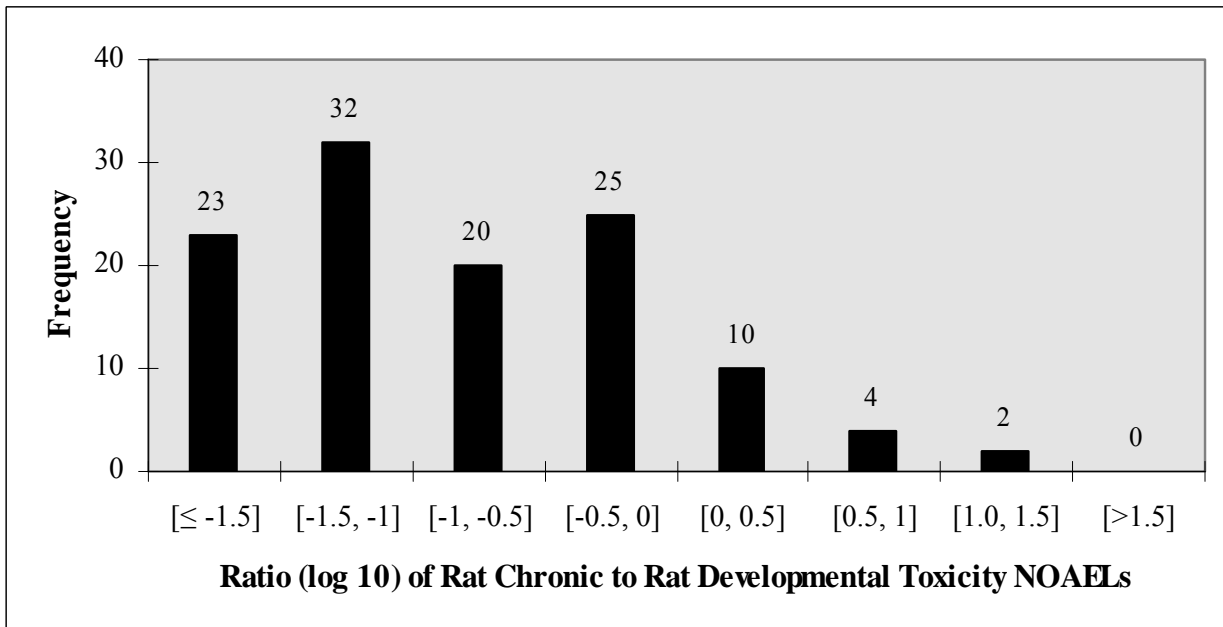


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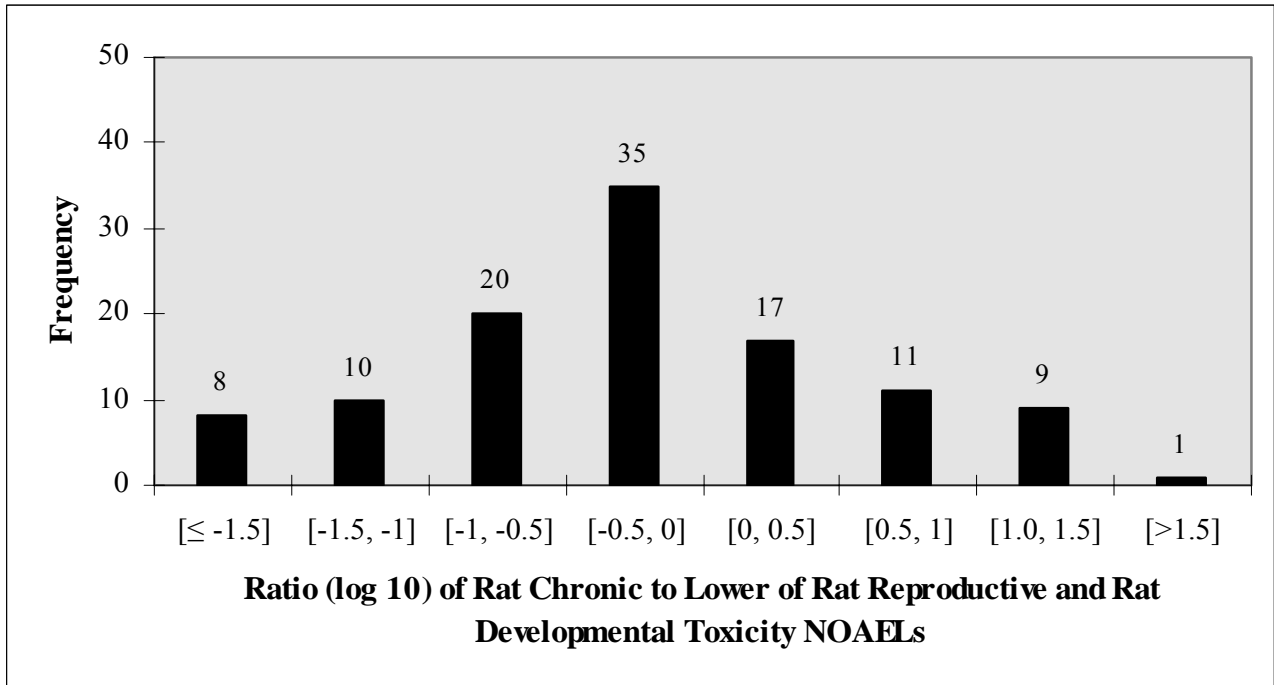
579 Figure 5B



580

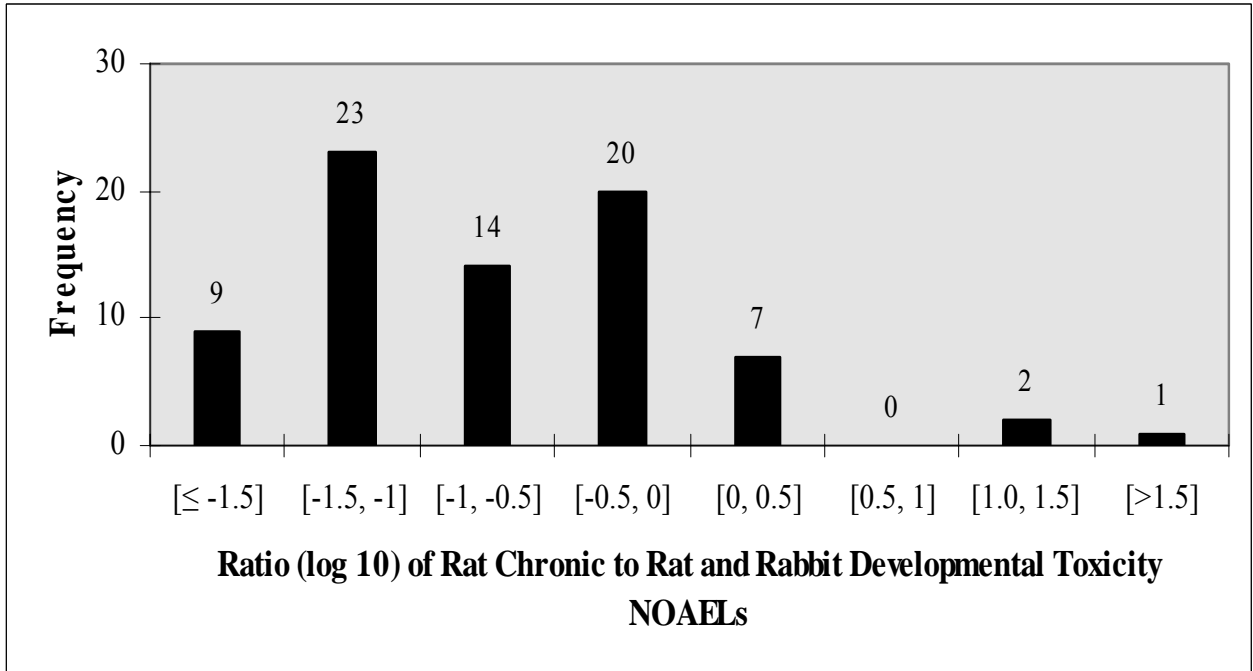
581

582 Figure 5C

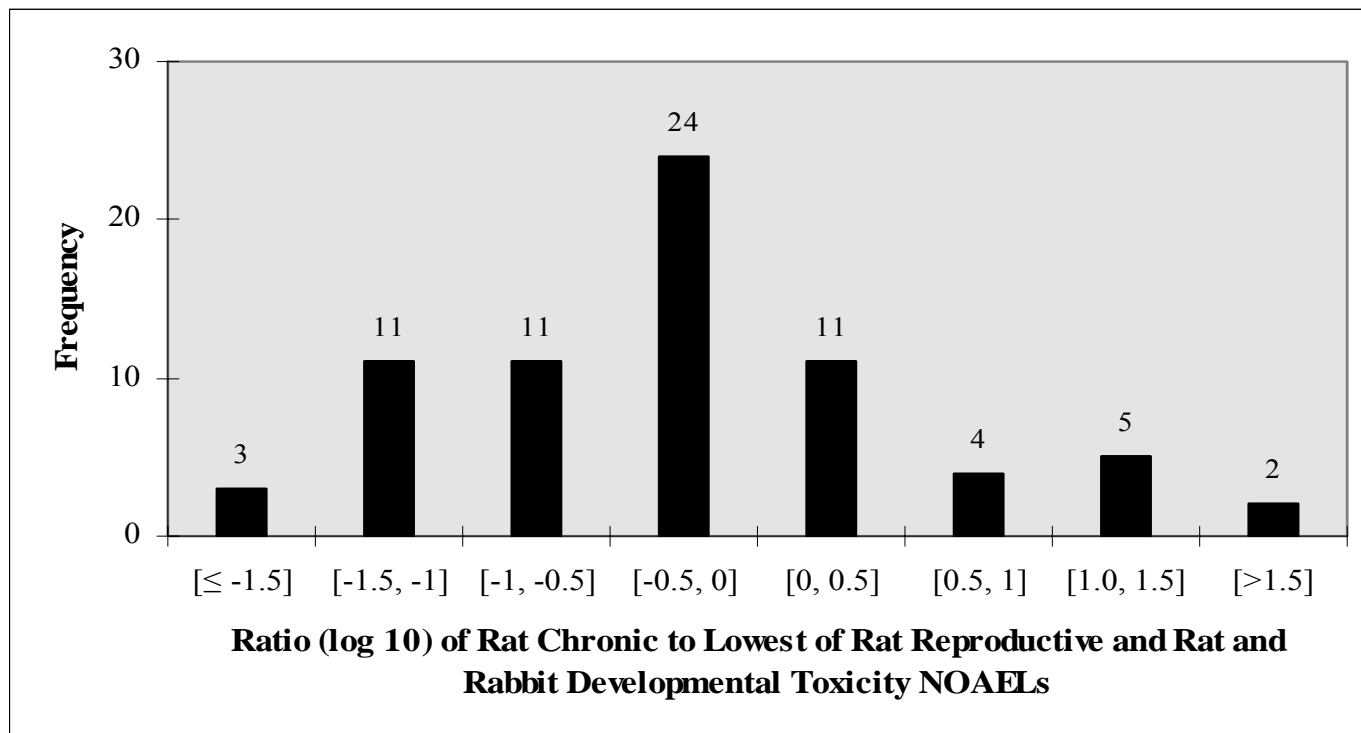


583
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585
586

Figure 5D



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588
589
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591



593
594

595 Based on Figure 5, the impact of missing a reproductive or developmental toxicity study or both
596 in developing RfDs is shown in Tables 1-6. In each table, the first column of UFs is U.S. EPA's
597 judgment based in part on the Dourson et al. (1992) analysis.¹¹ The next column shows the
598 probability of the default UF_D values adequately accounting for missing these toxicity studies
599 based on the distribution of the various NOAEL ratios from this current analysis. The last
600 column of UFs shows UF_D values, calculated from the present analysis, that are needed to
601 adequately cover the 95th percentile¹² of the distribution.

602

603 For 70 chemicals analyzed in rats, results indicated that when only the chronic rat study is
604 available, but a second chronic bioassay, a rat reproductive bioassay, and rat and rabbit
605 developmental toxicity studies are missing (the last 3 assays include younger animals),
606 application of the default UF_D of 10 will only account for 89% of the possible occurrences of
607 lower NOAELs (Table 1). The analysis shows that a UF_D of 20 accounts for 95% of such
608 occurrences. When only rat chronic and rabbit developmental NOAELs are available, UF_D of 10
609 will account for 92% of the possible occurrences that the missing second chronic bioassay, rat
610 reproductive and rat developmental NOAELs will provide a lower NOAEL. A UF_D of 17 will
611 account for 95% of such occurrences. However, when chronic rat, rat reproductive, and either

¹¹ These judgments were of U.S. EPA's RfD/RfC Work Group from 1985 to 1995.

¹² The 95th percentile of the distribution was calculated using Excel Spreadsheet and was based on the transformed NOAEL ratios. However, no differences in ratios were observed when the percentile was calculated using the untransformed ratios.

612 **Table 1. Impact of Missing Second Species, Reproductive and/or Developmental Toxicity Study and Adequacy of Default**
 613 **Database Uncertainty Factor Based on Toxicity Studies on 70 Chemicals in the Rat*, #**
 614

NOAELs Available	Missing NOAELs	EPA Default UF _D Needed to Address Data Gap	Percent of Chemicals Covered by Default UF _D	UF _D Needed To Cover 95 th Percentile
Rat Chronic	1 chronic, 1 reproductive, and 2 developmental	10	89	20
Rat Chronic and Rat Reproductive	1 chronic and 2 developmental	3	99	2
Rat Chronic and Rat Developmental	1 chronic, 1 reproductive, and 1 developmental	10	93	13
Rat Chronic and Rabbit Developmental	1 chronic, 1 reproductive, and 1 developmental	10	92	17
Rat Chronic, Rat Reproductive, and Rat Developmental	1 chronic and 1 developmental	3	99	1
Rat Chronic, Rat Reproductive, and Rabbit Developmental	1 chronic and 1 developmental	3	100	1
Rat Chronic, Rat Developmental, and Rabbit Developmental	1 chronic and 1 reproductive	10	94	10

615 *NOAEL ratios were calculated from “complete” database; *i.e.*, for each chemical in the database, there were NOAELs for the chronic, reproductive, and two
 616 developmental toxicity studies.

617 #UF_D values greater than the default were highlighted in bold.

618
 619
 620
 621

622 rat or rabbit developmental NOAELs are available, the default UF_D of 3 will account for $\geq 99\%$
623 of the possible occurrences of lower NOAELs identified from the missing second chronic
624 bioassay, rat or rabbit developmental bioassays. Our analysis suggests that a UF_D of 1 is needed
625 to account for 95% of such occurrences.

626
627 Table 2 shows results for NOAEL ratios for 45 chemicals studied in mice. The results show that
628 the default UF_D of 10 that is applied when only a chronic mouse NOAEL is available, will only
629 account for 84% of the possible occurrences of a lower NOAEL being identified from the
630 missing second chronic bioassay, rat reproductive bioassay, and rat and rabbit developmental
631 studies. A UF_D of 23 is needed to account for 95% of such occurrences. Similar to rats, a
632 default UF_D of 3 accounts for 100% of possible occurrences of identifying a lower NOAEL from
633 a missing second chronic bioassay, and rat or rabbit developmental bioassay ; a UF_D of 1
634 accounts for 95% of such occurrences.

635
636 Fifty-nine chemicals were analyzed for the dog (Table 3). A default UF_D of 3 when only a dog
637 chronic NOAEL¹³ is available will account for 93% of the possible occurrences of missing a
638 second chronic bioassay and reproductive and developmental studies. In order to account for
639 95% of such occurrences, a UF_D of 14 is needed. Availability of a chronic dog NOAEL and rat
640 reproductive NOAEL accounts for 97% of the possible occurrences of a lower NOAEL being
641 identified from the missing second chronic bioassay and rat and rabbit developmental studies.
642 The analysis suggested that a UF_D of 1 is adequate to account for 95% of such occurrences. A
643 UF_D of 1 instead of the default value of 3 is needed when rat reproductive NOAEL and one
644 developmental NOAEL are available in addition to the dog chronic NOAEL.

645
646 Table 4 shows for 41 chemicals that when only chronic rat and mouse NOAELs are available,
647 the default UF_D of 3 will account for 93% of the possible occurrences of a lower NOAEL being
648 identified from the missing rat reproductive and rat and rabbit developmental bioassays. Our
649 results suggested a UF_D of 13 to account for 95% of such occurrences. The default UF_D of 3 that
650 is applied when only the rat reproductive bioassay is missing from the dataset will account for
651 98% of the possible occurrences of a lower NOAEL being identified from the missing
652 reproductive bioassay, compared to the 95% coverage for the calculated UF_D of 2. When the rat
653 reproductive NOAEL and one developmental NOAEL are missing, the default UF_D of 3
654 accounts for $\geq 95\%$ of the possible occurrences of identifying a lower NOAEL from the missing
655 reproductive and developmental studies. For these chemicals, when only one or more of the
656 developmental NOAELs are missing, the default value of 1 is adequate to account for $>95\%$ of
657 the possible occurrences of a lower NOAEL being identified from the missing developmental
658 studies.

659
660 Table 5 shows that for 52 chemicals, when chronic rat and dog NOAELs are available, the
661 default UF_D of 3 will only account for 88% of the possible occurrences of a lower NOAEL being
662 identified from the missing rat reproductive and rat and rabbit developmental bioassays. The
663 results show that a UF_D of 13 is needed to account for 95% of such occurrences. When only the
664 rat reproductive bioassay is missing, *i.e.* chronic bioassays are available in the rat and dog in
665 addition to developmental bioassays in two species, a UF_D of 8 will account for 95% of the

¹³ For purposes of this analysis, a chronic dog NOAEL is defined as one in excess of 6 months to 1 year in duration, consistent with the definition used by U.S. EPA's OPP.

666 **Table 2. Impact of Missing Second Species, Reproductive and/or Developmental Toxicity Study and Adequacy of Default**
 667 **Database Uncertainty Factor Based on Toxicity Studies on 45 Chemicals in the Mouse*, #**
 668

NOAELs Available	Missing NOAELs	EPA Default UF _D Needed to Address Data Gap	Percent of Chemicals Covered by Default UF _D	UF _D Needed To Cover 95 th Percentile
Mouse Chronic	1 chronic, 1 reproductive, and 2 developmental	10	84	23
Mouse Chronic and Rat Reproductive	1 chronic and 2 developmental	3	100	1
Mouse Chronic and Rat Developmental	1 chronic, 1 reproductive, and 1 developmental	10	96	9
Mouse Chronic and Rabbit Developmental	1 chronic, 1 reproductive, and 1 developmental	10	91	13
Mouse Chronic, Rat Reproductive, and Rat Developmental	1 chronic and 1 developmental	3	100	1
Mouse Chronic, Rat Reproductive, and Rabbit Developmental	1 chronic and 1 developmental	3	100	1
Mouse Chronic, Rat Developmental, and Rabbit Developmental	1 chronic and 1 reproductive	10	96	9

669 *NOAEL ratios were calculated from “complete” database; *i.e.*, for each chemical in the database, there were NOAELs for the chronic, reproductive, and two
 670 developmental toxicity studies.

671 #UF_D values greater than the default are highlighted in bold.
 672
 673

674 **Table 3. Impact of Missing Second Species, Reproductive and/or Developmental Toxicity Study and Adequacy of Default**
 675 **Database Uncertainty Factor Based on Toxicity Studies on 59 Chemicals in the Dog*, #**
 676

NOAELs Available	Missing NOAELs	EPA Default UF _D Needed to Address Data Gap	Percent of Chemicals Covered by Default UF _D	UF _D Needed To Cover 95 th Percentile
Dog Chronic	1 chronic, 1 reproductive, and 2 developmental	10	93	14
Dog Chronic and Rat Reproductive	1 chronic and 2 developmental	3	97	1
Dog Chronic and Rat Developmental	1 chronic, 1 reproductive, and 1 developmental	10	95	10
Dog Chronic and Rabbit Developmental	1 chronic, 1 reproductive, and 1 developmental	10	95	10
Dog Chronic, Rat Reproductive, and Rat Developmental	1 chronic and 1 developmental	3	97	1
Dog Chronic, Rat Reproductive, and Rabbit Developmental	1 chronic and 1 developmental	3	100	1
Dog Chronic, Rat Developmental, and Rabbit Developmental	1 chronic and 1 reproductive	10	97	10

677 *NOAEL ratios were calculated from “complete” database; *i.e.*, for each chemical in the database, there were NOAELs for the chronic, reproductive, and two
 678 developmental toxicity studies.

679 #UF_D values greater than the default are highlighted in bold.
 680
 681
 682
 683

684 **Table 4. Impact of Missing Reproductive and/or Developmental Toxicity Study and Adequacy of Default Database**
 685 **Uncertainty Factor Based on Toxicity Studies on 41 Chemicals in Both Rat and Mouse^{*, #}**
 686

NOAELs Available	Missing NOAELs	EPA Default UF _D Needed to Address Data Gap	Percent of Chemicals Covered by Default UF _D	UF _D Needed To Cover 95 th Percentile
Rat and Mouse Chronic	1 reproductive and 2 developmental	3	93	13
Rat and Mouse Chronic and Rat Reproductive	2 developmental	1	97	1**
Rat and Mouse Chronic and Rat Developmental	1 reproductive and 1 developmental	3	100	2
Rat and Mouse Chronic and Rabbit Developmental	1 reproductive and 1 developmental	3	95	3
Rat and Mouse Chronic, Rat Reproductive, and Rat Developmental	1 developmental	1	98	1**
Rat and Mouse Chronic, Rat Reproductive, and Rabbit Developmental	1 developmental	1	100	1**
Rat and Mouse Chronic, Rat Developmental, and Rabbit Developmental	1 reproductive	3	98	2

687 *NOAEL ratios were calculated from complete database; *i.e.*, for each chemical in the database, there were NOAELs for the chronic, reproductive, and two
 688 developmental toxicity studies.

689 #UF_D values greater than the default are highlighted in bold.
 690
 691
 692
 693
 694
 695
 696

697 **Table 5. Impact of Missing Reproductive and/or Developmental Toxicity Study and Adequacy of Default Database**
 698 **Uncertainty Factor Based on Toxicity Studies on 52 Chemicals in Both Rat and Dog^{*, #}**
 699

NOAELs Available	Missing NOAELs	EPA Default UF _D Needed to Address Data Gap	Percent of Chemicals Covered by Default UF _D	UF _D Needed To Cover 95 th Percentile
Rat and Dog Chronic	1 Reproductive and 2 Developmental	3	88	13
Rat and Dog Chronic and Rat Reproductive	2 Developmental	1	98	1**
Rat and Dog Chronic and Rat Developmental	1 Reproductive and 1 Developmental	3	90	10
Rat and Dog Chronic and Rabbit Developmental	1 Reproductive and 1 Developmental	3	90	10
Rat and Dog Chronic, Rat Reproductive, and Rat Developmental	1 Developmental	1	98	1**
Rat and Dog Chronic, Rat Reproductive, and Rabbit Developmental	1 Developmental	1	98	1**
Rat and Dog Chronic, Rat Developmental, and Rabbit Developmental	1 Reproductive	3	92	8

700 *NOAEL ratios were calculated from complete database; *i.e.*, for each chemical in the database, there were NOAELs for the chronic, reproductive, and two
 701 developmental toxicity studies.

702 **NOAEL ratio is less than 1 (*i.e.*, available data listed cover the critical effect in all tested cases).
 703
 704
 705
 706
 707
 708
 709

710 possible occurrences of a lower NOAEL being identified from the missing reproductive bioassay,
711 compared to 92% of such occurrences when the default UF_D of 3 is applied. The default UF_D of 3 is
712 associated with 90% coverage when a sole developmental NOAEL is available in addition to the rat
713 and dog chronic NOAELs, compared to the calculated value of 10. The results show that a default
714 UF_D of 1 is consistent with >95% coverage when a reproductive NOAEL is available in addition to
715 the chronic bioassays or when chronic bioassays in 2 species are available in addition to the rat
716 reproductive and one developmental bioassays.

717
718 Thirty-four chemicals were analyzed in both dog and mouse (Table 6). The table shows that the
719 default UF_D of 3 accounts for 91% of the occurrence of a lower NOAEL being identified from
720 missing reproductive and developmental studies. To account for 95% of such occurrences, a
721 UF_D of 11 must be applied. A UF_D of 5 is needed for 95% coverage when only a rat
722 developmental study is available in addition to the dog and mouse chronic studies whereas a
723 value of 11 is needed for 95% coverage when only a rabbit developmental study is available in
724 addition to the chronic studies from these two species. The results also show that whether one or
725 more developmental NOAELs are missing, the default UF_D of 1 accounts for 100% of the
726 occurrence of a lower NOAEL being identified from the missing developmental NOAEL(s)
727 when chronic studies in the dog and mouse and a rat reproductive NOAEL are available.

728 729 **2.6 Discussion**

730
731 Health Canada, IPCS, U.S. EPA and other groups' risk assessments include the use of uncertainty
732 factors when identifying "safe" doses for limiting chemical exposures. Those factors are designed to
733 account for differences in susceptibility within and among species and to compensate for limited data
734 availability, when necessary. Proposals have been made to use an additional 10-fold uncertainty
735 factor for the extra protection of children when estimating safe exposure limits from a database that is
736 inadequate to determine whether children are more sensitive to a chemical's toxicity than are adults.
737 Use of such an additional uncertainty factor, as is presently stated by the Food Quality Protection Act
738 (FQPA) for pesticide safety evaluations, is meant to address the same issues already addressed by
739 U.S. EPA's database uncertainty factor, UF_D, as well as additional issues related to exposure
740 uncertainty. U.S. EPA states that the use of the FQPA factor should be modified when UF_D has
741 already been used (U.S. EPA, 2002b; Fenner-Crisp, 2001).

742
743 Table 7 shows the summary of the impact of missing studies that include testing in young
744 experimental animals when only two mammalian chronic bioassays are available. When chronic
745 studies in two species are available, but rat reproductive and developmental bioassays in two
746 species are missing, the default UF_D of 3 accounts for 88-93% of the occurrence of a lower
747 NOAEL being identified from the missing bioassays. This indicates that the default UF_D is not
748 adequately protective of the missing studies NOAELs at the 95th percentile of the distribution in
749 any case. This compilation also shows that, depending on the available two species, a UF_D of
750 11-13, instead of the default UF_D of 3, is more appropriate if 95% coverage is desired. If all
751 three chronic bioassays are available, however, then an uncertainty factor of 6 would cover 95%
752 of the missing NOAELS.

753
754 From Tables 4 through 6, when chronic bioassays in 2 species are available along with either one
755 or two of the remaining studies that include testing in young experimental animals, the

756 **Table 6. Impact of Missing Reproductive and/or Developmental Toxicity Study and Adequacy of Default Database**
 757 **Uncertainty Factor Based on Toxicity Studies on 34 Chemicals in Both Dog and Mouse^{*, #}**
 758

NOAELs Available	Missing NOAELs	EPA Default UF _D Needed to Address Data Gap	Percent of Chemicals Covered by Default UF _D	UF _D Needed To Cover 95 th Percentile
Dog and Mouse Chronic	1 Reproductive and 2 Developmental	3	91	11
Dog and Mouse Chronic and Rat Reproductive	2 Developmental	1	100	1**
Dog and Mouse Chronic and Rat Developmental	1 Reproductive and 1 Developmental	3	91	5
Dog and Mouse Chronic and Rabbit Developmental	1 Reproductive and 1 Developmental	3	91	11
Dog and Mouse Chronic, Rat Reproductive, and Rat Developmental	1 Developmental	1	100	1**
Dog and Mouse Chronic, Rat Reproductive, and Rabbit Developmental	1 Developmental	1	100	1**
Dog and Mouse Chronic, Rat Developmental, and Rabbit Developmental	1 Reproductive	3	94	4

759 *NOAEL ratios were calculated from complete database; *i.e.*, for each chemical in the database, there were NOAELs for the chronic, reproductive, and two
 760 developmental toxicity studies.

761 **NOAEL ratio is less than 1 (*i.e.*, available data listed cover the critical effect in all tested cases).
 762
 763

764 **Table 7. Impact of Missing Reproductive and/or Developmental Toxicity Study and Adequacy of Default Database**
 765 **Uncertainty Factor when Chronic Toxicity Studies Are Available for Rats, Mice, and/or Dogs. *, #**
 766

NOAELs Available	Missing NOAELs	Number of Chemicals Analyzed	EPA Default UF _D Needed to Address Data Gap	Percent of Chemicals Covered by Default UF _D	UF _D Needed To Cover 95 th Percentile
Rat and Dog Chronic	1 Reproductive and 2 Developmental	53	3	88	13
Rat and Mouse Chronic	1 Reproductive and 2 Developmental	41	3	93	13
Dog and Mouse Chronic	1 Reproductive and 2 Developmental	34	3	91	11
Rat, Mouse, and Dog Chronic	1 Reproductive and 2 Developmental	31	3	87	6

767 *NOAEL ratios were calculated from complete database; *i.e.*, for each chemical in the database, there were NOAELs for the chronic, reproductive, and two
 768 developmental toxicity studies.

769 #UF_D values greater than the default are highlighted in bold.
 770
 771
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 773

774 uncertainty factor for 95% coverage is greater than the current U.S. EPA default value of 3 for 9
775 out of 12 comparisons (75%), is less than the default value in 2 out of 12 cases (~17%), and is
776 equal to the default value in 1 out of 12 cases (~8%). These results suggest that the current
777 default UF_D of 3 is less often protective than overprotective. These tables also show that when
778 chronic bioassays in 2 species plus a rat reproductive bioassay are available, but one or both
779 developmental bioassays are missing, the uncertainty factor for 95% coverage is less than or
780 equal to the current U.S. EPA default value of 1 in all 9 cases (*i.e.*, 100% of the time), indicating
781 that the default value of 1 is sufficient. It, therefore, appears that availability of NOAELs from
782 two chronic studies and a rat reproductive study will adequately account for $\geq 97\%$ of the
783 possible occurrences of a lower NOAEL being identified from the missing rat and/or rabbit
784 developmental bioassay.

785
786 From Tables 1 through 3, when a chronic bioassay in 1 species is available and one or more of
787 the remaining studies that include testing in young experimental animals are missing in addition
788 to a chronic bioassay in a second species, the uncertainty factor for 95% coverage is greater than
789 the current U.S. EPA default value of 10 for 6 out of 12 comparisons (50%), is equal to the
790 default value in 4 out of 12 cases (~33%), and is less than the default in 2 out of 12 (~17%).
791 This indicates that the default value of 10 in this situation is about as unprotective as it is
792 protective. These tables also show that the uncertainty factor for 95% coverage is less than the
793 current U.S. EPA default value of 3 for all cases analyzed (9 out of 9; *i.e.*, 100% of the time)
794 when one chronic and rat reproductive bioassays are available and when one or none of the
795 developmental bioassays are missing.

796
797 Taken together, the present compilation conducted on a broader range of chemicals indicates that
798 a UF_D to provide 95% coverage of missing NOAELs would vary depending on the availability of
799 other bioassays and that rat reproductive bioassay is important in developing “safe” doses. The
800 analysis also confirms the earlier conclusions that an effect from a developmental study is only
801 occasionally the critical effect, but also that such studies yield useful information that is
802 important in any dose response assessment. Limitations of this analysis include the fact that only
803 NOAEL ratios of available studies were used, whereas benchmark dose (BMD) ratios would
804 have resulted in better defined differences. In addition, studies were used “as is,” and, thus for
805 example, 2-generation reproductive studies were included that may not have adequately tested
806 for the critical effect in rat “children,” thereby making comparisons between chronic bioassays
807 and 2-generation reproductive studies somewhat less likely to show large differences. In
808 contrast, maternal and young animal NOAELs from developmental toxicity and reproductive
809 toxicity studies were not distinguished, thereby making comparisons with chronic bioassays
810 more likely to show differences due to younger animal response.

811 812 **2.7 Future Steps**

813
814 Health Canada, IPCS, U.S. EPA and others commonly use an uncertainty factor for varying
815 degrees of data base incompleteness. When there are inadequate data on developmental effects,
816 reproductive effects, or a second species bioassay, U.S. EPA calls out a specific factor of either 3
817 or 10. As this conclusion is based on a fair number of pesticides, we compiled information on
818 other types and a larger number of chemicals. Based on the present compilation, the default
819 uncertainty factor of 10 does not appear to be consistent with 95% coverage when reproductive

820 and developmental NOAELs are missing and a chronic NOAEL is available in only one species.
821 Instead, values of 14, 20, and 23 are needed for such coverage based on analysis of studies
822 conducted in the dog, rat, and mouse, respectively. A factor of 1-3 is needed when a rat
823 reproductive NOAEL is available in addition to one chronic NOAEL. When a chronic NOAEL
824 (in rat, mouse, or dog), rat reproductive NOAEL, and one developmental NOAEL are available,
825 a UF_D of 1 ensures 95% coverage. However, when only two chronic NOAELs are available, a
826 UF_D value of 11-13, but not the default UF_D value of 3, provides 95% coverage.

827
828 Taken together, the present analysis suggests that the basis for the default UF_D values of 1, 3, and
829 10 might need to consider the experimental animal species of the systemic toxicity study prior to
830 the selection of a specific value.

831
832 Possible additional work that might be considered to extend this compilation includes

- 833
- 834 • Conducting similar analyses for additional classes of chemicals (e.g., those with acetyl
835 cholinesterase inhibition as the critical effect). Analyses of various chemical subclasses
836 also could be developed.
 - 837 • Conducting a similar analysis for inhalation exposures. This could be done by review of
838 inhalation dose-response assessment decisions from ATSDR (e.g., see *ITER*), U.S. EPA
839 (e.g., see IRIS) and Health Canada (e.g., see *ITER*). In addition, route-to-route
840 extrapolation could be attempted in which the estimated systemic dose resulting from
841 chronic inhalation studies could be compared with the estimated systemic dose in the
842 reproductive and developmental studies
 - 843 • Enhancing the present analysis using other possible approaches (e.g., Evans and Baird,
844 1998) to inform the decision to update the default UF_D values.

845 **3. Supplemental Guidance to the IPCS CSAF Methodology for Evaluating**
846 **Toxicodynamic Uncertainty for Reproductive and Developmental Endpoints**

847
848 **3.1 Introduction**

849
850 Within the International Programme on Chemical Safety (IPCS), Chemical-Specific Adjustment
851 Factors (CSAFs) are used as enhancements to the traditional uncertainty factors to account for
852 the variations between animals and humans or within human populations (Meek et al., 2002;
853 IPCS, 2001). As proposed by IPCS (2001), a subdivision of the 10-fold interspecies factor into
854 toxicokinetic¹⁴ and toxicodynamic¹⁵ components allows part of the traditional default uncertainty
855 factor to be replaced by relevant, chemical-specific data when they are available, thereby
856 advancing the scientific basis for dose-response characterization and the resulting acceptable or
857 tolerable reference intakes or concentrations. Part of the IPCS (2001) framework is designed to
858 provide guidance on developing CSAFs for toxicodynamic variations between animals and
859 humans (see Figure 1). We use this part of the framework to focus on endpoints related to the
860 developing fetus and/or young children.

861
862 As indicated by IPCS (2001), the adjustment factors for interspecies toxicodynamic aspects are
863 commonly based on results of *in vitro* studies using animal and human tissue. For most of
864 noncancer toxicity response, when the mechanism of causing the toxic effect in the target organ
865 has been identified, the dose that causes the toxic effect in 10% of animal target tissue (e.g.,
866 ED10) from an *in vitro* study can be compared to the ED10 for the same target tissue from
867 humans that is estimated from an *in vitro* study in order to estimate the interspecies
868 toxicodynamic variations. However, such data are usually not available for reproductive or
869 developmental endpoints, since no *in vitro* model or cell culture exists that can mimic the whole
870 process of reproduction and fetal development. Most of the data available on reproductive and
871 developmental toxicity come from *in vivo* studies, especially from experimental animal studies.
872 If there are adequate *in vivo* data in humans, the measure of dose-response would generally be
873 used directly and there would be no need to extrapolate from *in vivo* animal data using an
874 interspecies adjustment factor. However, analysis of combined *in vivo* dose/concentration-
875 response data (which reflect toxicokinetics and toxicodynamics) and toxicokinetic data by a
876 kinetic-dynamic link model is relevant to the development of a toxicodynamic adjustment factor;
877 this factor could then be modified based on quantitative differences between the animals in the

¹⁴ Toxicokinetics is the process of the uptake of potentially toxic substances by the body, the biotransformation they undergo, the distribution of these substances and their metabolites in the tissues, and their elimination from the body. Toxicokinetic data provide quantitative information about the active form of either the parent compound or its metabolites at the target tissue or organs. Such data on the comparative absorption, distribution, metabolism, and excretion of the potentially toxic substances in experimental animals and humans are increasingly available as a basis for definition of plasma and tissue toxicokinetics and, therefore, permit quantifying the variability between animals and humans in the internal or target organ dose.

¹⁵ Toxicodynamics is the process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects, and toxicodynamic data address any of the whole range of steps from molecular interaction up to the effect at the target site. CSAFs for interspecies variability may be derived from comparative response data for the toxic effect itself in the target organ or for a point in the chain of events that is considered critical to the toxic response based on understanding of mode of action. Hence, CSAFs could be derived from *in vitro* studies, from *in vivo* studies in which the toxicokinetic component has been delineated, or from *ex vivo* experimentation.

878 kinetic-dynamic study and the humans. This is particularly important because in reality, it is
879 very rare to have adequate *in vitro* human data on reproductive and developmental effects to
880 define the toxicodynamic difference between developing animals and humans.

881
882 The goal of this work is to identify examples where the concept of CSAFs for toxicodynamic
883 differences can be applied for reproductive and developmental endpoints. These examples will
884 be used to discuss the application of the IPCS CSAF framework to reproductive or
885 developmental toxicity endpoints. To the extent possible, a list of parameters that can be used to
886 develop quantitative estimates for intraspecies and interspecies differences in susceptibility will
887 be proposed. Difficulties and complications with using various types of endpoints will also be
888 included in the discussion.

889 **3.2 Methods for Reproductive and Developmental Response**

891
892 Developmental toxicity, defined in a wide sense to include any adverse effect on normal
893 development either before or after birth, has become of increasing concern in recent years.
894 Developmental toxicity can result from exposure of either parent prior to conception, from
895 exposure of the embryo or fetus *in utero* or from exposure of the progeny after birth. Developing
896 fetuses and children consist of unique human populations, which are sometimes significantly
897 different from average healthy adults. Throughout the entire life cycle, all aspects of
898 reproductive function are dependent on various endocrine communicating systems that employ a
899 wide variety of protein/peptide and steroid hormones, growth factors and other signaling
900 molecules that affect target cell gene expression and/or protein synthesis. This finely tuned
901 system of coordinated signals leads to the formation of gametes, their transport, release,
902 fertilization, implantation and gestation, and, ultimately, the development of offspring which are
903 eventually capable of successfully repeating the entire process under similar or different
904 environmental conditions.

905
906 Reproductive function, as a part of a continuum of reproductive and developmental processes,
907 directly relates to development of the fetus and child. Disorders of reproduction in humans are
908 included, but are not limited to, the following areas: onset of puberty, gamete production and
909 transport, impotence, menstrual disorders, sexual behavior, fertility, spontaneous abortion,
910 parturition, birth weight, lactation and other developmental (including heritable) defects,
911 premature reproductive senescence, and various genetic diseases affecting the reproductive
912 system and offspring.

913
914 The occurrence of adverse effects on the developing organism may result from exposure prior to
915 conception (either parent), during prenatal development, or postnatally to the time of sexual
916 maturation. However, adverse developmental effects may be detected at any point in the lifespan
917 of the organism. The major manifestations of developmental toxicity include death of the
918 developing organism, structural abnormality, altered growth, and functional deficiency (U.S.
919 EPA, 1991). Therefore, an understanding of mode of action and the mechanism of toxicity is
920 critical to the success of conducting a quantitative evaluation of interspecies toxicodynamic
921 difference because the knowledge of mode of action will aid in identifying the appropriate study
922 as well as the endpoint to be used in the analysis.

923

924 *In vitro* studies on developmental and reproductive toxicity are limited by many factors. *In vitro*
925 studies of the toxic response or a surrogate for the toxic endpoint in animal and human tissues
926 could provide relevant toxicodynamic data as a basis for development of the interspecies
927 toxicodynamic adjustment factor. Such data will define target site sensitivity directly, without
928 any toxicokinetic influences. However, most *in vitro* systems involve an interruption in normal
929 metabolism and the biological interrelationships found in the intact system (e.g., finely tuned
930 hormone regulation system in mother); therefore, the range of developmental effects that can be
931 produced and the power of the study to detect an effect are compromised as compared to those
932 obtained using standard study designs in whole animal systems. For these reasons, *in vitro*
933 developmental toxicity assays are rarely available for risk assessment purposes when there is no
934 prior knowledge about the potential for developmental toxicity.

935
936 A variety of *in vitro* test systems, including isolated perfused testis/ovary, primary cultures of
937 gonadal cells, investigation of subcellular fractions of different organs and cell types and *in vitro*
938 fertilization techniques, are available that can be used in supplementary investigational studies of
939 different aspects of the reproductive system. *In vitro* testing systems are especially useful for
940 screening for toxicity potential and for identifying potential mechanisms of action of potential
941 toxicants. However, these tests are limited in their ability to assess complex, integrative
942 reproductive functions; thus the use of data from these studies for risk assessment purposes is
943 limited. Again, the information on the mode of action will provide guidance in identifying the
944 appropriate *in vitro* study suitable to quantitative comparison of toxicodynamics.

945
946 Although both reproductive function and fetal or child development can be described as a
947 continuum of reproductive process, they have their own unique characteristics. For example,
948 evaluation of reproductive toxicity usually involves observations from mature male and female
949 animals or humans while the evaluation of developmental toxicity usually involves responses in
950 fetuses or children. For ease of discussion, the reproductive and developmental endpoints are
951 considered separately in this framework. The reproductive endpoints were grouped as sexual
952 behavioral responses and male- and female-specific reproductive responses. The developmental
953 endpoints were divided into maternal responses, fetal developmental responses, and post
954 parturition responses. Figures 6 and 7 describe the applicability of these responses within the
955 IPCS CSAF framework

956

957 **3.3 Results for Reproductive Endpoints**

958 *3.3.1 Male Specific Reproductive Response*

959 Due to the unique structure of male reproductive organs and continuing spermatogenesis, male
960 reproductive function is relative easy to examine. For example, sperm samples are readily
961 available from both experimental animals and men, which provide a unique opportunity for
962 evaluation of the male gamete cellular function. In addition, the main male reproductive organ,
963 testis, which produces sperm, is located externally; therefore, it can be clinically examined easily
964 without using invasive medical techniques. As a result, male reproductive endpoints from
965 animals and humans provide a relatively rich database that is suitable for evaluation of
966 interspecies toxicodynamic variation. Following are the male specific endpoints that are
967 commonly used for evaluation of male reproductive functions.

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1. Organ weight and size (e.g., testis weight and size). These parameters are easy to examine in both animals and humans. Humans have small testes relative to the rat (0.08 compared to 0.4% of the body weight). Absolute testis weight is more relevant than the relative testis weight for assessing male reproductive toxicity because available data indicate that weights of reproductive organs are independent of body weights. However, a change of absolute testis weight may be influenced by other effects that counterbalance the reduction in testicular weight resulting from germ cell loss. For example, tissue edema in the testes may mask a decreased weight effect. It is insufficient to evaluate testis weight alone to assess reproductive toxicity of an agent. The same is true for the testis size.
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 2. Organ structure and morphology – evaluation of organ (such as the testes) structure and morphology requires invasive techniques (e.g., biopsy); thus, it is not suitable for testing in healthy humans. As a result, a direct comparison between these endpoints between animals and humans is unlikely.
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 3. Sperm evaluation – Sperm samples can be obtained from the epididymis or testis of experimental animals, and from human ejaculate; therefore, these endpoints are very valuable in evaluation of male reproductive function. Several factors may influence the results of semen evaluation, especially for human samples, including the period of abstinence preceding collection of the sample, age, season, social habits (e.g., alcohol, drugs, smoking) and health status. Below are specific measurements for sperm,
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 - a. Sperm count: sperm count represents the amount of cell available to perform reproductive function. Ejaculated sperm number from any species is influenced by several variables, including the length of abstinence and the ability to obtain the entire ejaculate. The results of measurements of sperm counts are also strongly dependent on the time of measurement after the treatment. Humans have lower sperm reserve than animals in terms of number of sperm to maintain fertility. In contrast, males of most test animal species produce sperm in excess of the minimum requirements for fertility, and test animals can undergo multiple successive matings without a decrease in fertility. In some strains of rats and mice, production of sperm can be reduced by 90% or more without compromising fertility capability (Mangelsdorf and Buschmann, 2002). However, in human males, less severe reduction in sperm production can cause reduced fertility. A decrease of sperm counts of about 70% will result in considerably reduced fertility (MacLeod and Gold, 1951; Zukerman et al., 1977; David et al., 1979). Nevertheless, it should not be assumed that a reduction in sperm count (i.e., <90%) will have no effect on fertility in rodents. When sperm count is used in quantitative comparison of animal and human variation, a direct comparison of effective dose should be used.
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 - b. Sperm morphology: Sperm morphology is a weak indicator of effects on male fertility. The traditional approach of measuring rodent sperm morphology is subjective categorization of sperm head, midpiece, and tail defects. Similarly, the heterogeneity of sperm structure in humans and non-rodent species makes it difficult to define clearly the limits of normality. However, sperm morphology
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1015 profiles are relatively stable and characteristic in a normal individual over time,
1016 and are less sensitive to abstinence; therefore, this is one of the least variable
1017 sperm measures in normal individuals which can be used for interspecies
1018 comparison.

1019 c. Sperm motility: Quantitation of sperm motility is a common means for assessing
1020 the quality of semen samples collected during routine clinical studies.
1021 Progressive motility and straight-line velocity appear to be the most important
1022 parameters relevant to fertility in humans.
1023

1024 In both experimental animals and humans, fertility is dependent not only on having
1025 adequate numbers of sperm, but also on the degree to which those sperm are normal. If
1026 sperm quality is high, then sperm number must be substantially reduced before fertility is
1027 affected. Similarly, if sperm numbers are normal, a relatively large effect on sperm
1028 motility is required before fertility is affected.
1029

1030 4. Endocrine parameters – hormone levels can be measured in both animal and human
1031 samples. However, the endocrine requirements for the quantitative maintenance of
1032 spermatogenesis may be different in rats and men. For example, testosterone alone
1033 maintains spermatogenesis in rats, whereas both testosterone and FSH appear to be
1034 required for quantitative maintenance of spermatogenesis in men. Therefore, selection of
1035 the appropriate hormone to measure, and comparability of effects on different hormone
1036 measurements could be problematic.

1037 3.3.2 Female Specific Reproductive Response

1038
1039 The female reproductive system significantly differs from males in terms of organ location and
1040 function. All the female reproductive organs are located internally, and this makes organ
1041 examination more difficult. In addition, cyclic functional change of female reproductive system
1042 and its corresponding endocrine regulation also determine use of female characteristic endpoints
1043 in the reproductive function. Following are the common endpoints that could be used in
1044 evaluation of female reproductive function.
1045

1046 1. Organ weight – Female reproductive organs such as uterus and ovaries are located
1047 internally; therefore, these organs in healthy women are not accessible for conducting
1048 organ weight evaluation. New noninvasive techniques, such as ultrasonic measurement,
1049 may provide a way to conduct such evaluation, but more investigations are need to
1050 further correlate the results from these new methods to the weight changes observed from
1051 animals samples.
1052

1053 2. Organ histopathology – Since the organ histopathological examination requires taking
1054 samples from internal reproductive organs by using invasive biopsy techniques, it is
1055 usually not acceptable by most of healthy human subjects for research or epidemiology
1056 study purpose. In addition, ethical consideration also precludes using such techniques on
1057 healthy women, because of non replaceable existing gametes in mature ovaries. Nor is
1058 this endpoint routinely used for evaluation of reproductive function on healthy humans.
1059 Therefore, it is usually not available for analysis of interspecies or intra-human variation.

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3. Hormone levels – all functions of the female reproductive system are under endocrine control, and therefore, can be susceptible to disruption by effects on the reproductive endocrine system. Interpretation of endocrine effects is facilitated if information is available on a battery of hormones. However, in evaluating such data, it is important to consider that serum hormones such as FSH, LH, prolactin, and androgens exhibit cyclic variations within a 24-hour period. In addition, the hormone levels also depend on the estrous cycle and the age and strain of the animals. Thus, the time of sampling should be controlled rigorously to avoid excessive variability. Since hormonal levels fluctuate significantly in normal animals and humans; hormonal change may not be a highly sensitive indicator of reproductive toxicity. However, greater sensitivity can be obtained if multiple measurements of hormonal levels are made.
 4. Estrous cycle length – compared to female animals which physiological and living conditions are highly controlled, menstrual cyclicality in humans is affected by many parameters such as age, nutritional status, stress, exercise level, certain drugs, and the use of contraceptive measures that alter endocrine feedback. Therefore, these factors need to be controlled in human studies in order to identify the true effect caused by chemical exposure.
 5. Reproductive senescence – the principal cause of the loss of ovarian cycling in humans appears to be the depletion of oocytes. Oocyte depletion is difficult to examine directly in women because of the invasiveness of the tests required; however, it can be studied indirectly through evaluation of the age at reproductive senescence (menopause). Nevertheless, this effect may not be detectable until later in life long after exposure has ceased. Therefore, it could be very difficult to accurately characterize the original exposure condition.

1087 3.3.3 *Sexual Behavior and Fertility*

1088
1089 Sexual behavior reflects complex neural, endocrine, and reproductive organ interactions and is
1090 therefore susceptible to disruption by a variety of toxic agents and pathologic conditions. Data
1091 on sexual behavior are usually not available from studies of human populations exposed to
1092 potentially toxic agents, nor are such data obtained routinely in regular toxicity studies of
1093 environmental agents with test species. These data are usually obtained from epidemiology
1094 studies designed for evaluating reproductive toxicity, and animal reproductive studies.

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1. Mating index– In experimental animals, the evidence of mating can be obtained reliably as observations of copulation, copulatory plugs, or sperm in the vaginal fluid. However, the mating in humans is a very subjective and private activity, and it is also dependent on many factors such as mental status, as well as social and culture background. Therefore, the sexual activity in humans cannot be directly compared to that in experimental animals, and it is not suitable for interspecies or intra-human variation analysis.
 2. Libido, mounts, erection, impotence or ejaculation – similar to mating index, these behavioral changes are difficult to measure in animals and humans, and their

1105 comparability between humans and animals are questionable. Living in a tightly
1106 controlled environment, the sexual behavior in experimental animals can be objectively
1107 recorded. However, the sexual behavior in humans not only can be affected by
1108 reproductive toxicants, but also can be influenced by other factors such as mental or
1109 physical stress. Most of these endpoints are subjective evaluations. Thus, these sexual
1110 behavioral measurements are not suitable for interspecies or intra-human variation
1111 analysis.

1112
1113 3. Fertility index – this endpoint is not suitable for interspecies or intra-human variation
1114 analysis due to rather low sensitivity of this endpoint in laboratory animals, and the
1115 difficulty to quantify this endpoint in humans. Fertility assessment in test animals has
1116 limited sensitivity as a measure of reproductive injury, because, unlike humans, males of
1117 most test species produce sperm in excess of the minimum requirements for fertility. In
1118 addition, test animals can undergo multiple matings. In human males, less severe
1119 reduction in sperm production can cause reduced fertility.

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1121 **3.4 Results for Developmental Endpoints**

1122 *3.4.1 Maternal Toxicity Endpoints*

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1124 Maternal toxicity to pregnant dams or post-partum mothers can include systemic effects or
1125 effects related to the reproductive system. In this document, we will focus our discussion on
1126 only reproductive endpoints. For the evaluation of other systemic responses, please see other
1127 relevant risk assessment guidance documents.

1128

1129 1. Body weight in pregnancy -- Body weight and change in body weight during pregnancy
1130 can be viewed collectively as indicators of maternal toxicity for most species including
1131 humans, although these endpoints may not be as useful in rabbits, because body weight
1132 changes are usually more variable in rabbits. Nevertheless, changes in maternal body
1133 weight corrected for gravid uterine weight at sacrifice may be determined in animals, but
1134 not in humans for obvious reasons. Therefore, a different measurement of body weight
1135 has to be used.

1136

1137 2. Gestation length – changes in gestation length may indicate effects on the process of
1138 parturition. This endpoint can be determined in both humans and experimental animals
1139 that are allowed to deliver pups. However, in regular teratogenicity tests, this endpoint
1140 cannot be examined due to pre-term sacrifice of pregnant dams. While this endpoint can
1141 be measured in humans, it is not collected by registries, and is not frequently reported in
1142 epidemiology studies.

1143

1144 3. Number of corpora lutea – This parameter is used as an indicator of number of eggs
1145 ovulated from the ovaries, and it is used in calculation of the rate of preimplantation loss.
1146 Since the determination of this parameter requires invasive measure, it could not be done
1147 routinely on healthy women. In addition, the number of corpora lutea in humans is more
1148 likely to be a constant as women are more likely to bear a single child in pregnancy.

1149

1150 4. Resorptions, preimplantation and postimplantation loss – These endpoints can be of the
1151 ovaries and uterus. However, distinguishing these endpoints in human is difficult
1152 because accurate identification of these responses requires invasive techniques. In
1153 addition, any of these responses could be shown in humans as miscarriage. Thus, these
1154 endpoints in experimental animals should be considered together when they are
1155 compared to human miscarriage. Please note that clinical records of human miscarriage
1156 are not useful, because many early losses are either not recognized or not reported. For
1157 the same reason, use of hospital records to identify embryonic or early fetal loss will
1158 underestimate events. A better way of identifying human pregnancy is to measure human
1159 chorionic gonadotropin (HCG) levels. Therefore, more weight should be given to a study
1160 in which biological data such as HCG measurements on pregnancy status are available
1161 from study members. Evaluation of early resorption in humans has a special issue where
1162 human pregnancy can be influenced by medical intervention. Therefore, a comparison of
1163 interspecies variation between animals and humans is commonly confounded by such
1164 medical intervention.

1165 3.4.2 *Fetal Developmental Endpoints*

- 1166
- 1167 1. Fetal weight and length – fetal weight or length (birth weight or length in humans) in
1168 both animals and humans can be easily determined, and indicate general fetal
1169 development during gestation. It is worth noting that these parameters are inversely
1170 related to the number of pups in each litter. However, the number of children in humans
1171 is usually one. Therefore, while conducting a quantitative comparison of these endpoints
1172 between experimental animals and humans, one should control for litter size in animals
1173 and for multiple births in humans.
1174
 - 1175 2. Fetal deaths – In humans, this endpoint is also called stillbirth. It is relatively easy to
1176 identify in both experimental animals and humans.
1177
 - 1178 3. Live birth index – this is an endpoint for animal studies. There is no corresponding
1179 parameter in humans because humans usually bear a single fetus rather than multiple
1180 fetuses as seen in the commonly used experimental animals (e.g., mouse, rat, and rabbit).
1181
 - 1182 4. Offspring gender – This endpoint is easy to identify in both experimental animals and
1183 humans. However, the data presentation would be different because of multiple fetuses
1184 in animals vs. the usual single fetus in humans. In animal study, the offspring gender can
1185 be expressed as percentage of pups are male or female in each litter, but in humans, a
1186 single fetus pregnancy can only be expressed as male or female child in this pregnancy.,
1187 but a sex ratio can be obtained for the human population of interest. A special statistical
1188 data treatment is needed in order to conduct an appropriate interspecies comparison.
1189
 - 1190 5. Malformations and variations – Similar to the offspring gender, this endpoint is easy to
1191 be identified in both animals and humans, but a special statistical data analysis should be
1192 used in interspecies comparison. Information on malformations is readily collected for
1193 humans, due to the availability of birth defect registries.

1194 3.4.3 *Post Parturition Development*

1195
1196 Most of post parturition developmental endpoints, such as, offspring viability, pup growth
1197 indexes, pup developmental structural and functional delay, can all be evaluated in
1198 developmental animal studies where no medical intervention will take place. Therefore, these
1199 endpoints can truly reflect the neonatal development during the post natal period. In contrast,
1200 human neonatal development is usually monitored clinically, and medical intervention is
1201 common practice when abnormal neonatal development is noticed. In addition, human neonatal
1202 development is also affected by other environmental factors, such as smoking and drinking
1203 alcohol by lactating mothers. Thus, a quantitative comparison of these endpoints between
1204 experimental animals and humans is feasible, but an appropriate control for confounding factors
1205 is the key for the success of such analysis.

1206
1207 Onset of puberty can be identified in experimental animals. However, it can only be identified in
1208 humans through an epidemiology study. The major issue with this type of epidemiology study is
1209 the accuracy of the exposure identification. The unreliable exposure identification in case-
1210 control study or high cost of long-term cohort study makes it impossible to compare this
1211 endpoint between animals and humans.

1212 3.4.4 *In Vitro Studies and Biomarkers for Developmental and Reproductive Functions*

1213
1214 A number of *in vitro* developmental systems have been used to investigate the morphological
1215 and biochemical basis of normal and abnormal development (NRC, 2001). These study systems
1216 include whole mammalian embryo culture, non-mammalian embryo culture, organ (e.g., testis,
1217 ovary) perfusion, culture of isolated cells from the reproductive organs (e.g., Leydig cells, Sertoli
1218 cells, granulosa cells, oviductal or epididymal epithelium) and tissue cultures (seminiferous
1219 tubule segments). In addition, *in vitro* tests of sperm properties and function are also available
1220 that include evaluation of penetration of sperm through viscous medium, and capacitation and
1221 fertilization assays. However, most of these *in vitro* tests focus on a narrow range of
1222 developmental events; thus, *in vitro* studies should be based on previously characterized
1223 mechanism information from *in vivo* studies. The major limitation of using *in vitro* systems in
1224 evaluation of interspecies and intra-human variation is the very limited availability of *in vitro*
1225 systems using human organs, tissues or cells. In addition, ethical considerations may also
1226 prevent researchers from using human reproductive cells in *in vitro* tests. However, while
1227 validated human and corresponding animal *in vitro* systems are available, and if these systems
1228 represent the critical event in the mode of action of the chemical of interest, such systems can be
1229 used in calculation of CSAFs for interspecies and intra-human dynamic variations, as can be
1230 done for the *in vitro* studies used for evaluation of systemic toxicity.

1231
1232 Recently, significant progress has been made in early detection of developmental and
1233 reproductive defects in humans by using various biomarkers (Longo, 1987; Ewing and Mattison,
1234 1987; Clarkson, 1987; Glasser et al., 1987; Miller, 1987). These biomarkers have been used to
1235 detect compromised pregnancies, exposure to environmental toxicants during gestation, genetic
1236 damage in the human fetus, and neurodevelopmental effects etc. For example, sensitive detection
1237 of HCG has provided both the patient and the doctors the capability to detect a pregnancy before
1238 the clinical signs of a pregnancy. Therefore, it is a very useful tool in identifying early

1239 pregnancy loss in humans if there is any. However, almost all the biomarkers for developmental
1240 or reproductive effects are specifically designed for use in humans, and humans and animals
1241 differ significantly in many parts of the reproductive process. In order to conduct a quantitative
1242 comparison between animals and humans, more research is needed to identify corresponding
1243 biomarkers in experimental animals and their comparability with those of humans. Until such
1244 results are available, the use of biomarkers in quantitative evaluation of interspecies
1245 toxicodynamics is limited.

1246 **3.5 Case Studies**

1248 *3.5.1 Example/Case Study 1 - Lead*

1249
1250 Lead is a well-known reproductive toxicant in both experimental animals and humans. Observed
1251 adverse effects include changes in reproductive functions in both males and females. Numerous
1252 studies, both in animals and humans, have been performed. Please note that a risk value should
1253 be derived based on dose response information for critical effects, and the same is true in
1254 estimation of CSAF. In this example, we use the data on reproductive toxicity caused by lead
1255 exposure only to illustrate how to use this draft approach. In particular, we focus our comparison
1256 on adverse effect on sperm count.

1257 *3.5.1.1 Identification of Active Chemical Moiety*

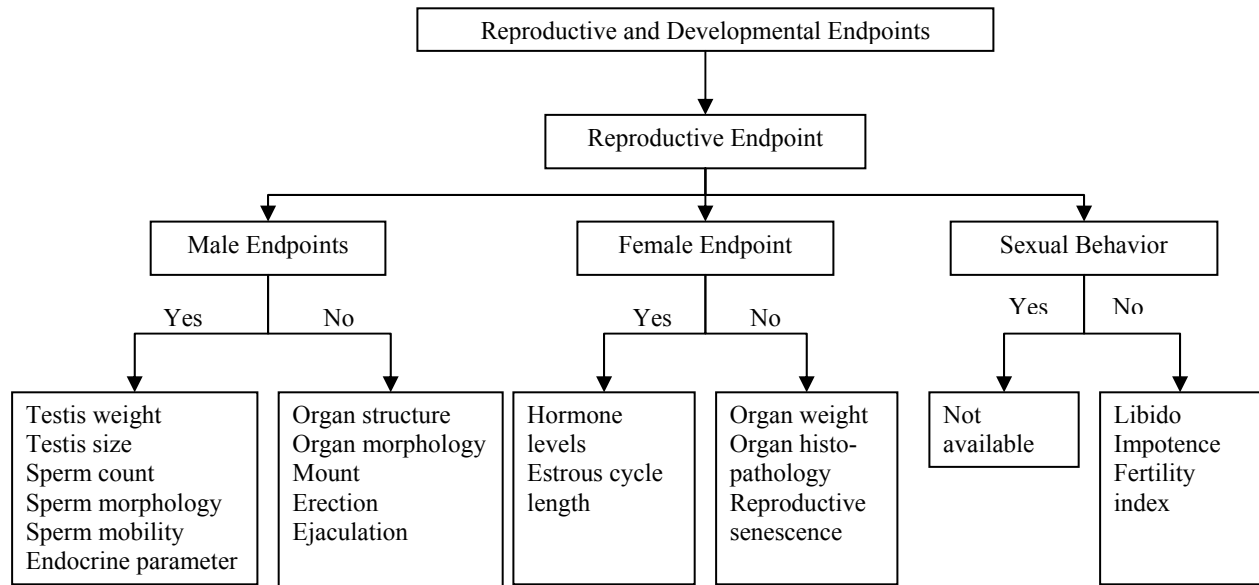
1258
1259 Lead can affect reproductive function in both males and females. Whether the mode of action of
1260 lead is a direct effect on reproductive organs, or the endocrine control of reproduction, or both, is
1261 still unclear. Regardless, of the exact mode of action, it is known that lead itself, rather than a
1262 lead metabolite, is the toxicologically active moiety. Therefore, based on current CSAF
1263 guideline, a default blood concentration of the parent compound would be an appropriate dose
1264 metric.

1265 *3.5.1.2 Consideration of End-Point*

1266
1267 To conduct a quantitative comparison of toxicodynamic variations between animals and humans,
1268 the most important step is to identify the endpoint that is available from both animals and
1269 humans and the measurements from animals that are comparable to those from humans. This
1270 example will focus on sperm measurement comparison. However, a similar approach can be
1271 applied to other endpoints or critical effects if they are identified. As shown in Figure 6 under
1272 male reproductive endpoints, sperm measurement can be used to quantitatively estimate
1273 toxicodynamic variations between animals and humans. Available animal data (see Table 8)
1274 indicate that lead can cause adverse changes in sperm count, sperm morphology as well as sperm
1275 function. An analysis of the threshold doses for these changes in rabbits exposed to lead

1276 **Figure 6. Reproductive Endpoints and Their Applicability to Use Within the IPCS**
 1277 **Framework for Compound Specific Adjustment Factors (CSAFs).**

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Table 8. Threshold dose levels in male rabbits associated with lead effects on sperm*

Parameter	Approximate Threshold Dose (ug/dl blood)
Percent normal cells	16.2
Percent normal acrosomes	17.1
Percent sperm motility	21.3
Percent sperm velocity (straight line)	22.1
Total sperm count	23.7
Sperm head perimeter	16.3

*Source: Moorman et al., 1998

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The threshold doses shown in this table were determined based on regressions, and they are very similar, ranging from 16.2 to 23.7 ug/dl blood, suggesting that a common mode of action for the effects on sperm cells and similar sensitivity for the different endpoints. In addition to the rabbit study, Apostoli et al. (1998) also reported a threshold dose of 35 µg/dl in rodents for changes in sperm count. This means that, relative to rodents, rabbits are more sensitive to lead-induced sperm count changes. From these data, the authors estimated that a blood lead of 30.2 µg/dl in rabbits would produce a 10% decrease in sperm concentration.

suggests a comparable sensitivity among these endpoints in rabbits. Since there are dose-response data of sperm count from both experimental animals and humans, this endpoint was used for quantitative comparison of toxicodynamic differences between animals and humans in effects on sperm.

1299 It is necessary to emphasize that when a CSAF based on direct measurement of sperm count is
1300 used in risk assessment, the same endpoint should also be used as the point of departure in dose
1301 response analysis. Since experimental animals, such as rodents, have sperm numbers in excess
1302 of minimum requirements for fertility while humans have a relatively small reserve, a
1303 comparison based on fertility function might have a significantly different result than that based
1304 on sperm count. However, as long as the same endpoint is used in both CSAF estimation and
1305 dose response analysis (e.g., point-of-departure), the resulting risk value should be the same.

1306 3.5.1.3 *Experimental Data in Animals (Moorman et al., 1998)*

1307
1308 Groups of 7-15 sexually mature male rabbits received subcutaneous administration of lead
1309 acetate for 10 weeks to achieve target blood lead concentrations of 0, 20, 40, 50, 70, 80, 90 or
1310 100 µg/dL. Blood and sperm samples were obtained from the rabbits weekly. Sperm samples
1311 were examined for sperm concentration, motility, morphology, and motility. The study results
1312 are summarized in the Table 8.

1313 3.5.1.4 *Epidemiology Data in Humans*

1314
1315 Moorman et al. (1998) identified five studies that reported blood lead levels and sperm
1316 concentration of lead exposed workers. The combined data from these articles revealed a
1317 decreasing linear relationship with a decrease sperm concentration of $47 \times 10^6/\text{ml}$ for an increase
1318 of 100 µg/dl in blood lead with a background sperm concentration of $90.3 \times 10^6/\text{ml}$. From these
1319 data, the authors estimated a blood lead level of 19.4 µg/dl would produce a 10% decrease in
1320 sperm concentration in men.

1321 3.5.1.5 *Calculation of a CSAF for Interspecies Differences in Toxicodynamics*

1322
1323 The ED10s for sperm count change are available from both rabbits and humans after exposure to
1324 lead. The effective dose for both species was internal blood lead concentration. Such internal
1325 dose from *in vivo* studies is ideal for quantitatively estimating interspecies dynamic variation.
1326 For lead exposure, the CSAF for interspecies differences in toxicodynamics AD_{AF} is calculated
1327 as $ED_{10}(\text{rabbit}) / ED_{10}(\text{human})$, and the resulting AD is 1.6. Thus, the interspecies
1328 toxicodynamic adjustment factor is 1.6 for lead-induced sperm toxicity.

1329 3.5.2 *Example/Case Study 2 – Methyl mercury*

1330
1331 Methyl mercury is a well-known neurotoxicant in both experimental animals and humans.
1332 Observed adverse effects include changes in many neurological functions in both males and
1333 females. Effects are known to be more severe in the developing fetus and young animal when
1334 compared to older animals. In this example, we use neurological clinical signs and symptoms
1335 measured in adults as indicators of delayed developmental toxic effect to develop interspecies
1336 toxicodynamic CSAF for developmental toxicants.

1337

1338 *3.5.2.1 Identification of Active Chemical Moiety*

1339
1340 The mode of action of methyl mercury is considered to be a direct effect on neurological organs
1341 by the parent compound. For purposes of this example, the brain concentration of the parent
1342 compound is considered to be the appropriate dosimeter.

1343 *3.5.2.2 Consideration of End-Point*

1344
1345 To conduct a quantitative comparison of toxicodynamic variations between experimental animals
1346 and humans, an important first step is to identify similar endpoints between experimental animals
1347 and humans; afterwards an attempt is made to find specific measurements from experimental
1348 animals that are comparable to the critical effect in humans. A wealth of information from
1349 epidemiology studies is available that demonstrate the critical effect in humans and from which
1350 risk assessment values have been derived. Independently, a large number of experimental
1351 animal studies are available that demonstrate similar neurological endpoints to those found in
1352 humans. However, the specific critical effects for methyl mercury in humans (i.e.,
1353 neuropsychological impairments measured by a number of different psychological tests) are not
1354 directly measurable in experimental animals. Because of this, we focus the development of a
1355 CSAF on general neurological clinical signs and symptoms, or in some cases histological lesions
1356 in the brain or peripheral nerves, as indicators of developmental delay as shown in the
1357 framework (Figure 7) under post parturition endpoints.

1358
1359 Since our choice of endpoint for the CSAF evaluation is not the critical effect and further, since
1360 we did not attempt to further define clinical signs and intoxication among species, our choice is
1361 only for demonstration of the proposed framework. The resulting CSAF would not be relevant
1362 for methyl mercury risk assessment.¹⁶

1363 *3.5.2.3 Summary of Experimental Data in Differing Species*

1364
1365 Table 9 shows brain concentrations of methyl mercury in different species at intoxication with
1366 neurological signs or symptoms. Each entry contains information regarding the species tested,
1367 number of animals exposed, mean mercury exposure level, range of mercury exposure, and
1368 reference information. In addition, mode of exposure is provided when available. When
1369 examined in its entirety, the table provides a broad spectrum of useful exposure information
1370 dealing with mercury. Based on the effective (intoxication) concentrations in the target organs
1371 of animals and humans, interspecies toxicodynamic CSAFs can be estimated as ratios between
1372 animal effective concentration to human effective concentration in the brain.

1373

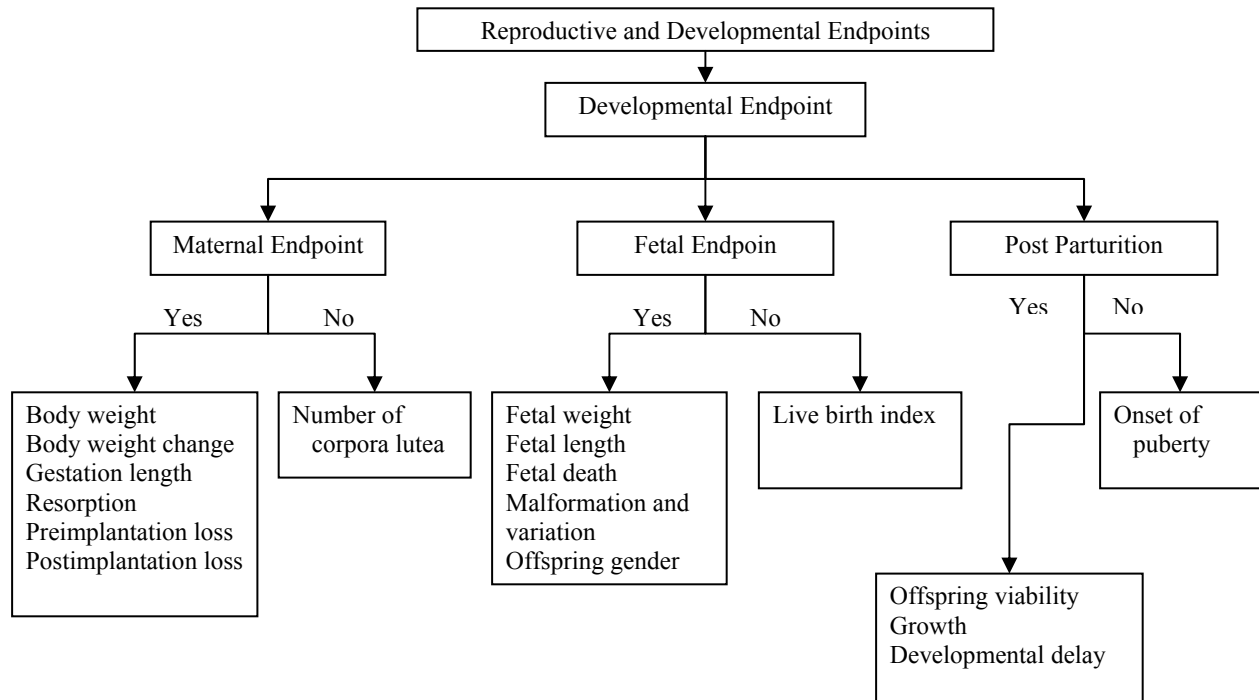
¹⁶ Note that potential CSAFs have been proposed by a number of investigators for within human variability in toxicokinetics for methyl mercury as summarized by Dourson et al., (2001).

1374 **Table 9. Brain concentrations of methyl mercury in different species at intoxication with**
 1375 **neurological signs or symptoms.***
 1376

Species	No.	Mean (µg/g)	Range (µg/g)	Reference
Cat	2	9		Albanus et al (to be published)
Cat	3	10	8-12	Kai 1963
Cat	2	9	8-10	Kitamura (Minamata Report 1968)
Cat	5	13	8-19	Kitamura (Minamata Report 1968)
Cat	2	28	23-32	Rissanen 1969
Cat	4	14		Takeuchi 1961
Cat	7	13	8-18	Takeuchi et al.
Cat	7	21	3-60	Yamashita 1964
Cat	3	6	2-12	Yamashita 1964
Cat	5	11	2-19	Yamashita 1964
Dog	5	29	8-50	Yoshino et al.
Dog	5	19	4-32	Yoshino et al. 1966
Ferret	4	27	7-39	Borg et al. 1970
Human	1	12	12	Hook et al
Human	2	6	3-9	Lundgren Swensson
Human	2	40	15-66	Okinaka et al
Human	3	16	9-24	Takeuchi et al.
Human	1	35	22-48	Tsuda et al
Monkey	2	13	12-14	Berlin et al.
Monkey (Siamiri sciurus)	4	15	12-19	Nordberg et al. (in press)
Mouse	10	28	10-61	Saito et al.
Mouse	8	28	11-61	Saito et al. 1961
Mouse	20	30	20-40	Suzuki 1969
Mouse	10	40	25-55	Suzuki 1969
Rat	8	16	11-19	Berglund et al. (to be published)
Rat	12	49		Takeshita and Uchida 1963
Rat	10	49	49	Takeshita et al.

1377 * Information taken from personal communication of Maths Berlin (2005). Please note that some of these
 1378 references may be overlapping. Bolded and italicized values have been estimated by authors of this text.
 1379

1380 **Figure 7. Developmental Endpoints and Their Applicability to Use Within the IPCS**
 1381 **Framework for Compound Specific Adjustment Factors (CSAFs).**
 1382
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 1385 *3.5.2.4 Calculation of a CSAF for Interspecies Differences in Toxicodynamics*
 1386

1387 Table 10 illustrates species-specific averages for methyl mercury brain concentrations, and
 1388 corresponding ranges of these averages, and a CSAF estimated for humans from each species.
 1389 The CSAF value was estimated as the mean of averages (across studies) for the experimental
 1390 animals, divided by the mean of human averages. The species-specific mean of these averages
 1391 was computed on a sample-size weighted scale, based on the number of individuals examined,
 1392 using the averages found in Table 9. The human mean of averages was also derived in this
 1393 manner.

1394
 1395 **Table 10. Mean Values of Methyl Mercury Levels for Each Species and Resulting CSAFs.**
 1396

Species	Range of Averages	Mean of Averages	CSAF
Cat	6 - 28	14	0.7
Dog	19 - 29	24	1.2
Ferret	27	27	1.3
Human	6 - 40	21	1.0
Monkey	13 - 15	14	0.7
Mouse	28 - 40	31	1.5
Rat	16 - 49	40	1.9

*Information taken from Table 9.

1397
1398 The choice of an appropriate CSAF would depend in large part on the choice of species used as
1399 the basis of the risk assessment value. For example, if the rat was considered in the development
1400 of a RfD, then the CSAF would likely be 1.9 (i.e., $40/21=1.9$) to cover the interspecies
1401 toxicodynamic variations. With monkeys, the value of the CSAF would likely be 0.7 (i.e.,
1402 $14/21=0.7$). Of course, in the case of methyl mercury, this choice is not necessary, since human
1403 data form the basis of the risk value. Thus, this example again serves to illustrate the framework
1404 and should not be used in a specific risk assessment for methyl mercury.

1405 1406 **3.6 Discussion**

1407
1408 When data are available, CSAFs are used in place of traditional uncertainty factors to account for
1409 the variations between animals and humans or within human populations. The IPCS (2001)
1410 guideline has provided a framework for developing CSAFs for toxicodynamic variations
1411 between animals and humans. In the present analysis, part of this framework was used to focus
1412 on the developing fetus and/or young children. Parameters that can be used to develop
1413 quantitative estimates for intraspecies and interspecies differences in susceptibility were
1414 proposed. Examples were identified where the concept of CSAFs for toxicodynamic differences
1415 can be applied for reproductive and developmental endpoints. Our compilation suggests that the
1416 IPCS scheme is easily enhanced, in this case with a list of parameters that can be used to develop
1417 quantitative estimates for interspecies toxicodynamic differences in susceptibility for
1418 reproductive and developmental toxicity. However, not all parameters allow quantitative
1419 comparisons among experimental animals and humans, as discussed below.

1420
1421 Developing fetuses and children comprise a unique human population, which is often
1422 significantly different from average health adults. Throughout the entire life cycle, all aspects of
1423 reproductive function are dependent on various endocrine communicating systems that affect
1424 target cell gene expression and/or protein synthesis. In the IPCS CSAF guidelines, the
1425 adjustment factors for interspecies toxicodynamic aspects are commonly based on results of *in*
1426 *vitro* studies using animal and human tissue. However, while such data are suitable for systemic
1427 toxicity, they are usually not available for reproductive or developmental endpoints because
1428 there is no *in vitro* model or cell culture that can mimic the whole process of reproduction and
1429 fetal development. Most of the data available on reproductive and developmental toxicity come
1430 from *in vivo* studies, especially from experimental animal studies or human epidemiology
1431 studies. These *in vivo* data reflect variations in both toxicokinetics and toxicodynamics.

1432
1433 As shown in Figure 6, male reproductive function is relatively easy to evaluate because the male
1434 reproductive organs can be examined directly without invasive techniques, and semen samples
1435 are easy to obtain. In contrast, female reproductive endpoints cannot be evaluated easily because
1436 the female reproductive organs are located internally, and evaluation of these organs requires
1437 invasive techniques. In addition, cyclic functional change in female reproductive system also
1438 makes the evaluation even less reliable, unless care is taken to control for the position in the
1439 reproductive cycle.

1440
1441 In addition to the gender specific endpoints, sexual behavior can also be evaluated. However,
1442 human sexual behavior can be affected not only by reproductive toxicants, but also by other

1443 factors, such as mental and physical stress. In addition, human sexual behavior is also a
1444 subjective activity, which is not expected in experimental animals. Accurately identifying
1445 effects in sexual behavior in exposed humans is difficult.

1446
1447 Developmental toxicity can be evaluated as maternal response and fetal response. For maternal
1448 response, the major issue is that in humans, any of the resorption, pre-implantation loss, and
1449 post-implantation loss can be shown as miscarriage. Thus, these endpoints in experimental
1450 animals should be considered together when they are compared to human miscarriage. *In utero*
1451 fetal effects are relatively easy to evaluate. However, since humans usually have a single fetus in
1452 each pregnancy while experimental animals usually have multiple fetuses, a special statistical
1453 analysis should be used for data comparison between animals and humans. Evaluation of post-
1454 parturition fetal development has a special issue where human newborn development can be
1455 influenced by medical intervention. Therefore, a comparison of interspecies variation between
1456 animals and humans is commonly confounded by such medical intervention.

1457 1458 **3.7 Future Steps**

1459
1460 The goal of this work is to identify examples where the concept of a CSAF for toxicodynamic
1461 interspecies differences can be applied for reproductive and developmental endpoints. A list of
1462 parameters is developed that might allow for quantitative estimates of these CSAFs. Possible
1463 additional work might be considered to extend this compilation, such as:

- 1464
- 1465 • Determine the most likely reproductive or developmental critical effects from previous
1466 assessed chemicals and more fully research quantitative toxicodynamic comparisons for
1467 these effects between the experimental animal specie and human.
- 1468 • Develop additional, and perhaps more relevant, case studies that extend the suggested
1469 enhancements of the existing IPCS framework for other effects shown in Figures 6 and 7.
- 1470 • Conduct a similar exercise for comparing toxicodynamic variability among experimental
1471 animals and humans for other relevant endpoints, such as liver toxicity.

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Appendix A

**Highest No-Observed-Adverse-Effect Levels
(NOAELs) for 154 chemicals (mg/kg-day)**

Substance Name	CASRN	Last Significant Revision	STUDY TYPE					
			Chronic	Chronic	Chronic	Reproduction	Developmental	Developmental
			Rat	Dog	Mouse	Rat	Rat	Rabbit
Acephate	30560-19-1	May-89	2.5E+00	7.5E-01	-	2.5E+00	2.0E+02	1.0E+01
Acetochlor	34256-82-1	Sep-93	6.4E+00	2.0E+00	1.3E+01	2.1E+01	2.0E+02	3.0E+02
Acifluorfen, sodium	62476-59-9	Mar-87	2.5E+01	7.5E+00	-	1.3E+00	2.0E+01	3.6E+01
Acrolein	107-02-8	Dec-90	5.0E-02	2.0E+00	5.0E-01	2.5E+00	6.0E+00	5.0E-01
Acrylic acid	79-10-7	Apr-94	7.8E+01	-	-	5.3E+01	2.5E+02	-
Acrylonitrile	107-13-1	Dec-90	4.2E+00	1.0E+01	-	1.4E-01	1.0E+01	-
Alachlor	15972-60-8	Sep-93	2.5E+00	1.0E+00	-	1.0E+01	1.5E+02	1.5E+02
Aldicarb	116-06-3	Nov-93	3.0E-01	1.0E-01	-	3.0E-01	1.3E-01	2.5E-01
Aldicarb sulfone	1646-88-4	Nov-93	-	1.1E-01	-	2.4E+00	9.6E+00	-
Aluminum	7429-90-5	Jul-99	6.0E-01	-	1.2E+00	5.2E+01	1.1E+02	7.5E+00
Ally	74223-64-6	Jun-88	2.5E+01	1.3E+02	-	2.5E+02	1.0E+03	7.0E+02
Amdro	67485-29-4	Sep-87	2.5E+00	3.3E-01	2.8E+00	2.5E+00	1.0E+01	5.0E+00
Amitraz	33089-61-1	Aug-88	2.5E+00	2.5E-01	-	1.6E+00	-	1.2E+01
Apollo	74115-24-5	Jun-91	2.0E+00	1.3E+00	7.5E+01	2.0E+01	3.2E+03	1.0E+03
Assure	76578-14-8	Jun-91	9.0E-01	1.0E+01	1.5E+00	1.3E+00	3.0E+02	-
Asulam	3337-71-1	Jun-88	3.6E+01	3.0E+01	-	-	1.5E+03	7.5E+02
Atrazine	1912-24-9	Oct-93	3.5E+00	5.0E+00	3.8E+01	3.5E+00	1.0E+01	5.0E+00
Avermectin B1	65195-55-3	Jul-89	1.5E+00	2.5E-01	4.0E+00	1.2E-01	1.6E+00	-
Baygon	114-26-1	Sep-87	1.0E+01	-	1.1E+02	-	5.0E+01	1.0E+01
Bayleton	43121-43-3	Mar-88	2.5E+00	2.5E+00	7.5E+00	2.5E+00	5.0E+01	5.0E+01
Baythroid	68359-37-5	Mar-88	2.5E+00	4.0E+00	-	2.5E+00	3.0E+01	1.5E+01
Benomyl	17804-35-2	Jan-87	1.3E+02	1.3E+01	2.3E+02	5.0E+00	3.0E+01	-
Bentazon	25057-89-0	Mar-98	9.0E+00	3.2E+00	-	1.5E+01	1.0E+02	3.8E+02
Biphenthrin	82657-04-3	Aug-88	2.5E+00	1.5E+00	-	1.5E+00	1.0E+00	8.0E+00
Boric acid	11113-50-1	Oct-89	1.8E+01	8.8E+00	-	8.4E-01	9.6E+00	2.2E+01
Bromate	15541-45-4	Jun-01	1.1E+00	-	6.0E+01	7.7E+00	2.2E+01	-
Bromoxynil	1689-84-5	Jun-88	5.0E+00	-	-	1.5E+01	1.5E+01	3.0E+01
Bromoxynil octanoate	1689-99-2	Sep-88	7.3E+00	-	-	1.5E+01	1.5E+01	3.0E+01
Butylate	2008-41-5	Oct-94	5.0E+01	5.0E+00	2.0E+01	1.0E+01	5.0E+01	5.0E+02
Captafol	2425-06-1	Sep-87	2.8E+00	-	1.5E+01	6.0E+01	3.0E+01	1.7E+01
Captan	133-06-2	Mar-89	1.0E+02	-	-	1.3E+01	-	6.0E+01

Substance Name	CASRN	Last Significant Revision	STUDY TYPE					
			Chronic	Chronic	Chronic	Reproduction	Developmental	Developmental
			Rat	Dog	Mouse	Rat	Rat	Rabbit
Carbofuran	1563-66-2	Sep-87	1.0E+00	5.0E-01	1.9E+01	1.0E+00	1.0E+00	2.0E+00
Carbosulfan	55285-14-8	Jan-87	1.0E+00	-	-	1.0E+00	2.0E+00	5.0E+00
Carboxin	5234-68-4	Jan-87	1.0E+01	-	-	1.0E+01	4.0E+01	3.8E+02
Chloral hydrate	302-17-0	Sep-00	1.6E+02	-	1.5E+02	5.5E+01	1.5E+02	-
Chlorimuron-ethyl	90982-32-4	Nov-89	1.3E+01	6.3E+00	1.9E+01	5.0E+00	3.0E+01	1.3E+01
Chlorine	7782-50-5	Jun-94	1.4E+01	-	-	5.0E+00	1.5E+01	-
Chlorine dioxide	10049-04-4	Oct-00	1.0E+01	-	-	3.0E+00	3.0E+00	-
Chlorobenzene	108-90-7	Nov-90	6.0E+01	-	6.0E+01	1.7E+02	2.2E+02	1.3E+02
Chlorobenzilate	510-15-6	Dec-89	-	1.3E+01	-	1.0E+02	1.0E+02	8.0E+01
Chloromethane*	74-87-3	Dec-98	2.2E+02	-	5.1E+01	2.2E+02	4.8E+02	-
Chlorophenols	-	Jul-99	5.0E+01	-	4.3E+02	3.0E+00	1.0E+02	-
Chlorpyrifos	2921-88-2	Jan-87	3.0E+00	3.0E+00	-	1.0E+00	1.5E+01	-
Chlorsulfuron	64902-72-3	Jan-87	5.0E+00	6.3E+01	7.5E+01	2.5E+01	1.3E+02	2.5E+01
Chromium (VI)	18540-29-9	Sep-98	2.5E+00	-	-	3.7E+01	3.7E+01	-
Cyhalothrin/Karate	68085-85-8	Jun-88	2.5E+00	1.0E+00	-	5.0E-01	1.5E+01	3.0E+01
Cypermethrin	52315-07-8	Mar-89	7.5E+00	1.0E+00	-	2.5E+00	-	3.0E+01
Cyromazine	66215-27-8	Sep-87	1.5E+00	7.5E-01	-	5.0E+01	-	5.0E+00
Dacthal	1861-32-1	Aug-94	1.0E+00	2.5E+02	4.4E+02	1.8E+01	2.5E+03	5.0E+02
Danitol	39515-41-8	Oct-94	7.2E+00	2.5E+00	5.6E+01	3.0E+00	1.0E+01	3.6E+01
Di(2-ethylhexyl)adipate	103-23-1	Oct-89	7.0E+02	-	-	1.7E+02	1.7E+02	-
Diazinon	333-41-5	Aug-96	1.0E+01	2.5E+00	-	5.0E-02	2.0E+01	1.0E+02
Dicamba	1918-00-9	Aug-88	1.3E+02	5.2E+01	-	2.5E+01	4.0E+02	3.0E+00
DDD/DDE/DDT	-	Sep-02	-	1.6E+01	-	6.0E+00	1.9E+00	-
1,2-Dichloroethane	75-34-3	Jun-05	4.3E+01	-	1.5E+02	4.3E+01	1.6E+02	-
1,1-Dichloroethylene	75-35-4	Feb-98	9.0E+00	-	1.0E+01	3.0E+01	4.0E+01	-
1,2-Dichloropropane	78-87-5	Dec-89	6.0E+01	-	-	1.0E+02	3.0E+01	-
1,3-Dichloropropene*	542-75-6	Sep-92	2.0E+01	3.0E+00	5.0E+00	9.0E+01	1.5E+02	1.5E+02
1,4-Dichlorobenzene	106-46-7	Dec-98	1.5E+02	-	-	1.0E+03	2.5E+02	-
Dichlorvos	62-73-7	Jun-94	2.3E-01	5.0E-02	7.5E+00	2.5E+01	2.1E+01	-
Diiflubenzuron	35367-38-5	Sep-87	-	2.0E+00	2.4E+00	8.0E+00	4.0E+00	4.0E+00
Dimethipin	55290-64-7	Aug-88	2.0E+00	-	6.0E+00	1.0E+01	1.6E+02	4.0E+01
Dimethoate	60.51.5	Jan-87	1.3E+00	-	3.8E+00	7.5E+00	1.8E+01	-

Substance Name	CASRN	Last Significant Revision	STUDY TYPE					
			Chronic	Chronic	Chronic	Reproduction	Developmental	Developmental
			Rat	Dog	Mouse	Rat	Rat	Rabbit
2,4-Dinitrotoluene	121-14-2	Jun-92	3.9E+00	2.0E-01	-	5.0E+00	-	-
2,4 & 2,6-Dinitrotoluene	121-14-2	Dec-98	6.0E-01	2.0E-01	-	3.5E+01	5.1E+00	-
Di-2-ethylhexylphthalate	117-81-7	Sep-02	1.4E+01	5.9E+01	1.2E+02	5.8E+00	3.7E+00	-
Diquat	85-00-7	Mar-87	1.9E-01	1.7E+00	-	2.5E+01	2.5E+01	5.0E+00
Disulfoton	298-04-4	Aug-95	1.8E-01	1.4E-01	2.5E+00	9.0E-03	9.0E-03	1.5E+00
Endosulfan	115-29-7	Oct-94	7.0E-01	5.7E-01	8.4E-01	1.1E+00	-	1.8E+00
Endothall	145-73-3	Mar-87	-	2.0E+00	-	5.0E+00	1.0E+01	-
Endrin	72-20-8	Aug-96	5.0E-02	2.5E-02	-	3.0E-01	5.0E-01	-
Ethion	563-12-2	Sep-89	2.0E-01	-	2.3E-01	1.3E+00	6.0E-01	2.4E+00
Ethylbenzene*	100-41-4	Jul-99	2.5E+02	-	2.5E+02	2.5E+02	9.7E+01	9.6E+02
S-Ethyl dipropylthiocarbamate	759-94-4	Sep-87	5.0E+00	1.5E+01	-	2.5E+00	1.0E+02	3.0E+02
Ethylene oxide	75-21-8	Dec-90	-	-	1.0E+02	3.3E+01	3.3E+01	1.5E+02
Ethylene-propylene glycols	-	Sep-97	4.0E+01	-	8.1E+02	1.3E+03	5.0E+02	2.0E+03
Express	101200-48-0	Jan-89	1.3E+00	7.9E-01	3.0E+00	1.3E+00	2.0E+01	2.0E+01
Fenamiphos	22224-92-6	Sep-87	5.0E-01	2.5E-02	-	5.0E-01	-	3.0E-01
Fluridone	59756-60-4	Jan-87	8.0E+00	7.5E+01	1.5E+01	3.3E+01	3.0E+02	1.3E+02
Flurprimidol	56425-91-3	Jul-89	3.6E+00	7.0E+00	-	1.8E+00	1.0E+01	4.5E+01
Flutolanil	66332-96-5	May-89	1.0E+02	5.0E+01	-	-	-	4.0E+01
Fluvalinate	69409-94-5	Jun-88	1.0E+00	5.0E+00	-	1.0E+00	1.0E+01	2.0E+01
Folpet	133-07-3	Aug-88	-	1.0E+01	-	3.5E+01	6.0E+01	-
Formaldehyde*	50-00-0	Jul-99	2.0E+00	-	1.4E+01	4.0E+01	1.0E+01	-
Fosetyl-al	39148-24-8	Aug-88	1.0E+02	2.5E+02	-	3.0E+02	1.0E+03	5.0E+02
Gasoline*	8006-61-9	Jun-95	2.9E+02	-	2.9E+02	2.1E+03	1.6E+03	-
Glyphosate	1071-83-6	Oct-89	1.4E+00	2.0E+01	-	1.0E+01	1.0E+03	3.5E+02
Haloxypop-methyl	69806-40-2	May-90	6.5E-02	5.0E-02	6.5E-02	5.0E-03	1.0E+00	7.5E+00
Harmony	79277-27-3	Sep-88	1.3E+00	1.9E+01	3.8E+00	1.3E+02	1.6E+02	5.1E+02
Hexachlorophene	70-30-4	Aug-88	1.0E+00	-	1.5E+01	1.0E+00	-	-
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4	Sep-88	3.0E-01	-	-	5.0E+00	2.0E+00	2.0E+00
Hexazinone	51235-04-2	Sep-87	1.0E+01	-	3.0E+01	1.3E+02	-	1.3E+02
Imazalil	35554-44-0	Mar-87	-	1.3E+00	-	4.0E+01	1.0E+01	-
Imazaquin	81335-37-7	Jan-87	5.0E+02	2.5E+01	1.5E+02	1.0E+03	5.0E+02	5.0E+02
Iprodione	36734-19-7	Jun-88	5.0E+01	4.2E+00	1.9E+03	2.5E+01	-	6.0E+01

Substance Name	CASRN	Last Significant Revision	STUDY TYPE					
			Chronic	Chronic	Chronic	Reproduction	Developmental	Developmental
			Rat	Dog	Mouse	Rat	Rat	Rabbit
Isoxaben	82558-50-7	Sep-91	5.0E+00	1.0E+01	1.4E+01	1.3E+02	3.2E+02	1.0E+03
Lactofen	77501-63-4	Jun-88	2.5E+01	5.0E+00	-	2.5E+00	5.0E+01	2.0E+01
Londax	83055-99-6	Sep-88	3.0E+01	2.0E+01	2.3E+02	3.1E+02	1.3E+03	3.0E+02
Malathion	121-75-5	Sep-01	3.5E+01	-	1.7E+01	8.0E+02	1.5E+02	2.5E+01
Mepiquat chloride	24307-26-4	Aug-88	5.0E+00	-	1.5E+02	3.4E+02	3.4E+02	
Metalaxyl	57837-19-1	Jan-87	1.3E+01	6.3E+00	3.8E+01	6.3E+01	5.0E+01	3.0E+02
Methidation	950-37-8	Aug-88	2.0E-01	1.0E-01	1.6E+00	2.5E-01	2.3E+00	1.2E+01
Methomyl	16752-77-5	Jan-87	5.0E+00	2.5E+00	7.5E+00	5.0E+00	-	1.6E+01
Metolachlor	51218-45-2	Oct-90	1.5E+01	-	1.5E+02	1.5E+01	3.6E+02	3.6E+02
Methoxychlor	72-43-5	Sep-02	7.7E+01	-	6.0E+02	5.0E+00	5.0E+00	5.0E+00
Methyl tert-butyl ether*	1634-04-4	Aug-96	4.0E+02	-	4.0E+02	2.5E+03	4.0E+02	8.0E+03
Metribuzin	21087-64-9	Dec-93	5.0E+00	2.5E+00	1.2E+02	-	-	1.5E+01
Mirex	2385-85-5	Aug-95	7.5E-02	-	-	3.1E-01	3.1E-01	-
Monochloramine	10599-90-3	Mar-94	9.5E+00	-	-	1.0E+01	1.5E+01	-
Naled	300-76-5	Mar-87	-	2.0E-01	1.5E+01	6.0E+00	4.0E+01	-
Napropamide	15299-99-7	Jul-89	3.0E+01	-	-	3.0E+01	4.0E+02	-
Nickel [#]	7440-02-0	Sep-97	1.0E-01	-	3.9E+00	3.9E+00	8.0E-01	-
Nitrate	14797-55-8	May-91	2.0E+00	-	-	4.1E+01	4.1E+01	4.1E+01
Norflurazon	27314-13-2	Jan-87	1.9E+01	3.8E+00	5.0E+01	1.9E+01	4.0E+02	1.0E+01
NuStar	85509-19-9	Sep-88	4.6E-01	2.0E-01	-	-	2.0E+00	1.2E+01
Oryzalin	19044-88-3	Jul-89	1.5E+01	5.0E+00	7.5E+01	1.3E+01	2.3E+02	2.5E+01
Oxyfluorfen	42874-03-3	Jan-87	2.0E+00	2.5E+00	3.0E-01	5.0E-01	1.0E+02	1.0E+01
Paraquat	1910-42-5	Aug-88	1.3E+00	4.5E-01	1.9E+00	7.5E+00	1.0E+00	-
Pendimethalin	40487-42-1	Jun-88	-	1.3E+01	-	2.5E+01	5.0E+02	6.0E+01
Pentachlorophenol	87-86-5	Sep-01	3.0E+00	-	-	1.0E+01	4.0E+00	3.0E+01
Permethrin	52645-53-1	Mar-87	5.0E+00	5.0E+00	-	-	2.0E+02	4.0E+02
Phenmedipham	13684-63-4	Jun-90	2.5E+01	2.5E+01	2.5E+01	2.5E+01	-	-
Phenol	108-95-2	Mar-91	2.6E+02	-	4.5E+02	7.1E+01	1.2E+02	-
Phosmet	732-11-6	Jan-87	2.0E+00	1.0E+00	-	4.0E+00	-	6.0E+01
Picloram	1918-02-1	Sep-87	2.0E+01	7.0E+00	-	-	1.0E+03	-
Pirimiphos-methyl	29232-93-7	Sep-87	1.5E+01	2.0E+00	-	5.0E+00	1.5E+02	1.6E+01
Prochloraz	67747-09-5	Oct-89	1.9E+00	9.0E-01	2.0E+02	7.5E+00	5.2E+00	-

Substance Name	CASRN	Last Significant Revision	STUDY TYPE					
			Chronic	Chronic	Chronic	Reproduction	Developmental	Developmental
			Rat	Dog	Mouse	Rat	Rat	Rabbit
Propargite	2312-35-8	May-90	-	2.3E+01	1.5E+02	-	6.0E+00	2.0E+00
Propazine	139-40-2	Aug-87	5.0E+00	-	1.5E+01	5.0E+00	-	-
Propiconazole	60207-90-1	Aug-88	5.0E+00	1.3E+00	1.5E+01	2.5E+01	3.0E+01	1.8E+02
Pursuit	81335-77-5	Jan-90	5.0E+02	2.5E+01	7.5E+02	-	1.1E+03	1.0E+03
Quinalphos	13593-03-8	Mar-87	1.0E+00	5.0E-02	7.5E-02	5.0E-01	-	4.0E+00
Resmethrin	10453-86-8	Sep-88	-	1.0E+01	-	-	4.0E+01	1.0E+02
Rotenone	83-79-4	Sep-88	-	4.0E-01	-	3.8E-01	3.0E+00	-
Savey	78587-05-0	Sep-88	2.3E+01	2.5E+00	3.8E+01	3.5E+01	2.4E+02	1.1E+03
Sethoxydim	74051-80-2	Nov-89	1.8E+01	8.9E+00	1.8E+01	5.4E+01	2.5E+02	1.6E+02
Simazine	122-34-9	Sep-93	5.2E-01	7.6E-01	5.3E+00	2.9E+01	-	5.0E+00
Styrene	100-42-5	Sep-92	2.1E+01	2.0E+02	-	2.0E+02	3.0E+02	-
Sythane	88671-89-0	Sep-88	2.5E+00	3.1E+00	3.0E+00	2.3E+00	2.9E+01	6.0E+01
Tebuthiuron	34014-18-1	Aug-88	-	2.5E+01	-	7.0E+00	-	2.5E+01
Terbacil	5902-51-2	Jan-87	-	1.3E+00	-	1.3E+01	1.3E+01	2.0E+02
Terbutryn	886-50-0	Sep-88	1.0E-01	1.0E+01	-	1.5E+01	5.0E+01	5.0E+01
Tetrachlorovinphos	961-11-5	Mar-87	6.3E+00	3.1E+00	-	1.7E+01	-	1.5E+02
Thiobencarb	28249-77-6	Sep-87	1.0E+00	8.0E+00	-	-	2.5E+01	-
Thiophanate-methyl	23564-05-8	Mar-88	8.0E+00	5.0E+01	2.3E+01	8.0E+00	1.3E+02	-
Thiram	137-26-8	Sep-87	5.0E+00	-	-	3.0E+01	-	-
Tralomethrin	66841-25-6	Jul-89	7.5E-01	1.0E+00	7.5E-01	7.5E-01	1.8E+01	3.2E+01
Triallate	2303-17-5	Aug-90	-	1.3E+00	3.0E+00	7.5E+00	3.0E+01	5.0E+00
Triasulfuron	82097-50-5	Jan-91	-	2.5E+00	1.2E+00	5.0E+01	3.0E+02	2.4E+02
Tributyltin oxide	56-35-9	Aug-88	1.9E-01	-	-	4.4E+00	3.4E-01	-
Tridiphane	58138-08-2	Jan-87	3.0E+00	1.0E+01	-	3.3E-01	3.0E+01	-
Trifluralin	1582-09-8	Jul-89	1.0E+01	7.5E-01	-	1.0E+02	4.8E+02	2.3E+02
1,3,5-Trinitrobenzene	99-35-4	Oct-97	2.7E+00	-	-	3.0E+00	4.5E+01	-
Vinyl acetate	108-05-4	Jul-92	2.4E+02	-	-	4.8E+02	1.2E+02	-

*Inhalation exposures, with chronic NOAELs based on systemic toxicity endpoints; values are in ppm

Inhalation exposures, with chronic NOAEL based on systemic toxicity endpoint; value is in mg/m³

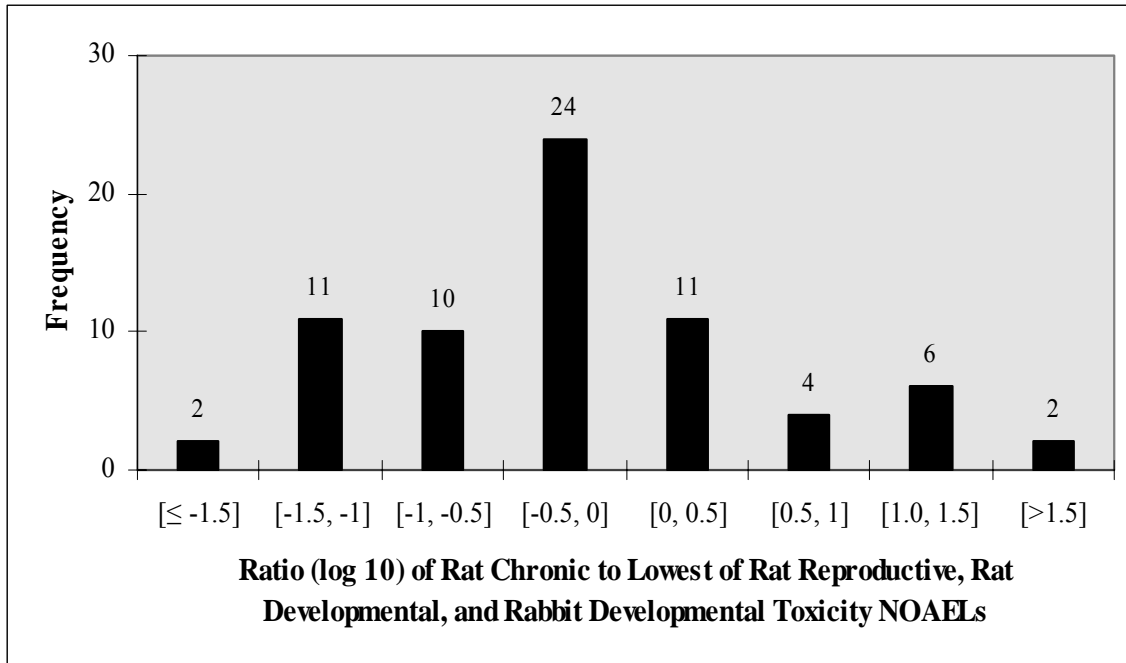
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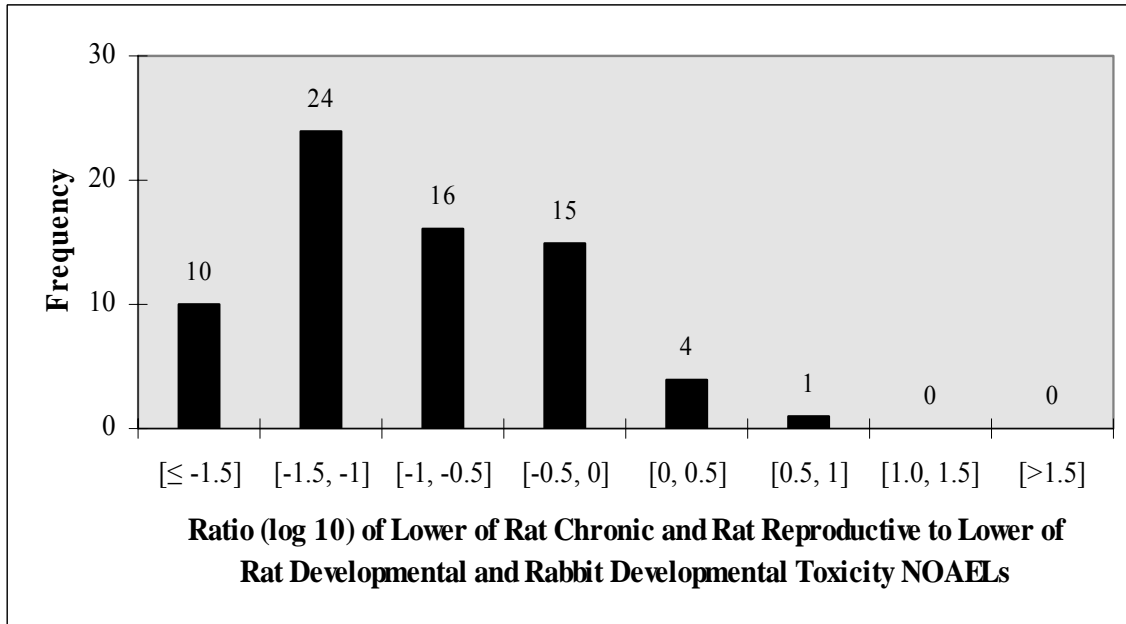
Appendix B

**Frequency Histograms of Ratios of NOAELs for Chronic,
Reproductive, and Developmental Toxicity Studies in the Rat, Dog, and Mouse**

1676 **Rat**
1677
1678 R1

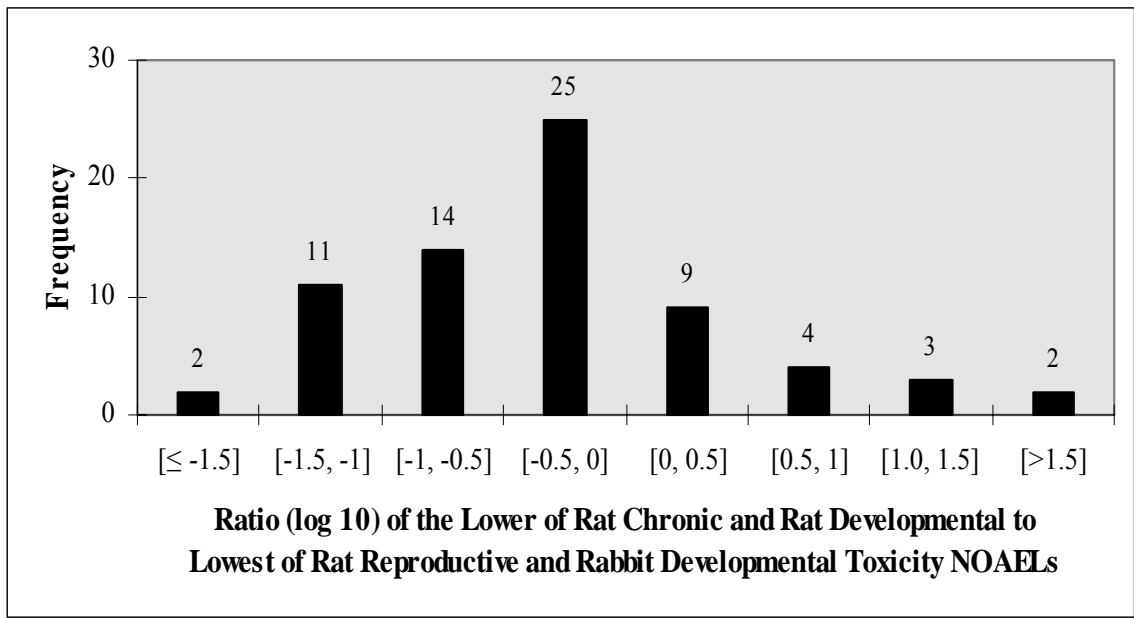


1679
1680
1681 R2

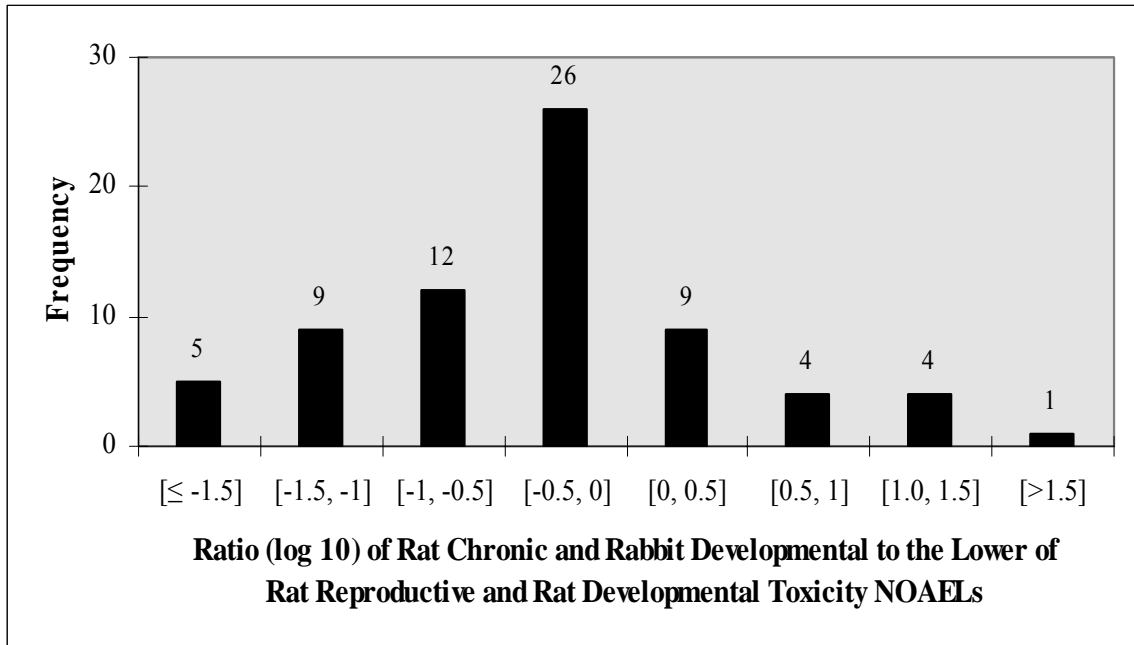


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1686
1687

1688
1689
1690 R3

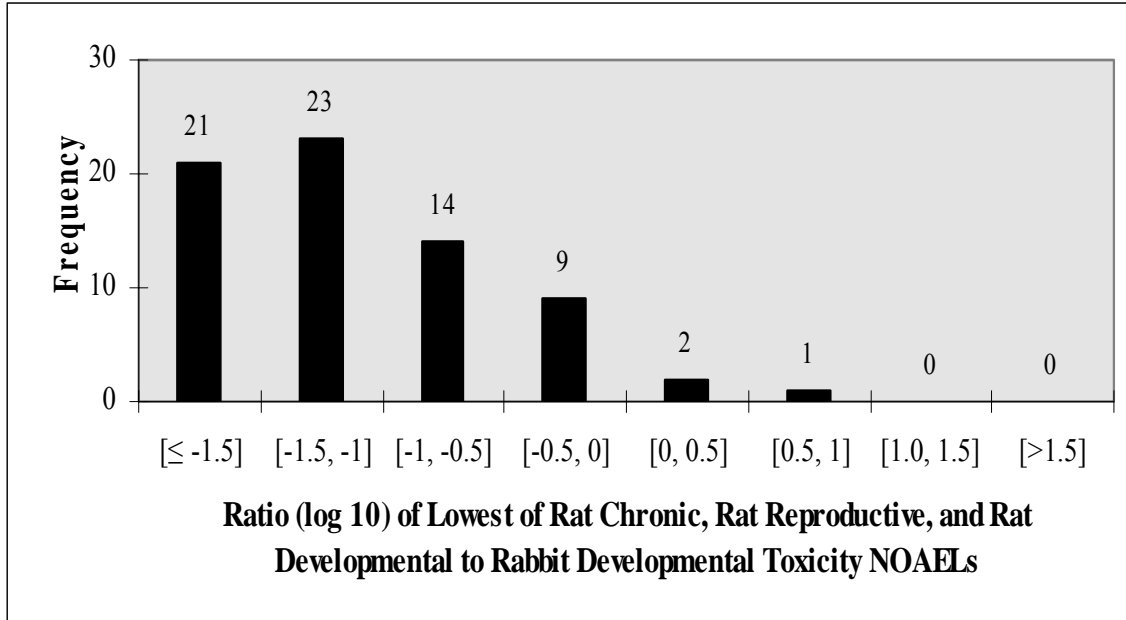


1691
1692
1693
1694
1695 R4

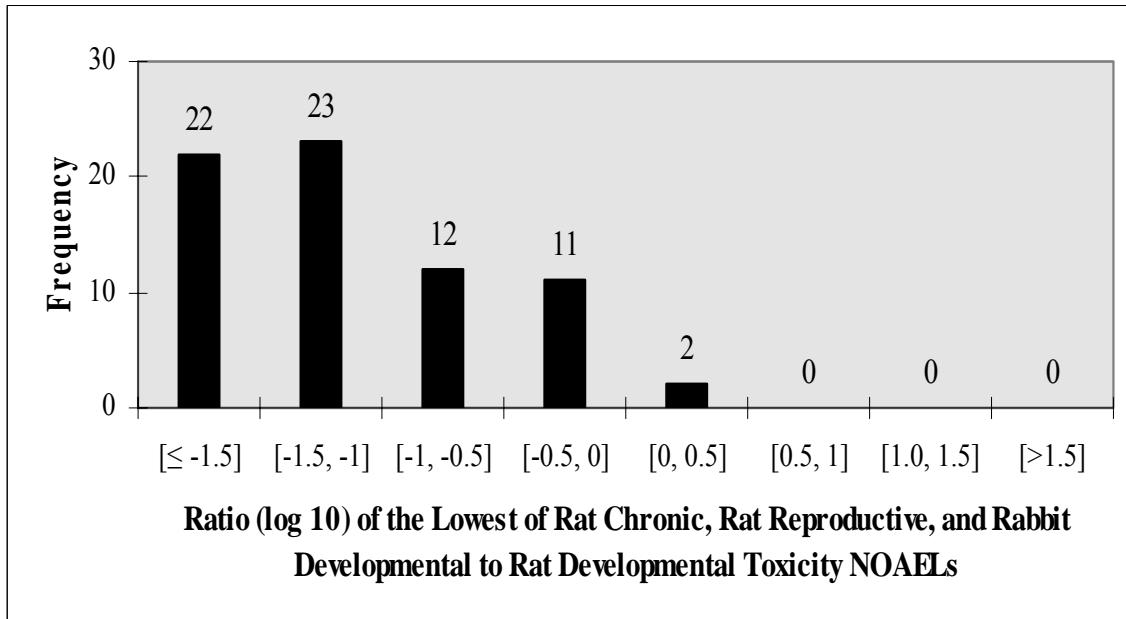


1696
1697
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1699
1700

1701
1702 R5

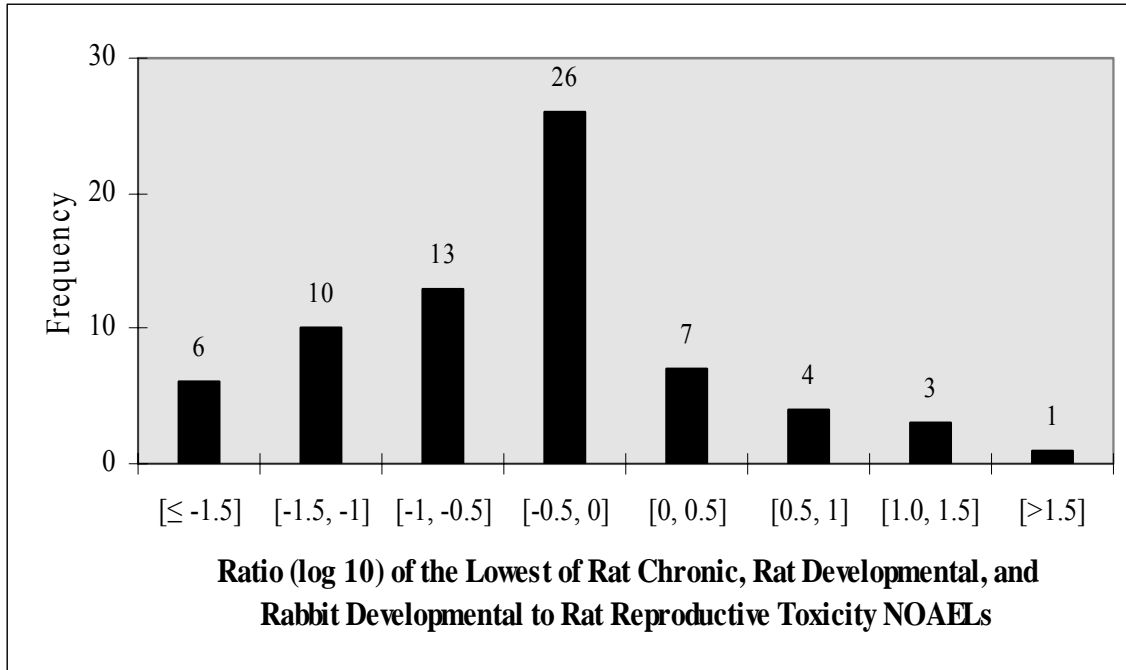


1703
1704
1705
1706
1707 R6



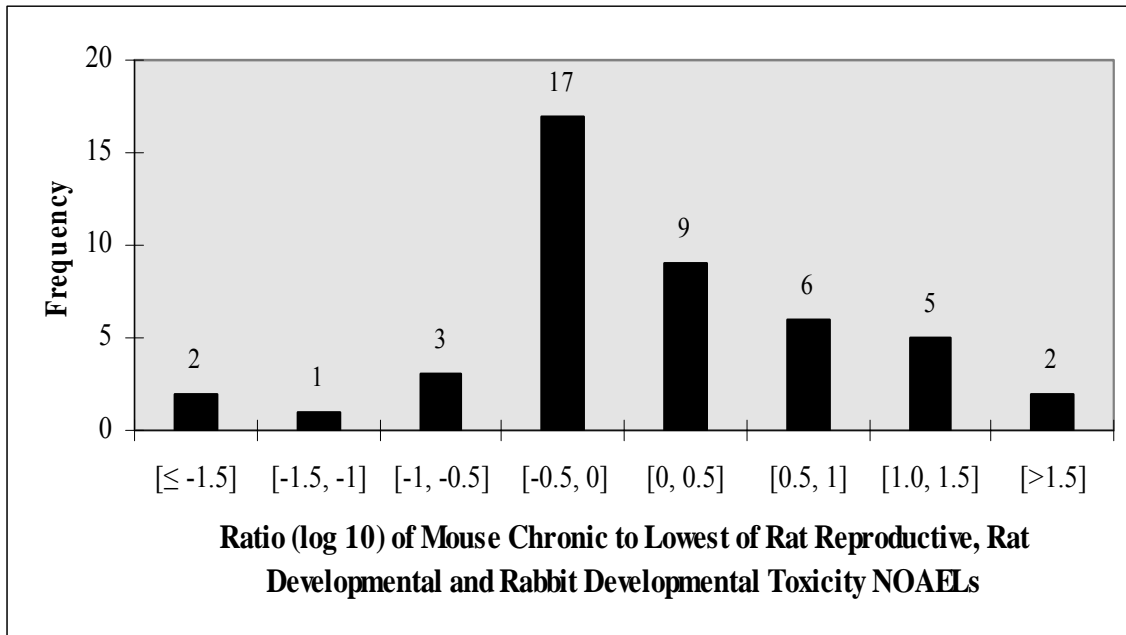
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1711
1712
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1714
1715 R7

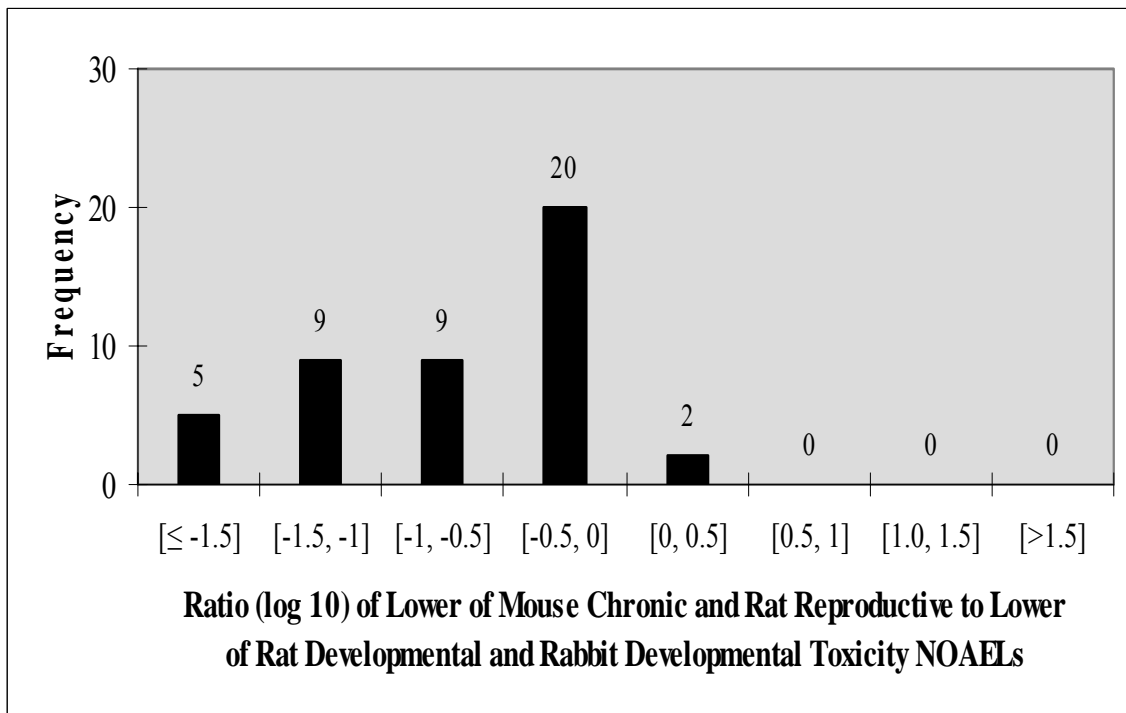


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1742 **Mouse**
1743
1744 M1

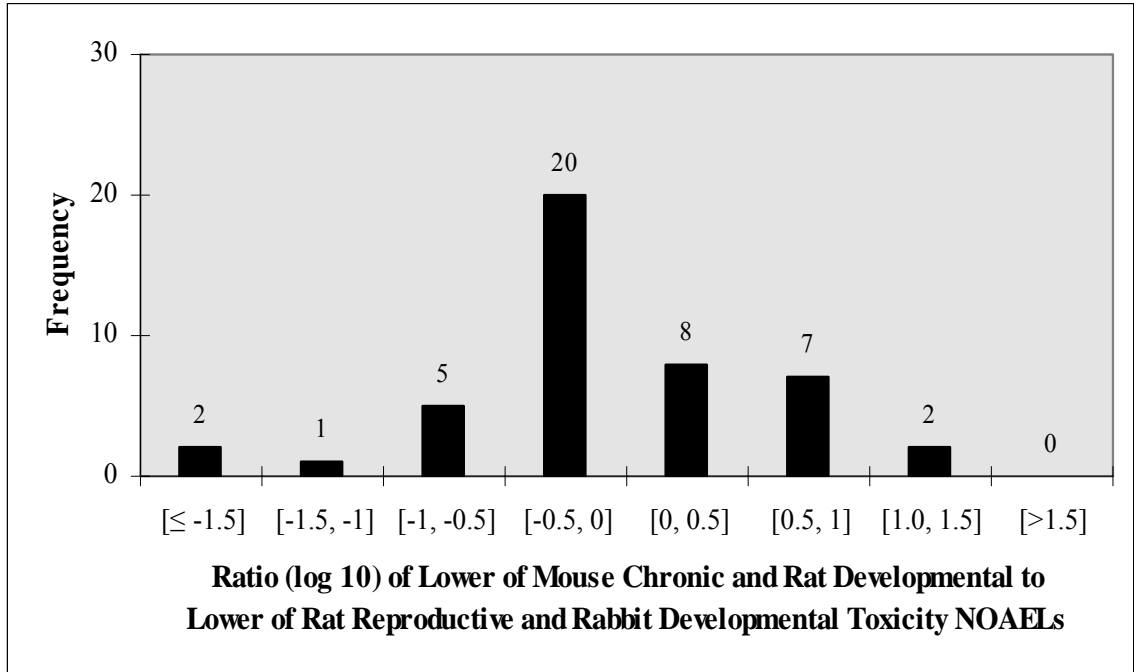


1745
1746
1747
1748
1749 M2

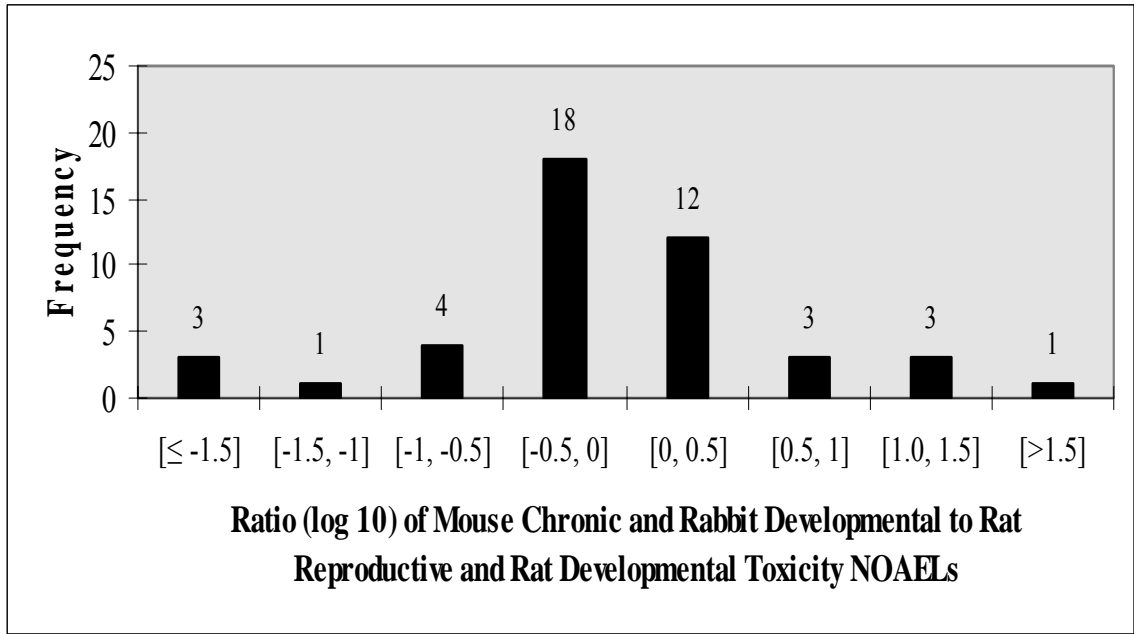


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1751

1752
1753 M3

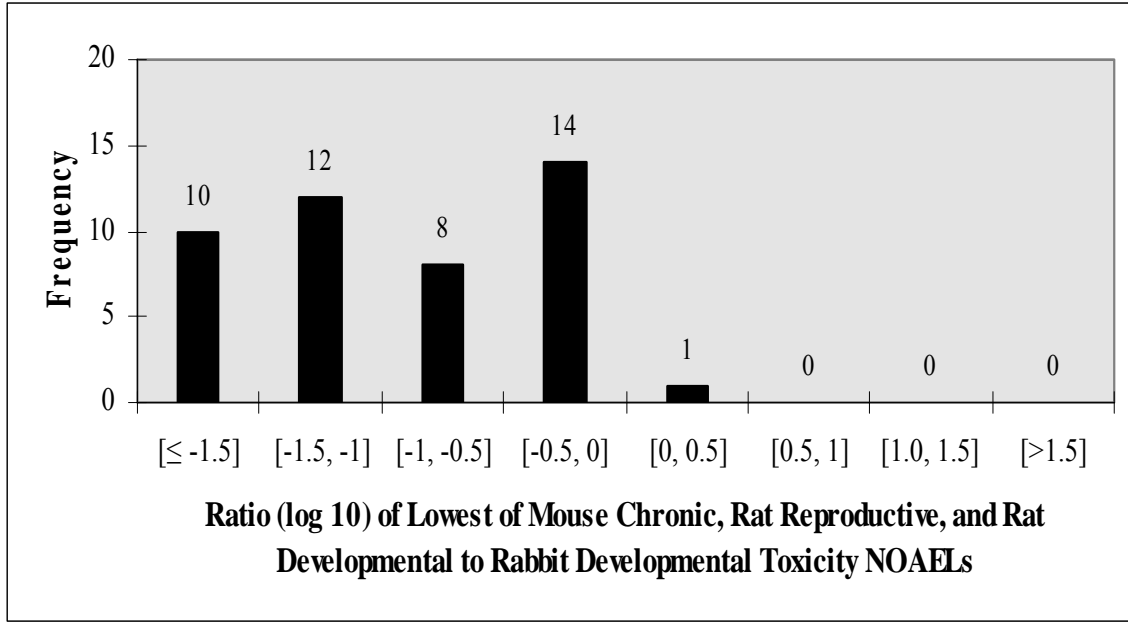


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1755
1756
1757 M4

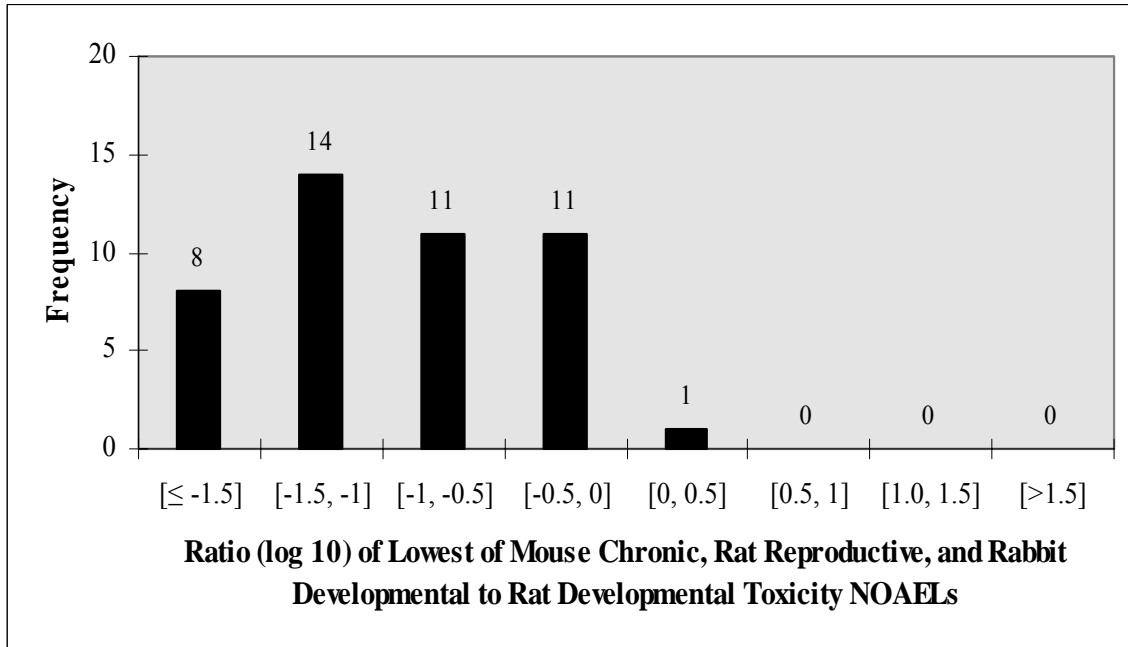


1758
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1764
1765 M5

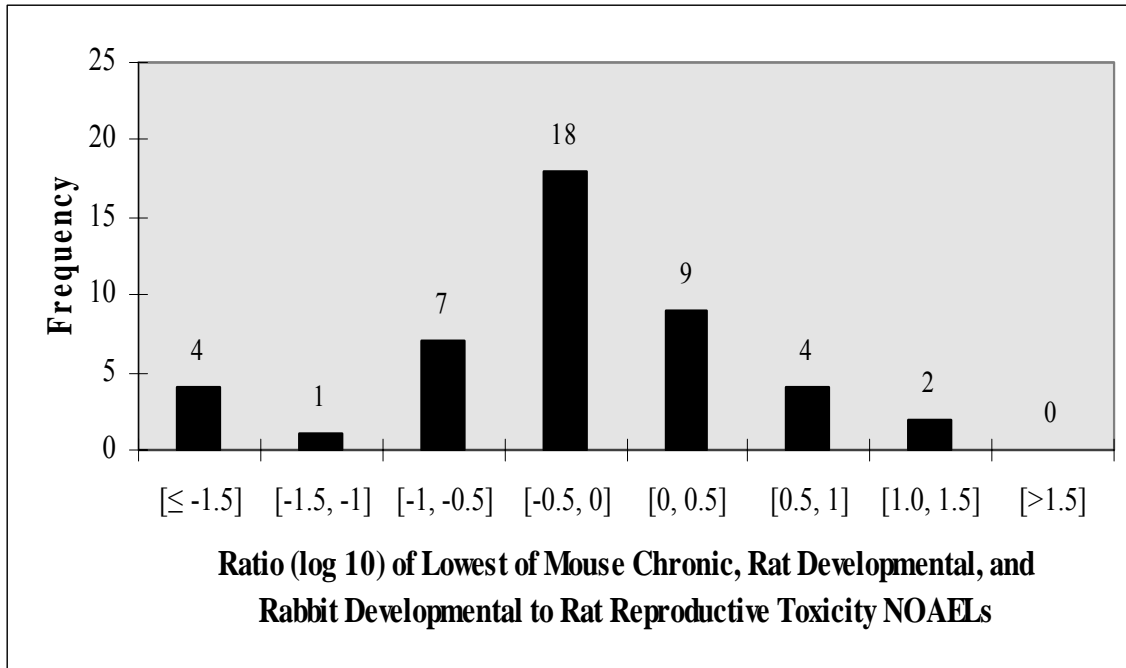


1766
1767
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1769
1770 M6



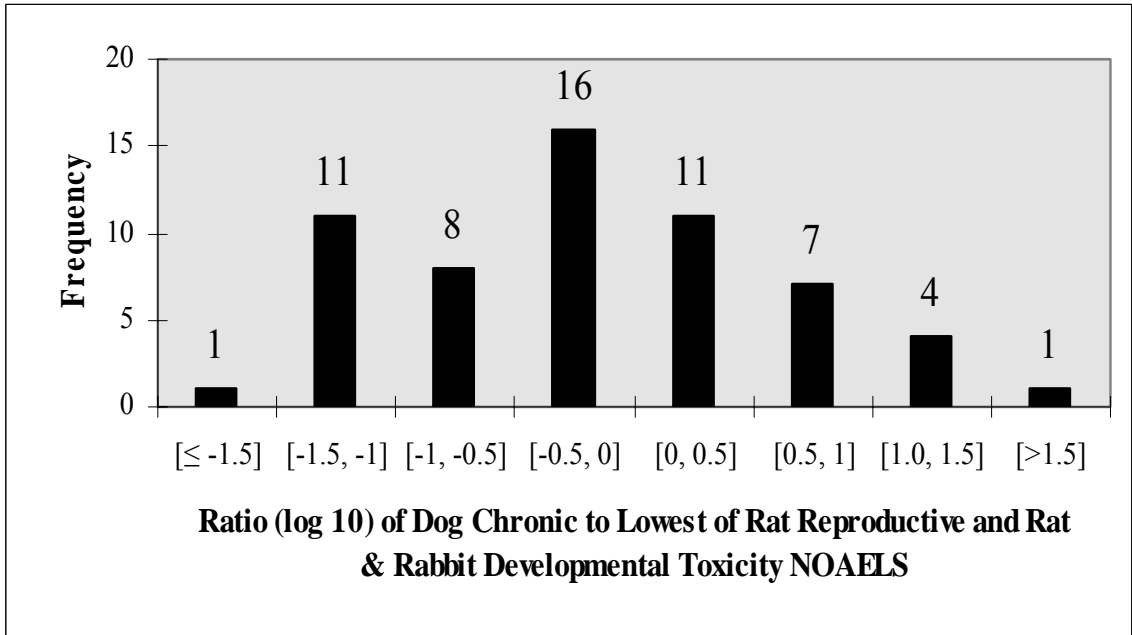
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1778 M7

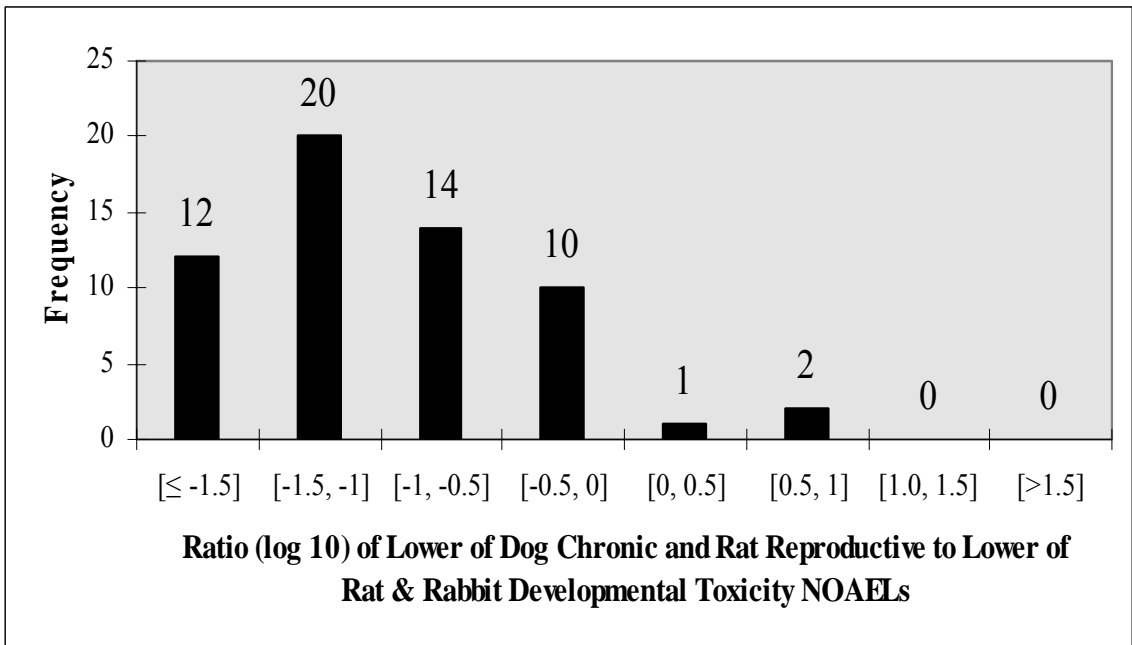


1779
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1782 **Dog**
1783
1784 D1

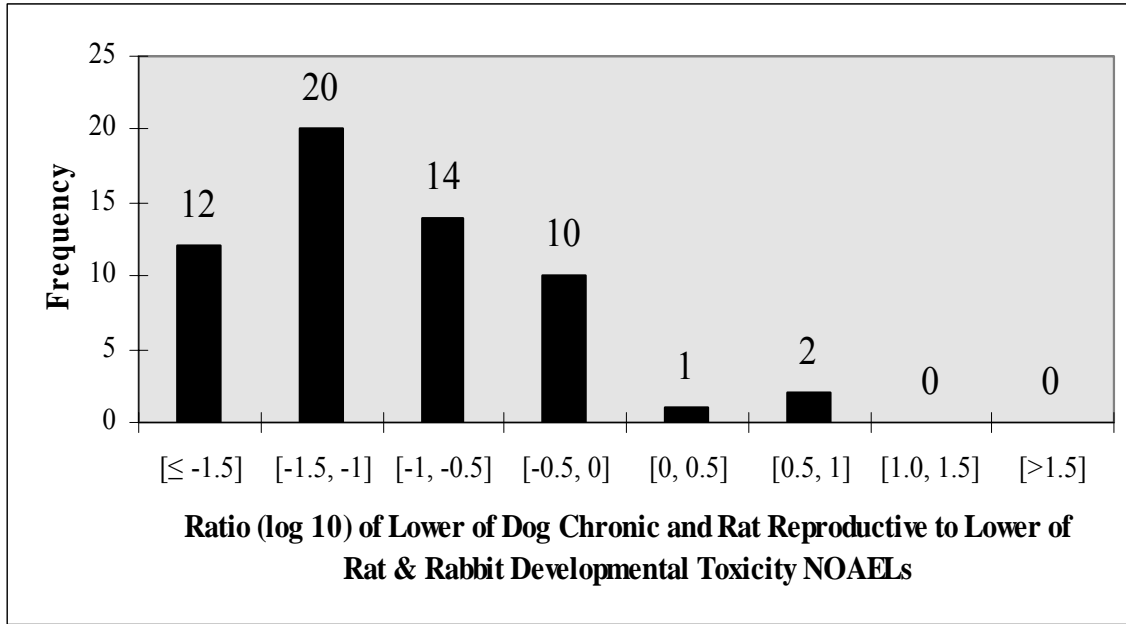


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1786
1787
1788
1789 D2

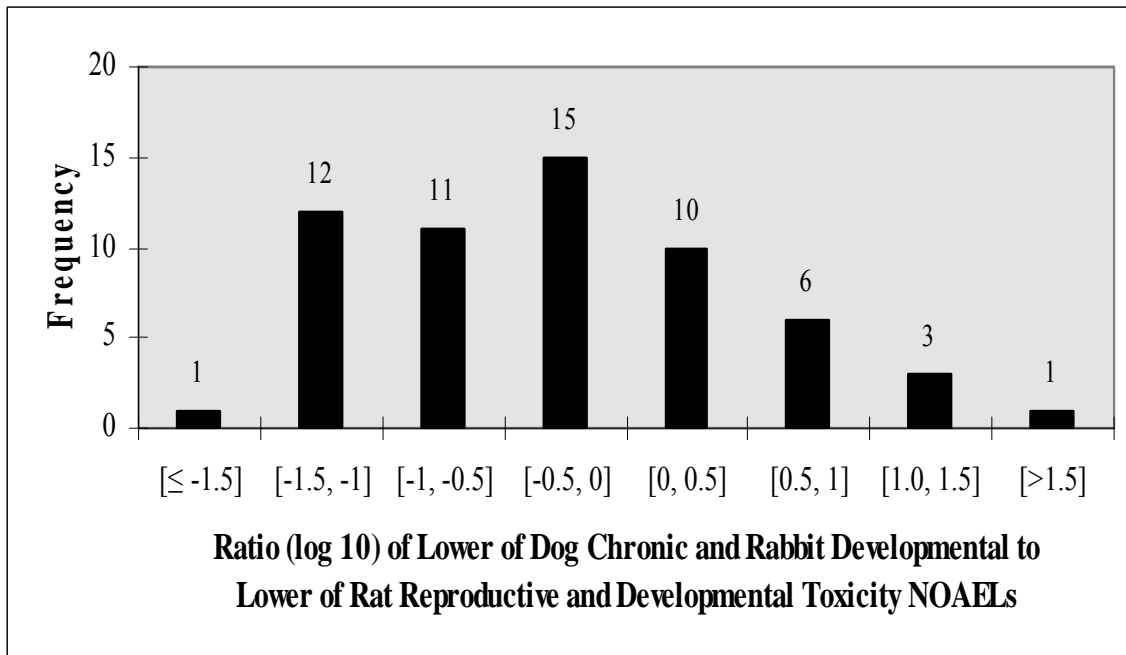


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1791
1792
1793

1794
1795 D3

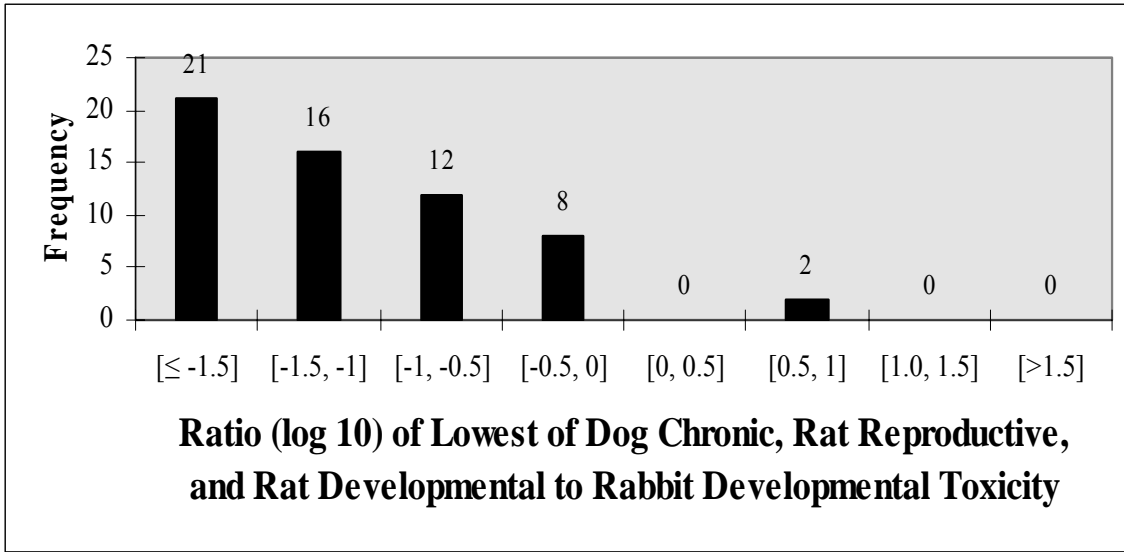


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1800
1801 D4

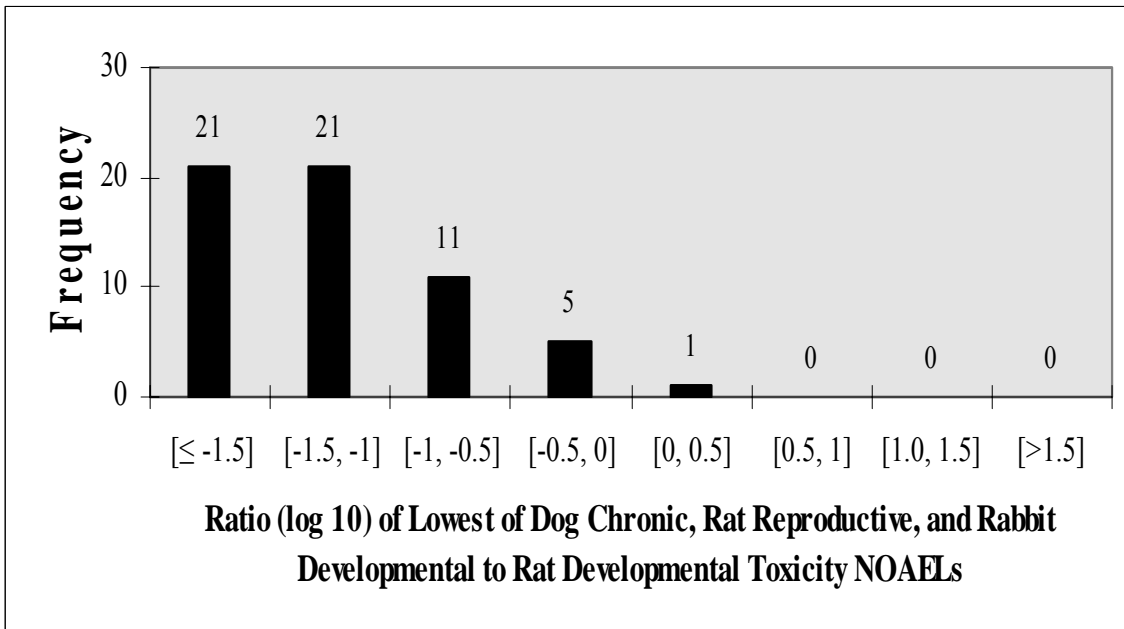


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1804
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1806
1807 D5

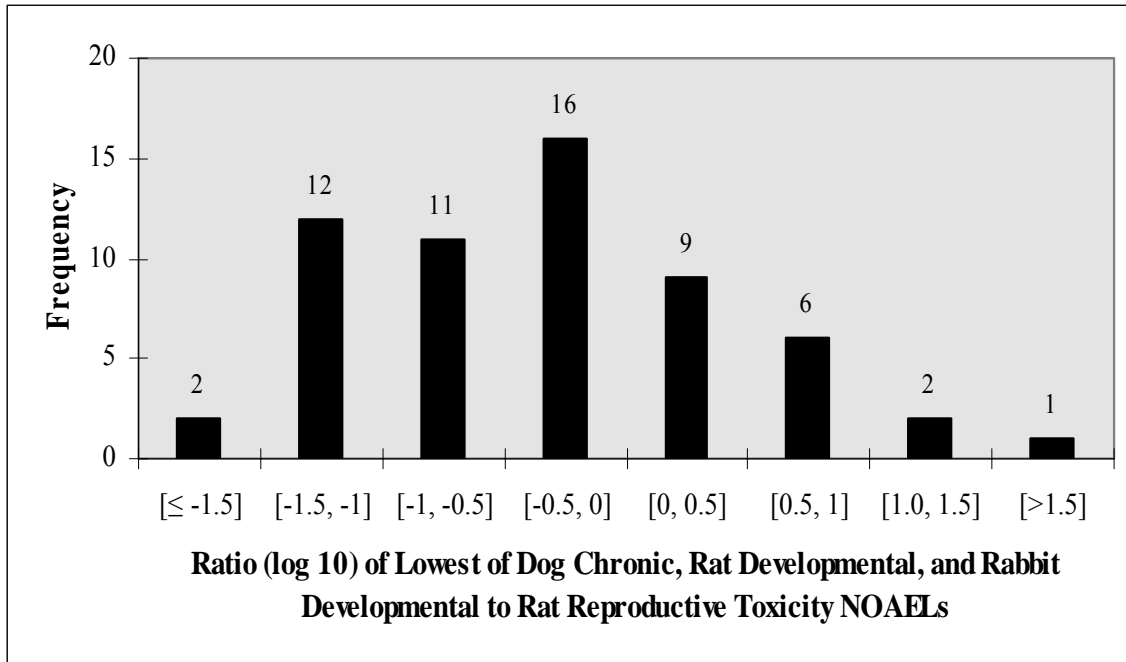


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1809
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1812
1813 D6



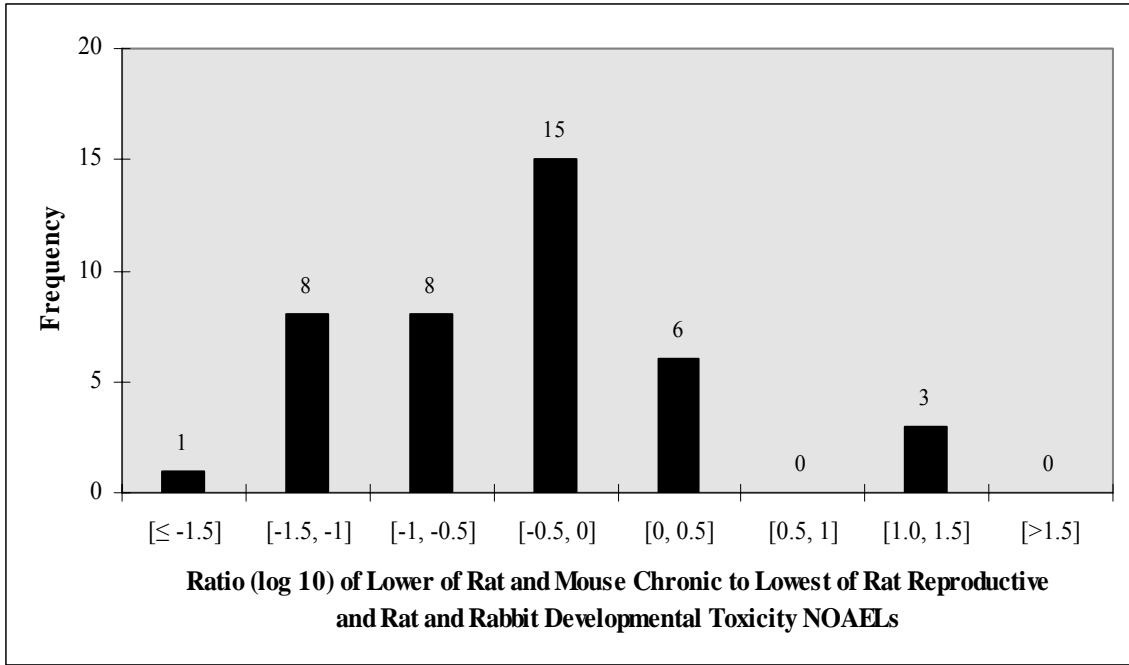
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1821 D7

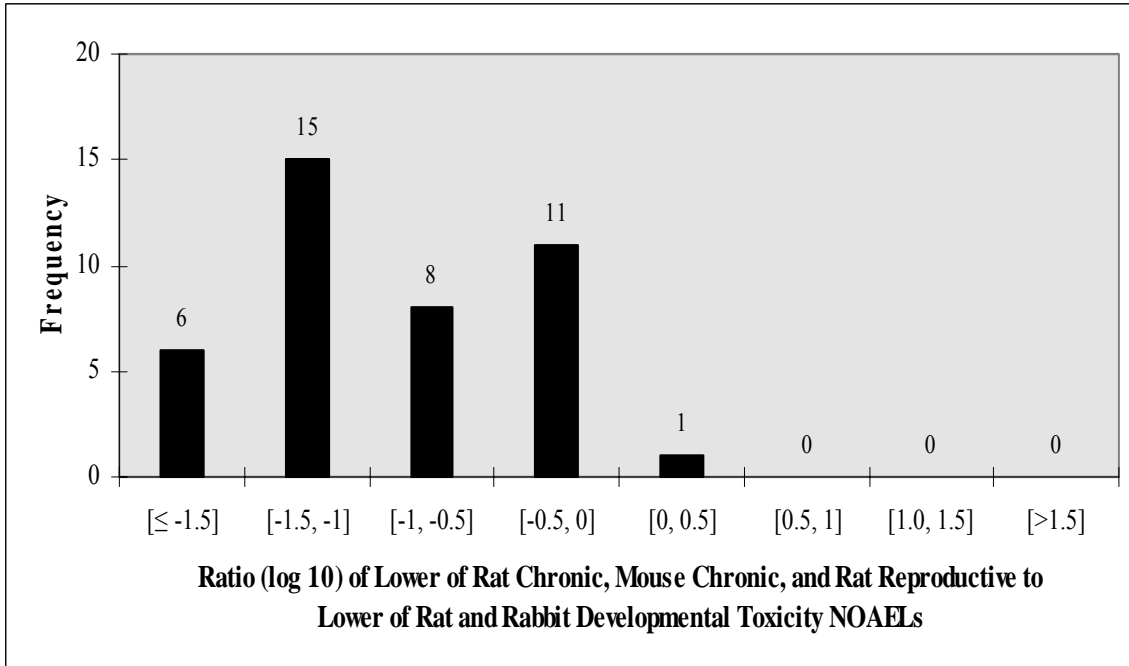


1822
1823
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1825 **Rat and Mouse**
1826
1827 RM1

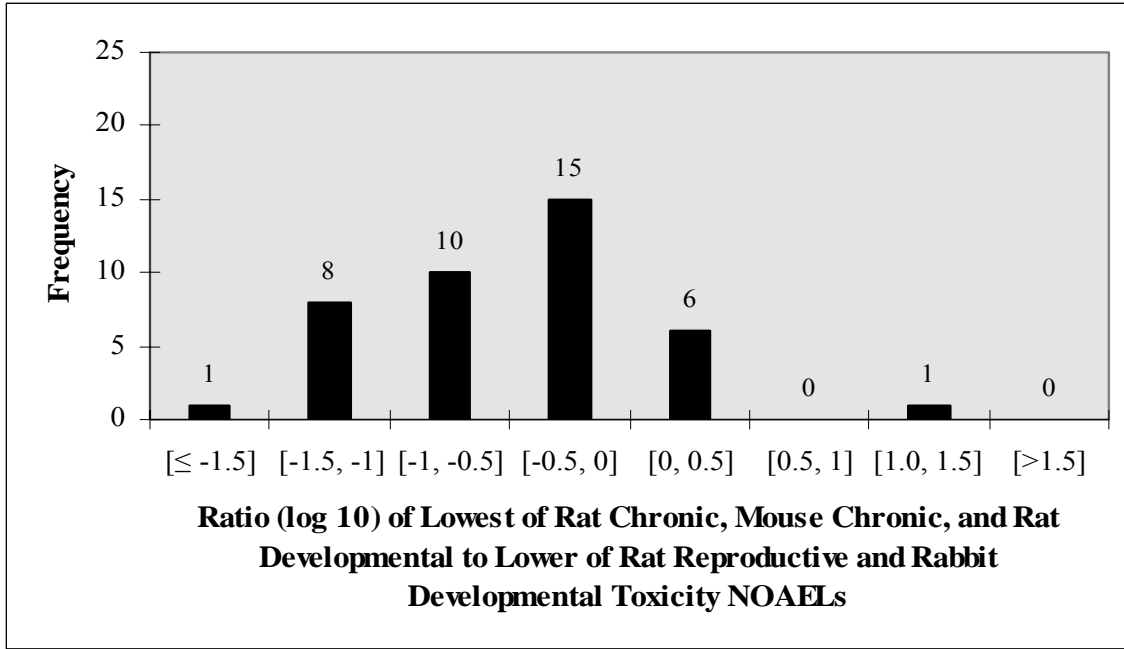


1828
1829
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1831
1832 RM2

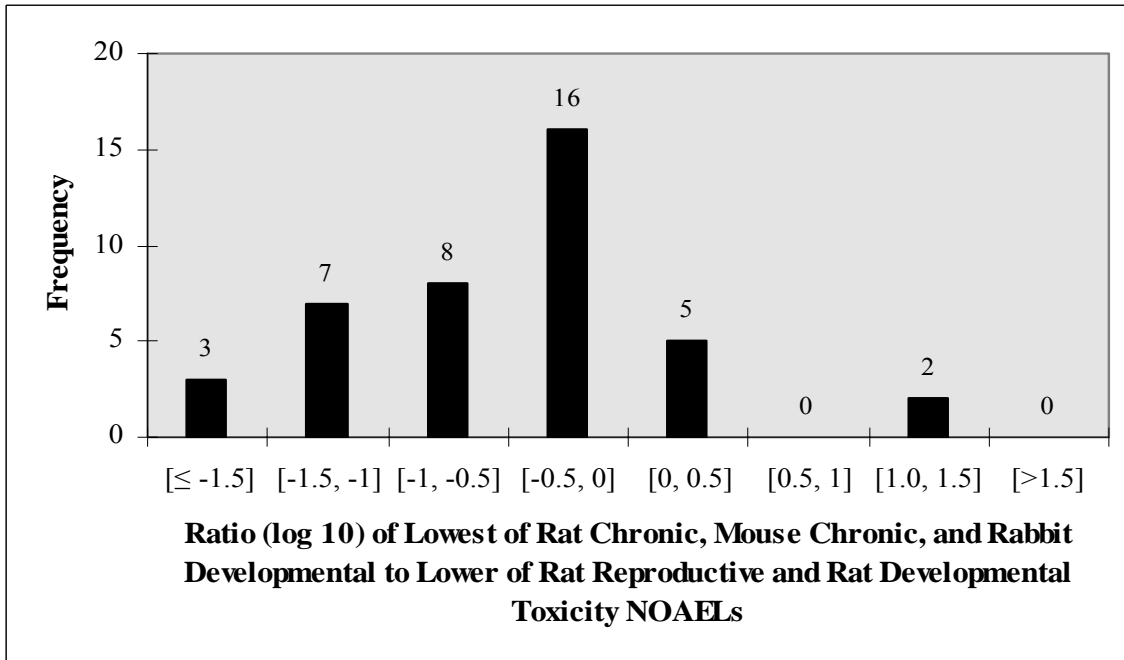


1833
1834
1835

1836
1837 RM3

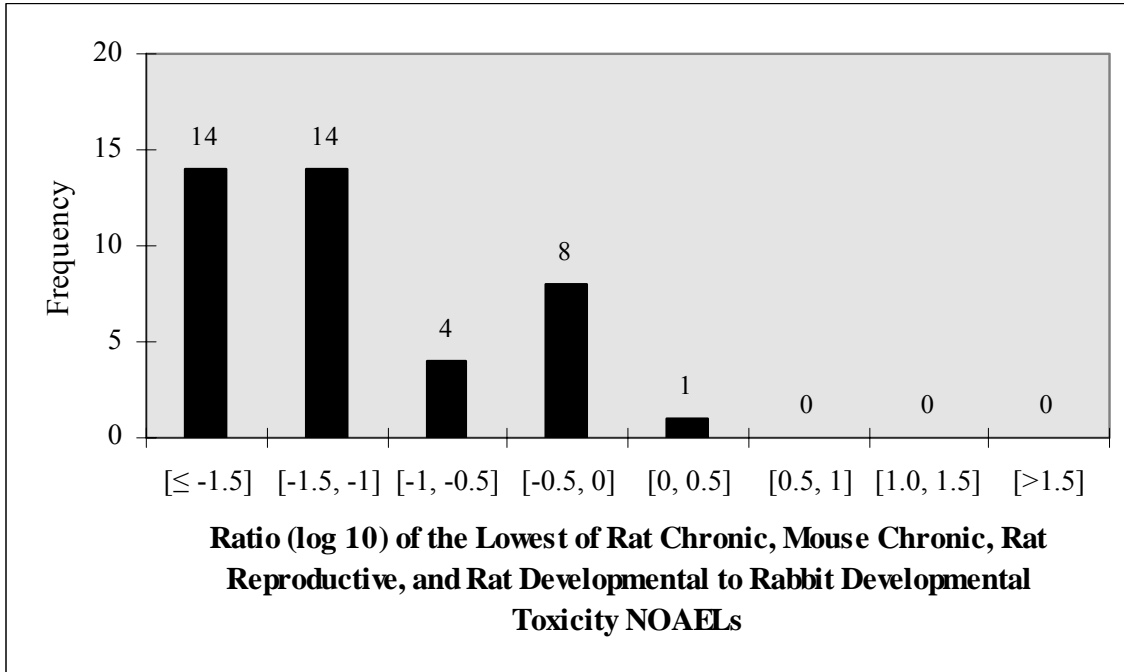


1838
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1841
1842
1843 RM4

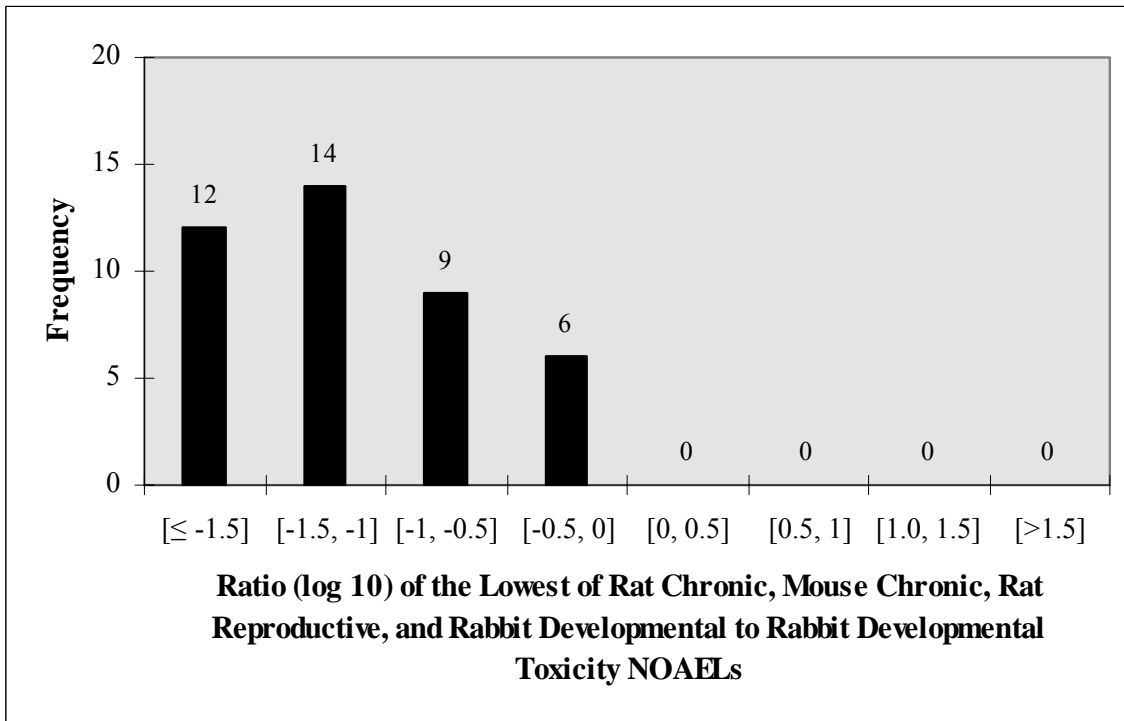


1844
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1846
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1848
1849 RM5

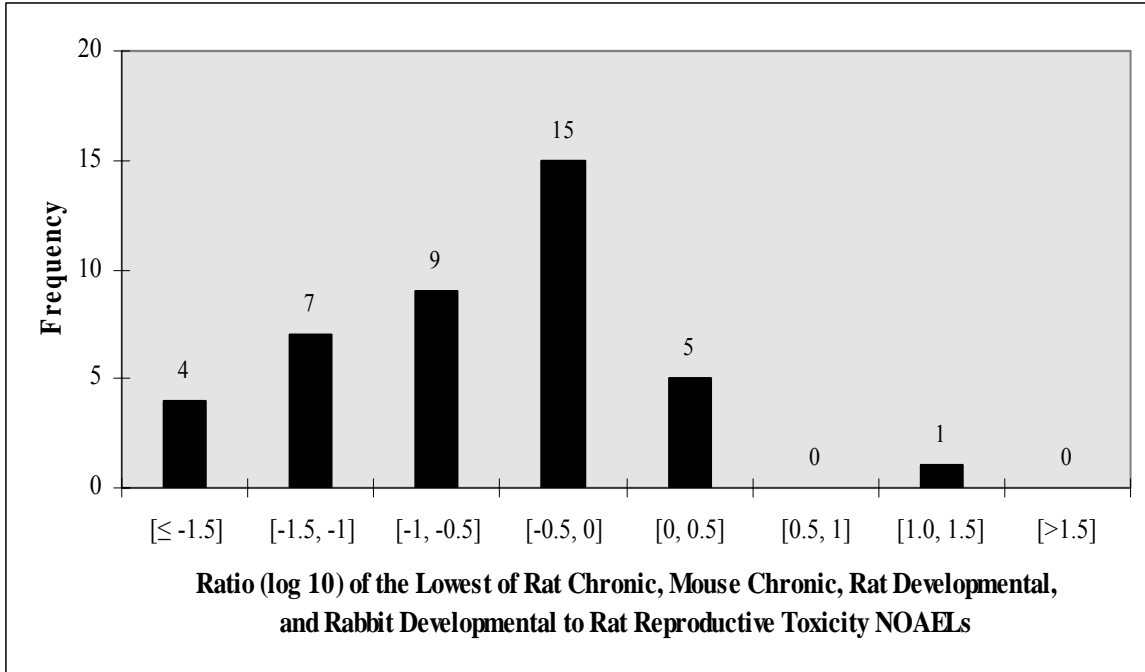


1850
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1854 RM6



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1856

1857
1858 RM7



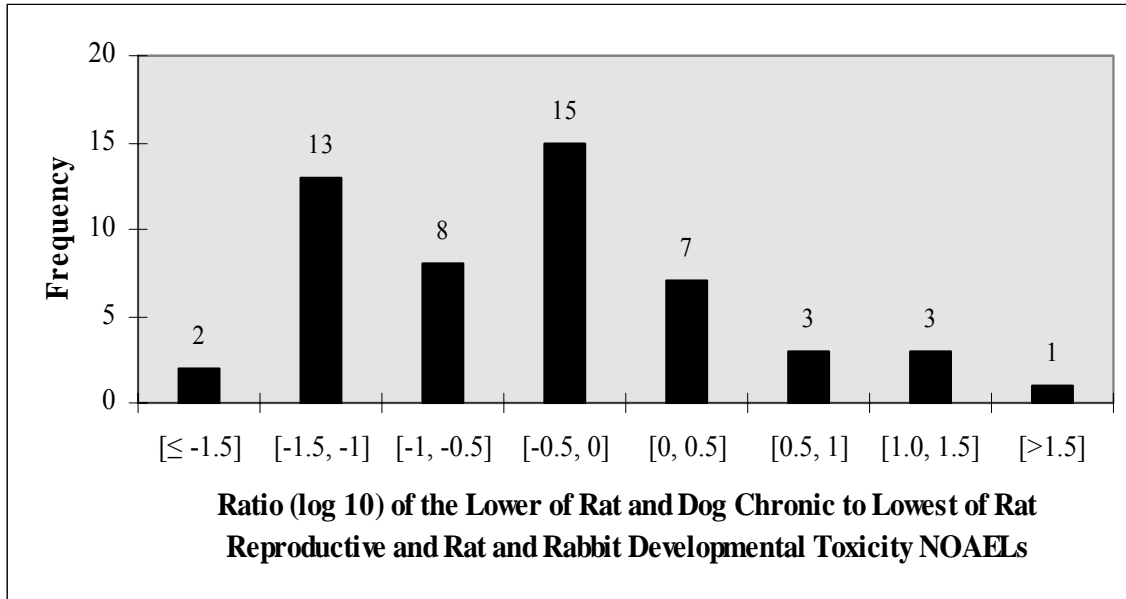
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1863 **Rat and Dog**

1864

1865

1866 RG1

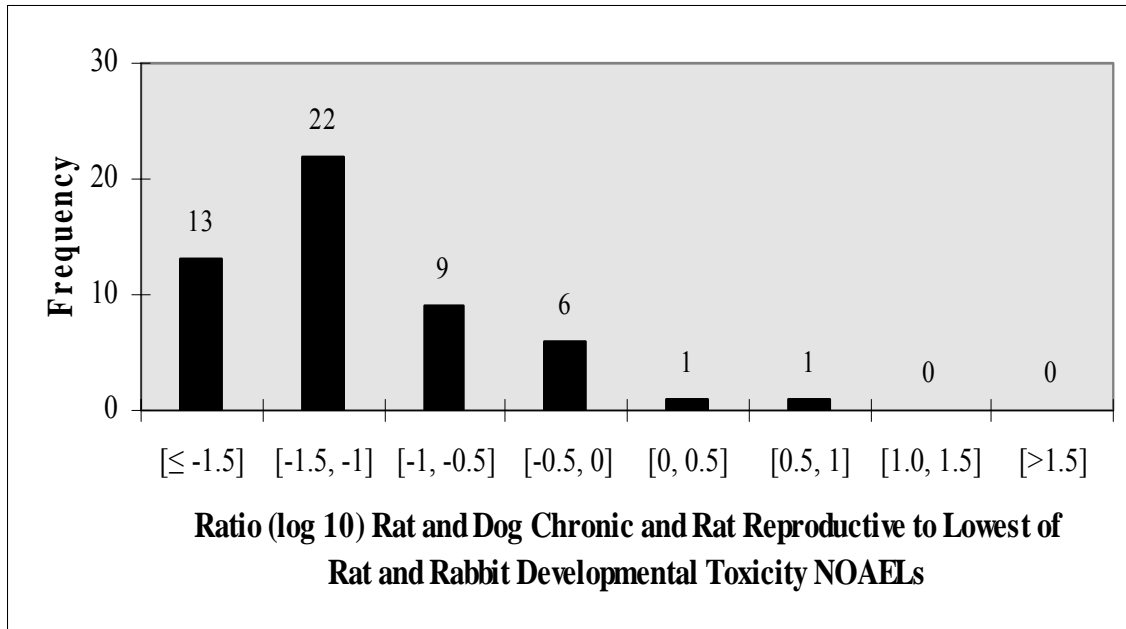


1867

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1870 RG2

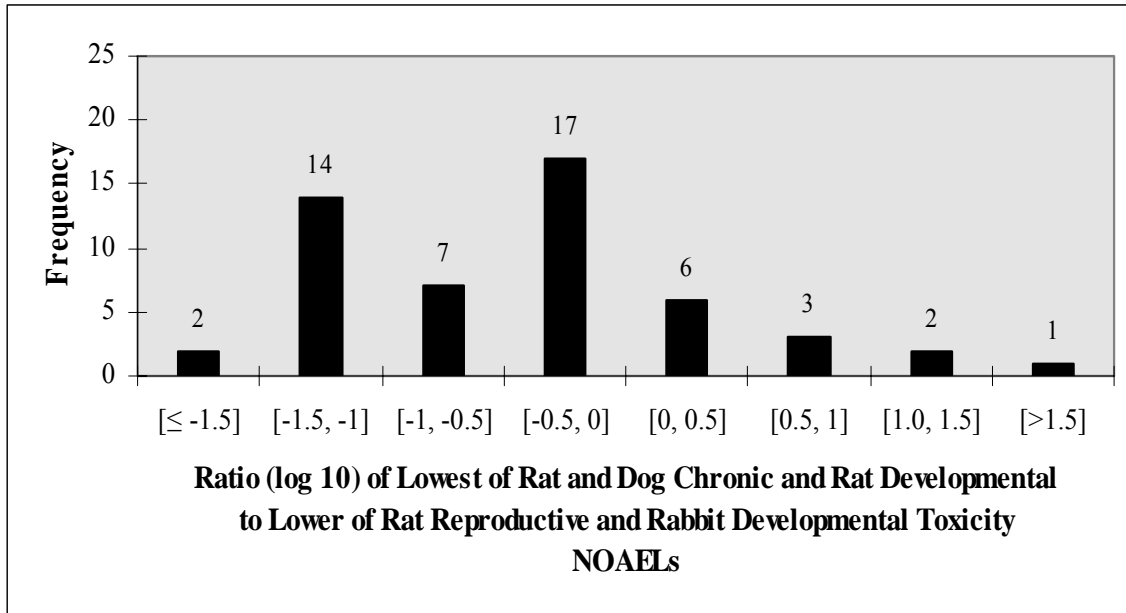


1871

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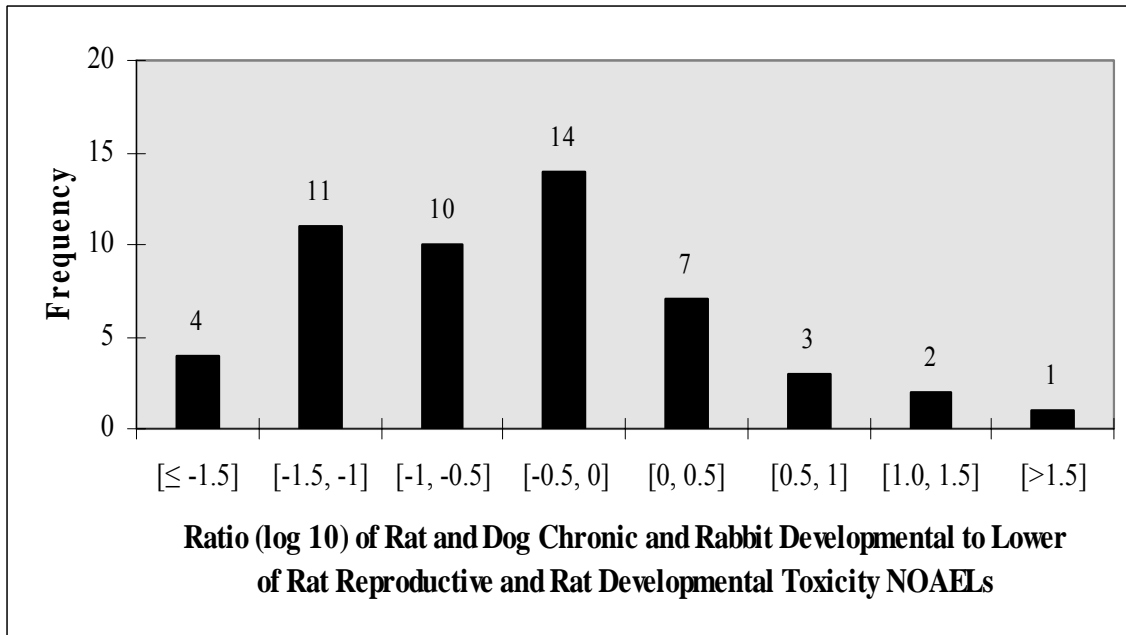
1873

1874 RG3



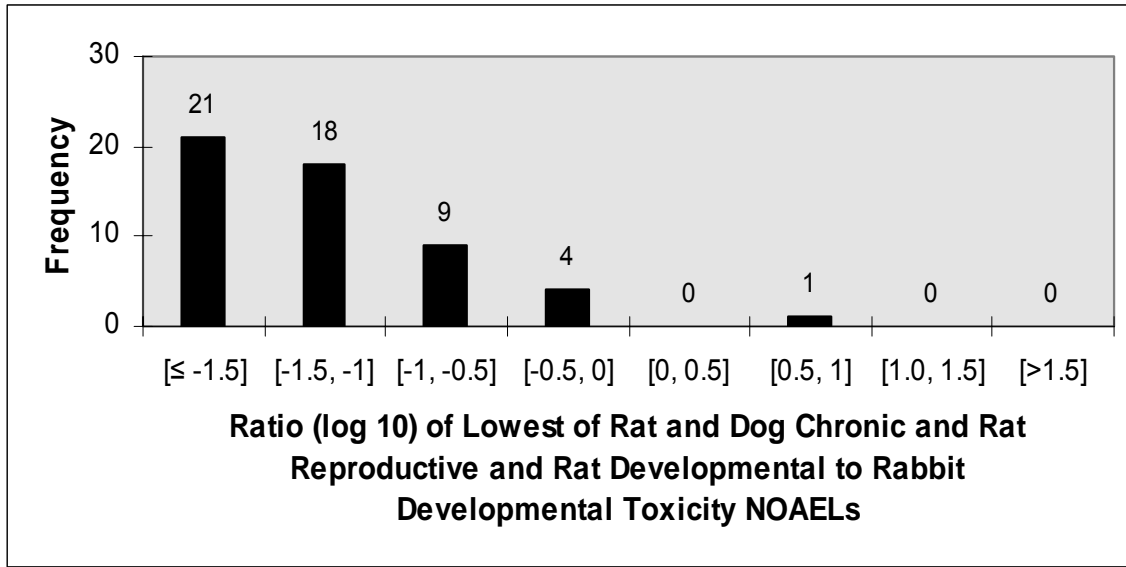
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RG4



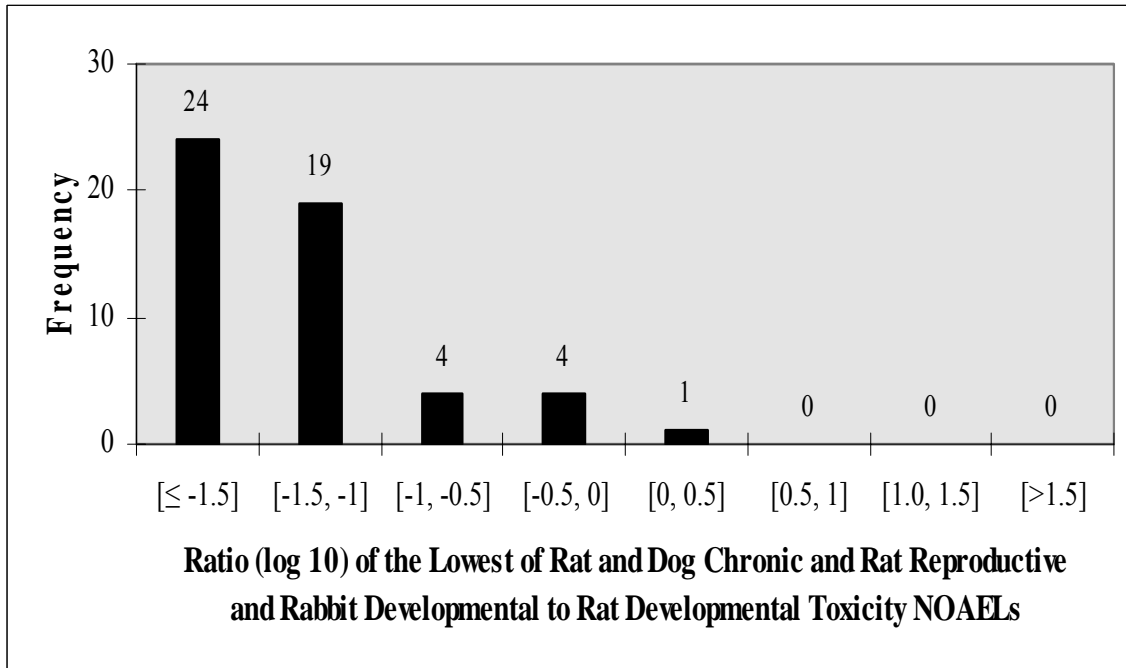
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1882 RG5



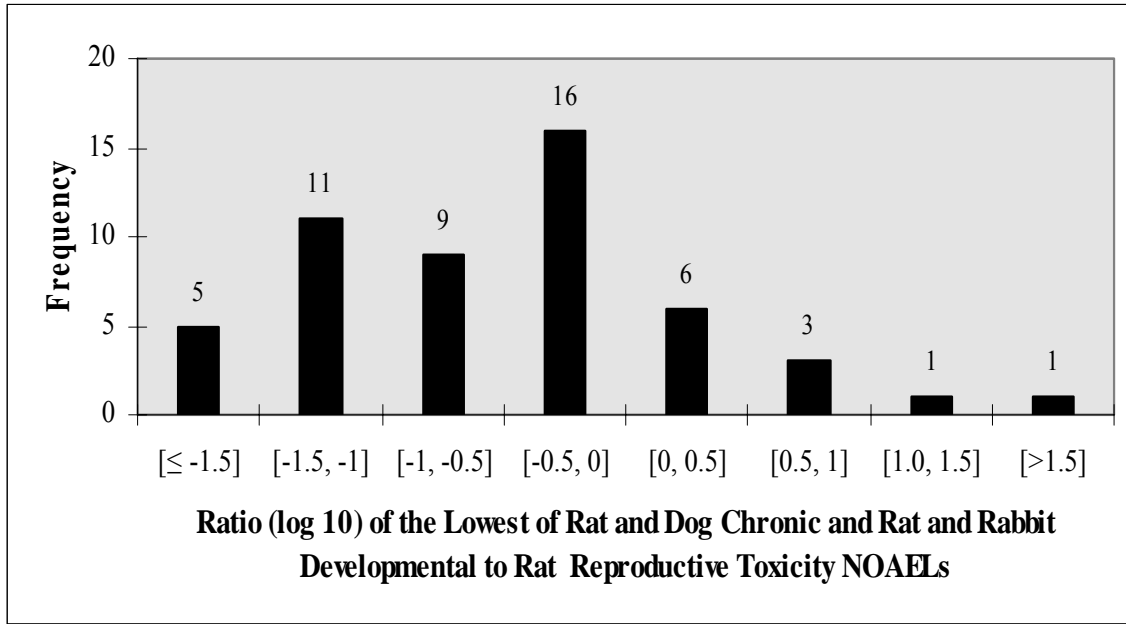
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1887

RG6



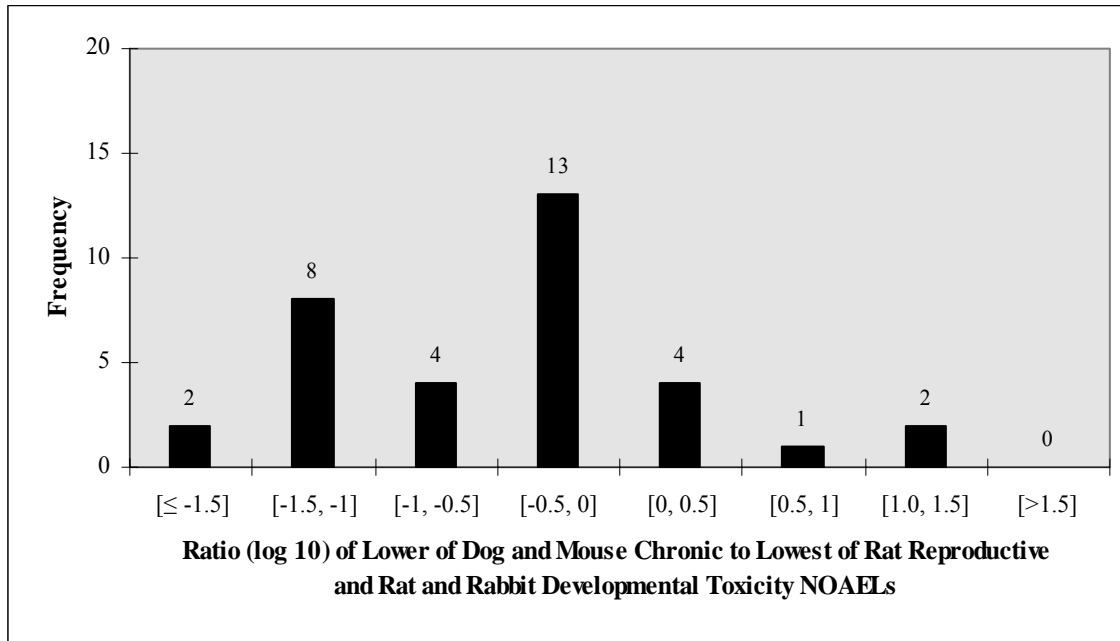
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1889
1890

1891
1892 RG7

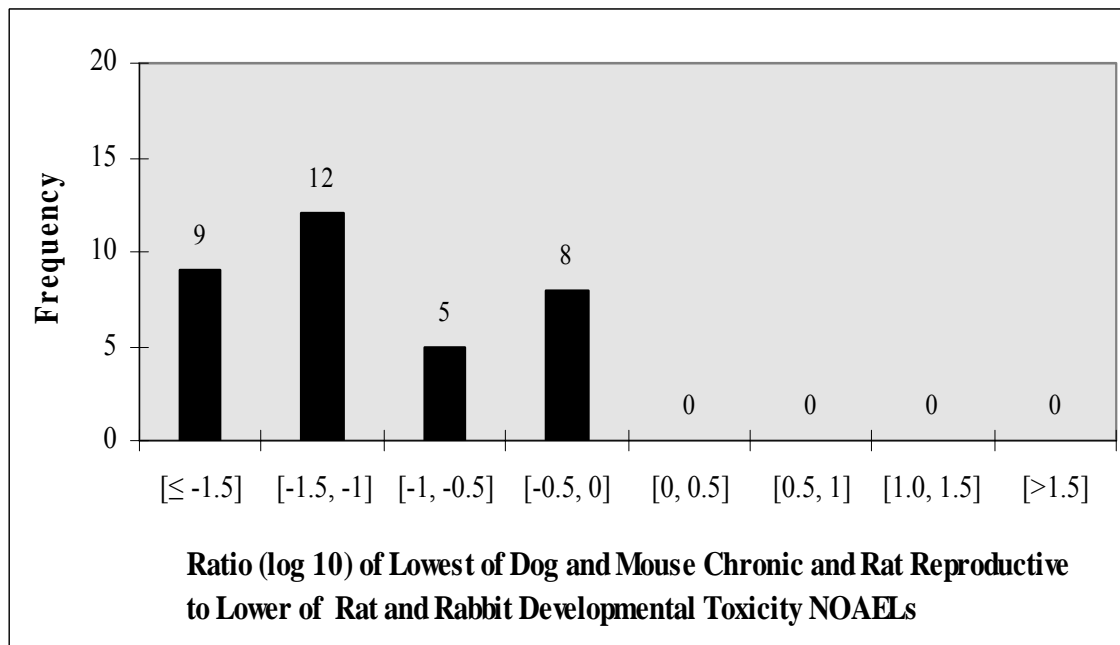


1893
1894
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1897 **Dog and Mouse**
1898
1899 DM1

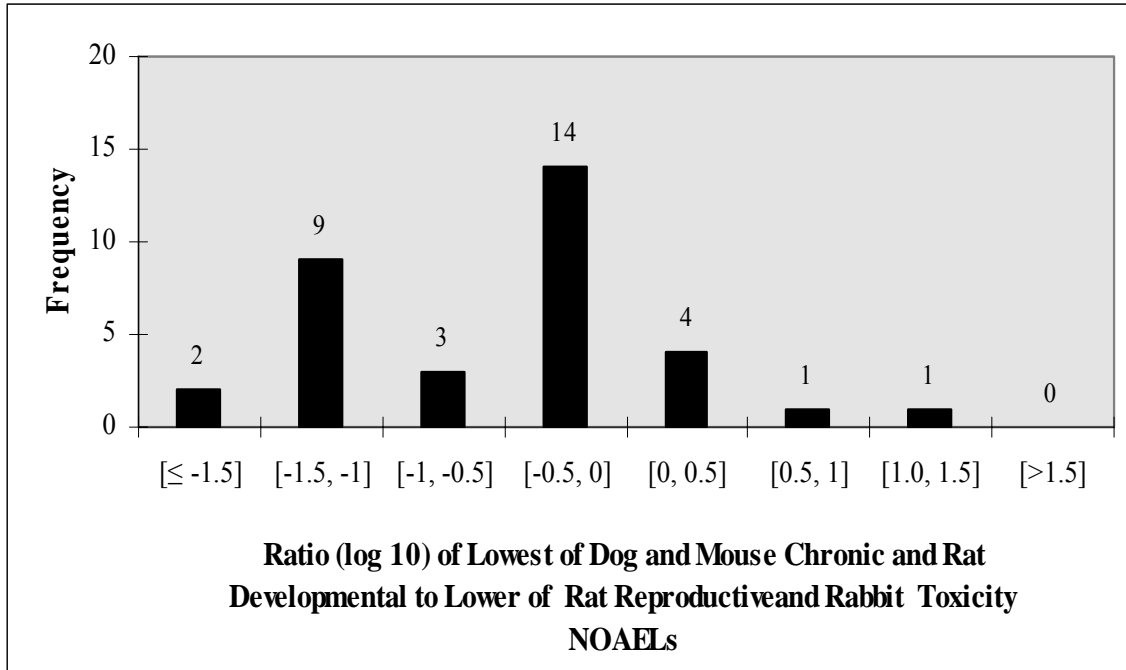


1900
1901
1902
1903
1904 DM2

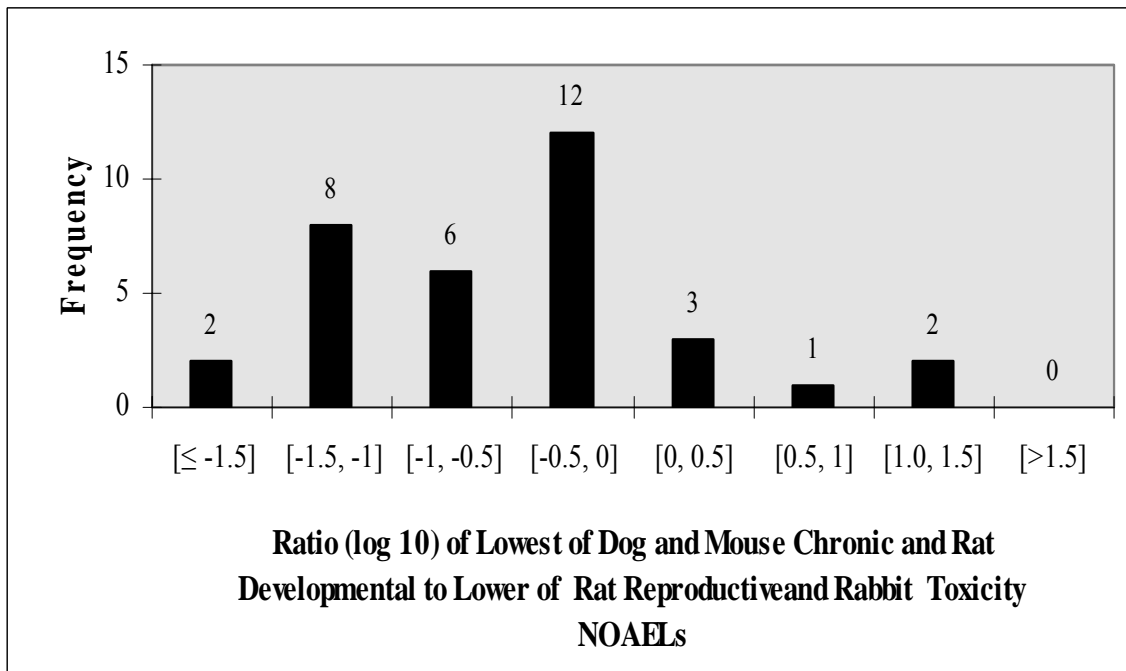


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1906
1907
1908

1909
1910 DM3

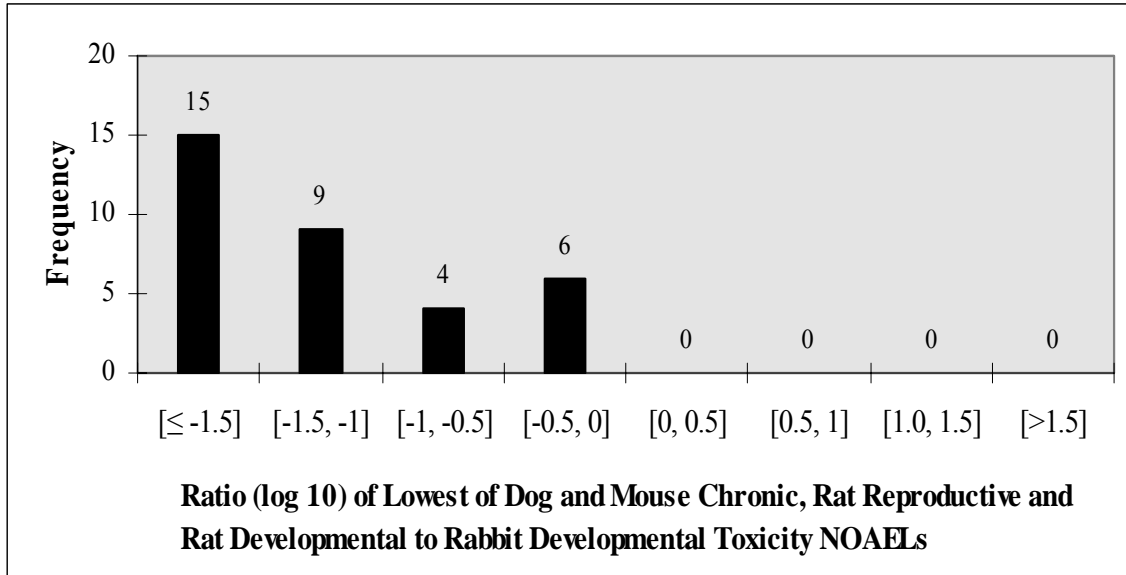


1911
1912
1913
1914
1915 DM4

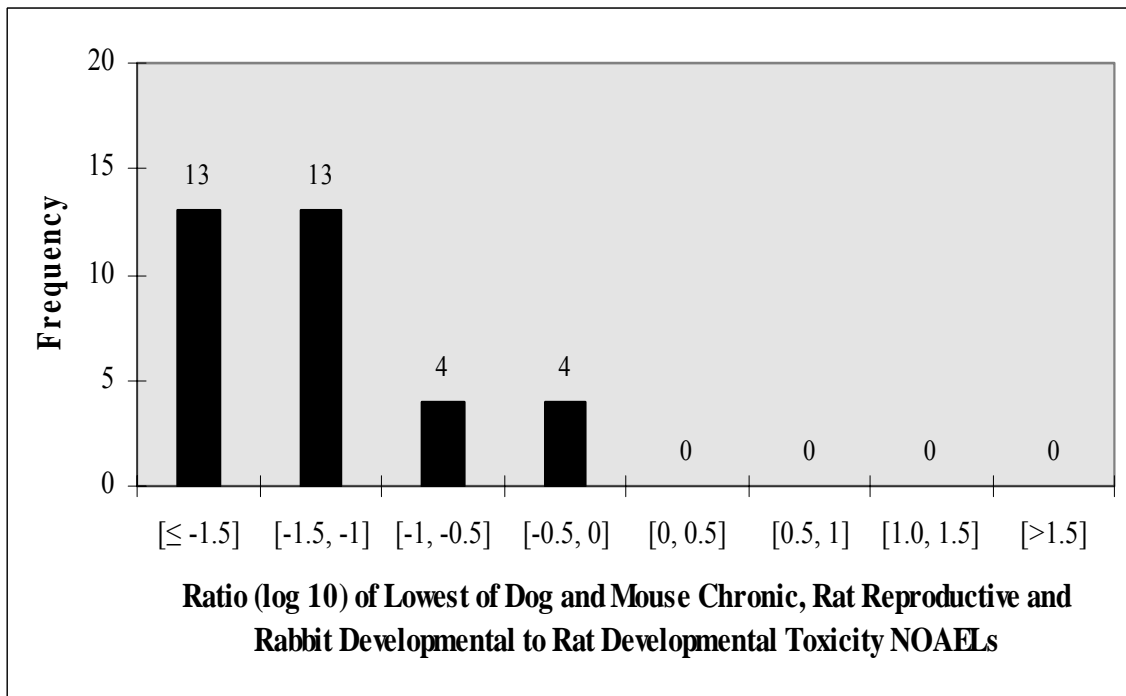


1916
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1918
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1920
1921 DM5

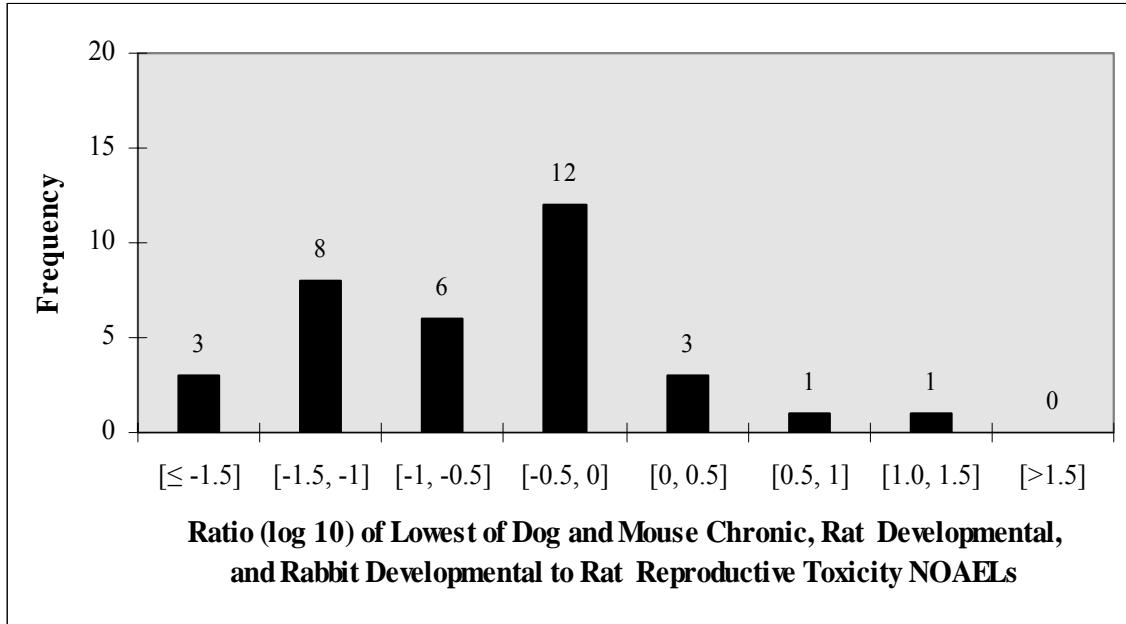


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1925
1926 DM6



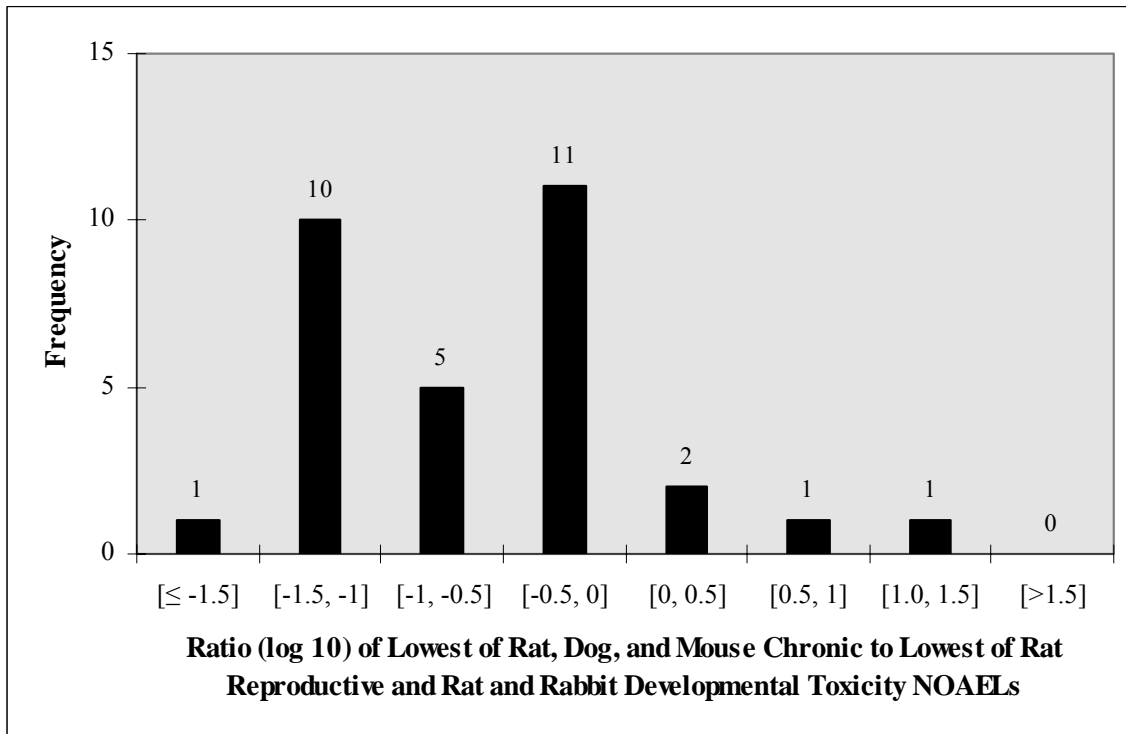
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1932

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1934 DM7

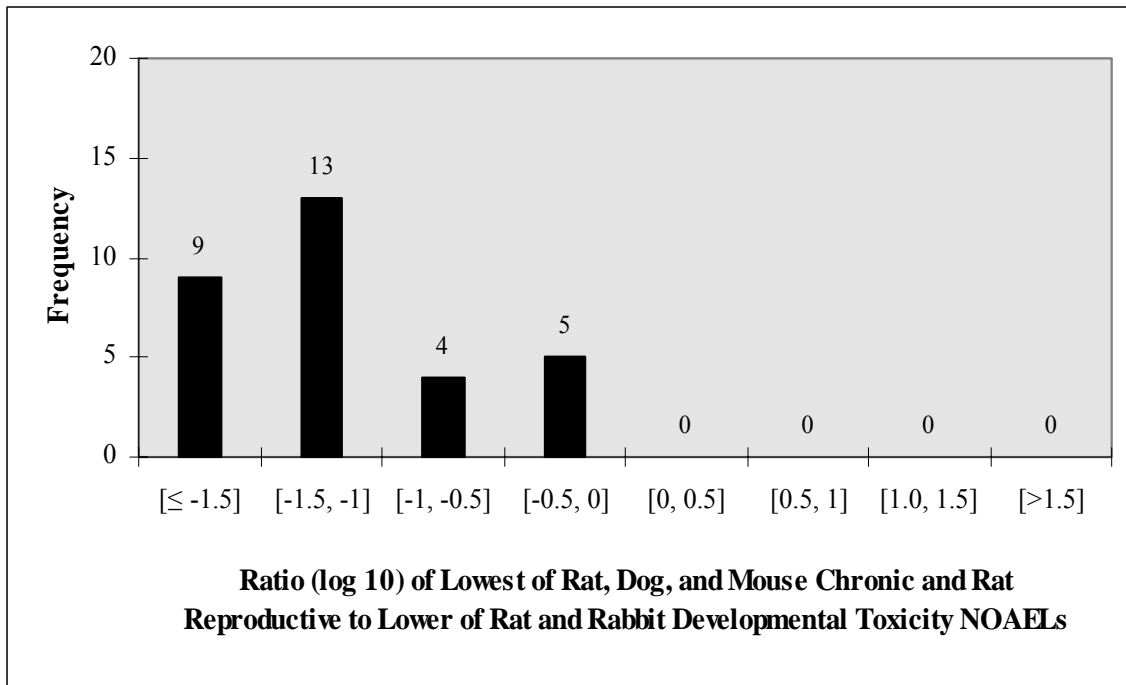


1935
1936
1937
1938
1939

1940 **Rat, Mouse, and Dog**
1941
1942 RMD1

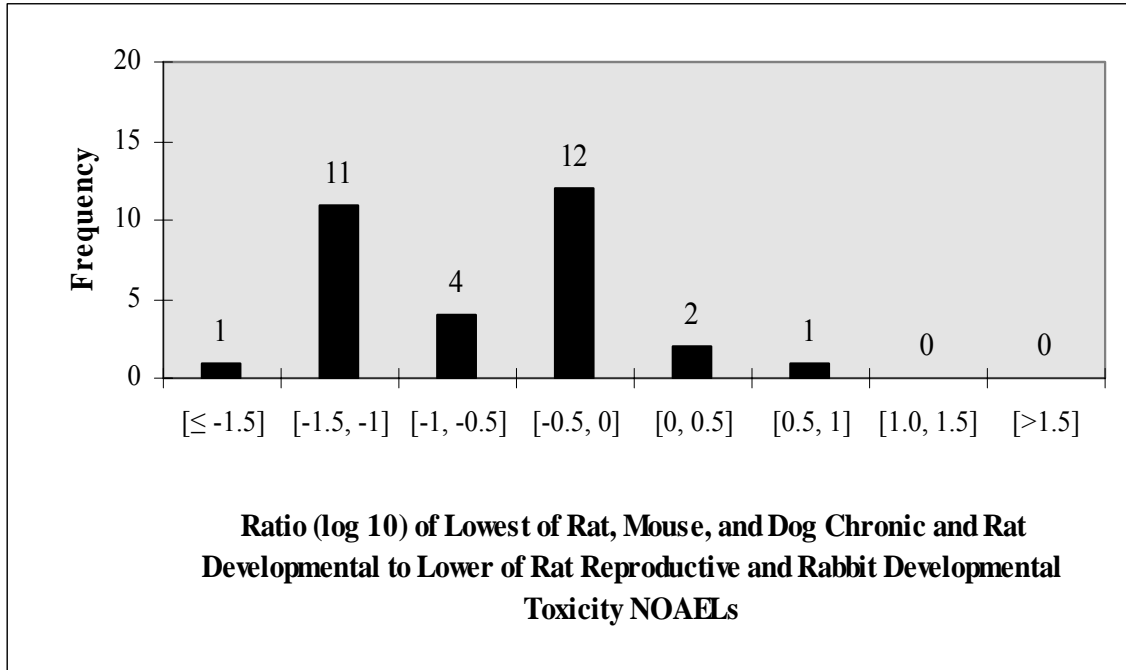


1943
1944
1945
1946 RMD2

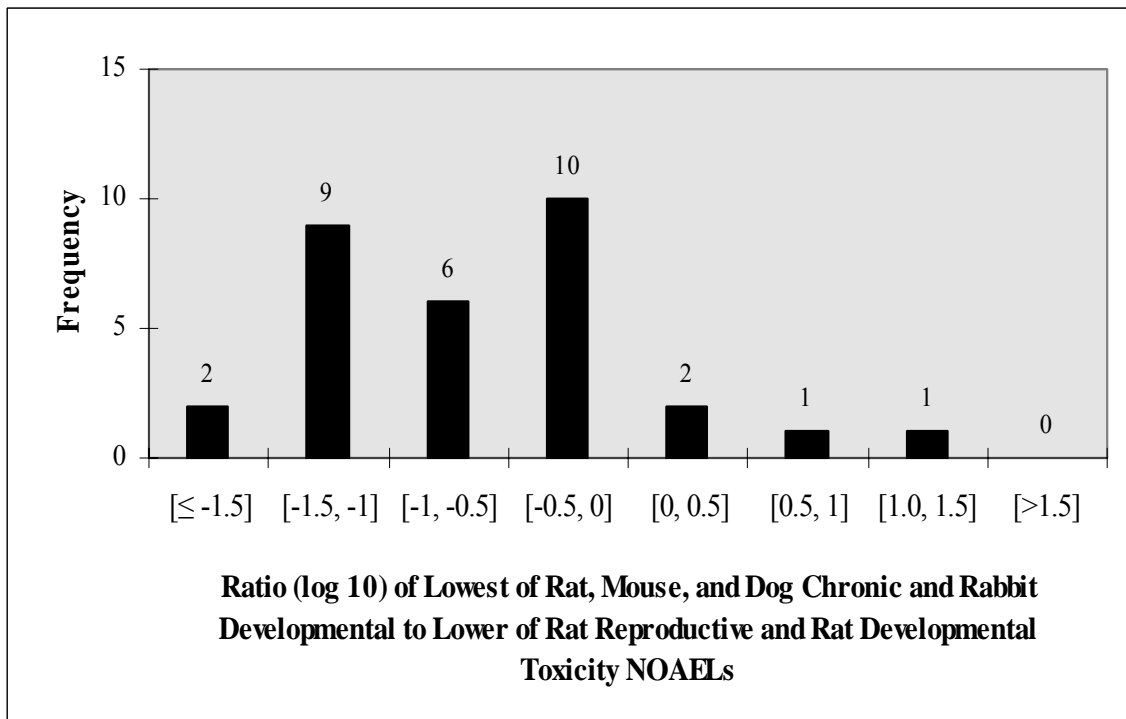


1947
1948

1949
1950 RMD3

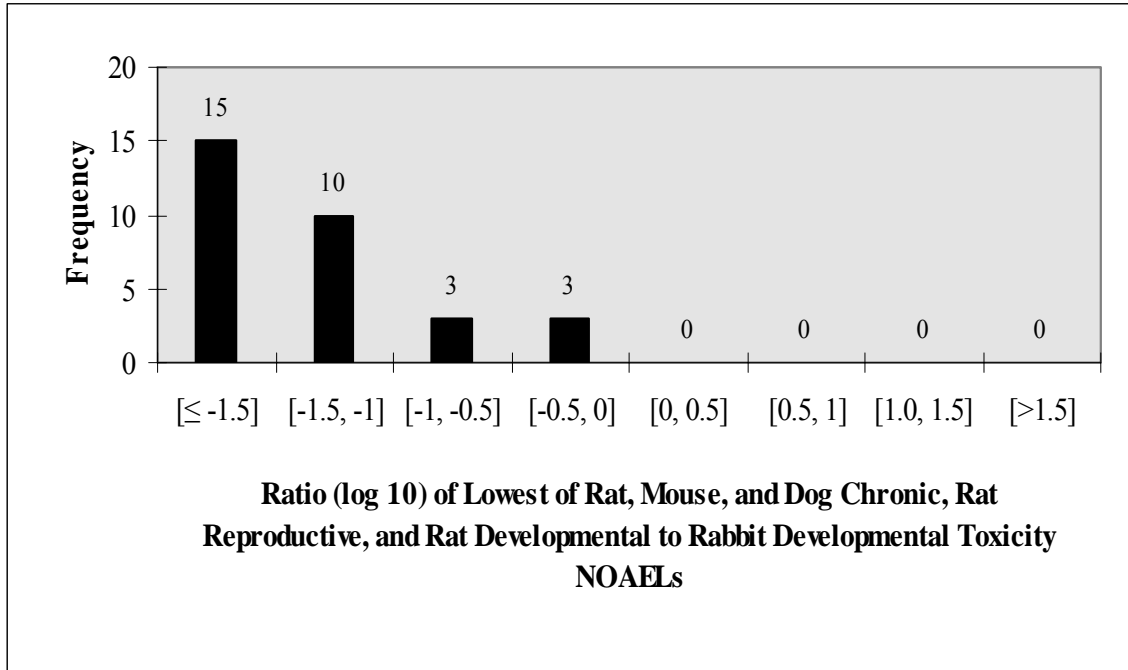


1951
1952
1953
1954
1955 RMD4

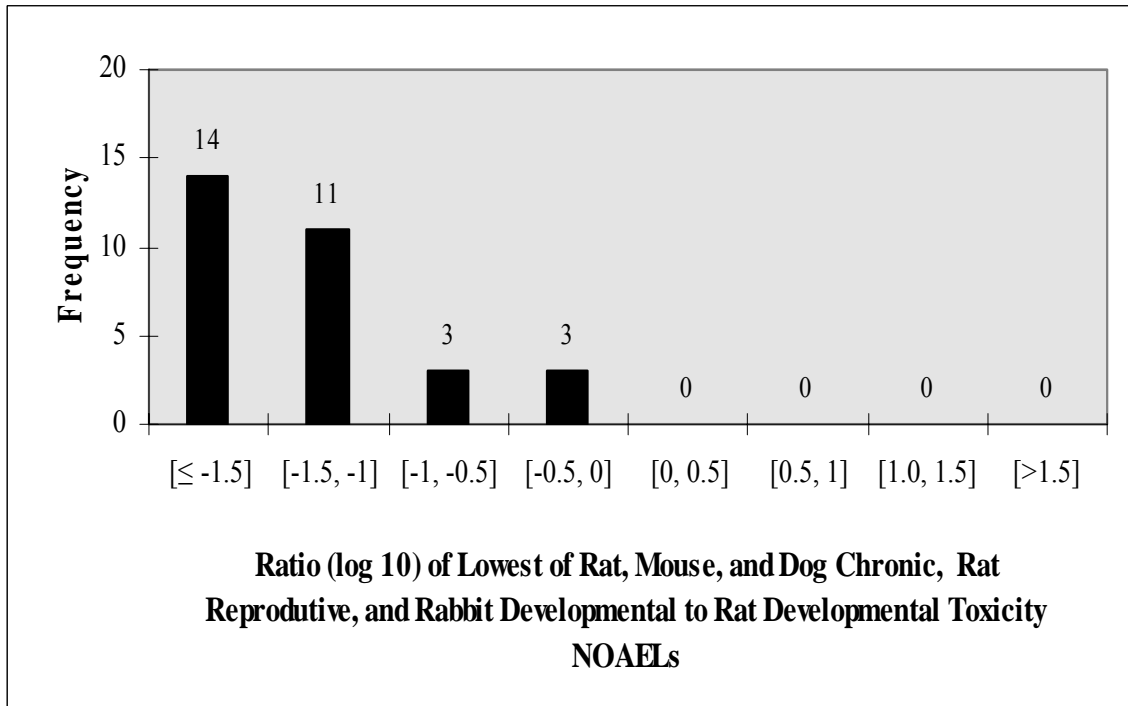


1956
1957

1958
1959 RMD5

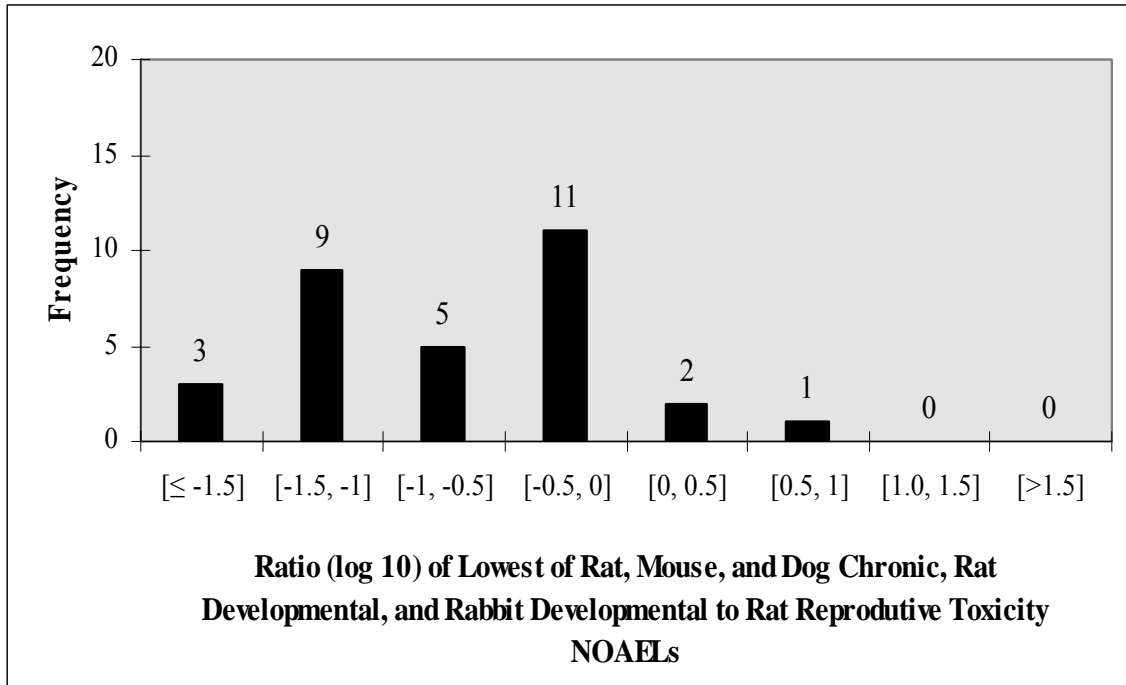


1960
1961
1962
1963 RMD6



1964
1965
1966
1967

1968 RMD7



1969
1970