COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Assessment of the adequacy of the 10-fold uncertainty factor to allow for interspecies variation in developmental toxicity

Introduction

1. At the February 2012 and 2013 COT meetings, as part of the horizon scanning agenda items, the possibility of evaluating the adequacy of the 10-fold uncertainty factor for interspecies variation was discussed in relation to developmental toxicity. This arose from the observation that humans appeared to be substantially more susceptible than the usual laboratory species used for developmental toxicity testing - rats and rabbits - to the teratogenic effects of some substances such as thalidomide. Indeed it was noted back in 1973 that "it is a disquieting fact that the animals most widely used in the testing of teratogenic effects of drugs, i.e., mouse, rat, rabbit, are relatively insensitive to this most potent human teratogen" (Wilson, 1973). In contrast, the difference was much smaller between non-human primates and humans, but most chemicals in food and the environment are not routinely studied in non-human primates. Reviews in the literature had indicated that humans were typically more susceptible than rats, mice and rabbits to known human developmental toxicants (Brown and Fabro, 1983; Newman et al., 1993; Schardein and Keller, 1989), and a 10-fold uncertainty factor applied to the most sensitive of these species did not appear to be adequate for a number of chemicals, i.e. this was not limited to thalidomide.

2. The Committee agreed that it would be useful to investigate this subject. It was agreed that estimates of LOAELs for developmental toxicity in humans, rats, rabbits and non-human primates would be compared for known human developmental toxicants. Data from mice would not be considered because developmental toxicity is not routinely studied in mice and because in considering information in a previous review, in which a 10-fold uncertainty factor applied to data from rats or rabbits did not appear to be adequately protective for humans for three substances (Schardein and Keller, 1989), additionally including data from studies in mice did not affect the outcome.

3. It was intended that consideration also be given to whether cases in which humans appear to be more than 10 times more susceptible than the most sensitive of rats or rabbits may be due to the human data also reflecting interindividual variation in addition to interspecies variation, if the available data allowed this.

4. Members' comments at the February 2013 meeting regarding differences in susceptibilities between strains of the same species, and the possibility of benchmark dose modelling of epidemiological data were considered during the preparation of this paper.

Background

5. It has long been recognised that there are large species differences in the teratogenicity of chemicals, and that no one laboratory species is a perfect model for humans (Nau, 1986). As a result the approach used in the hazard and risk assessment of most environmental chemicals and those used in the food chain is to test developmental toxicity (embryo and fetal development) in two species, usually the rat and rabbit.

6. An assessment of data accumulated for veterinary medicines showed that testing in both rats and rabbits identified 100% of chemicals which were teratogenic in rats, rabbits or mice and 99% of chemicals which were fetotoxic in one of the three species (Hurtt et al., 2003). This indicates that there is no need to additionally test substances in mice. As defined by the authors, teratogenicity was the induction of malformations; fetotoxicity included visceral or skeletal variations, embryo or fetal mortality, or interauterine growth retardation.

7. The results of studies in rats and rabbits of 29 human developmental toxicants showed that testing in these two species allowed identification of 100% of the human developmental toxicants provided that all types of developmental toxicity (intrauterine growth retardation, fetal/neonatal death and malformation in this exercise) were considered (Schardein and Keller, 1989). In addition, correlation between the types of developmental toxicity observed was improved when testing in both rats and rabbits. Thus, of 29 substances causing malformations in humans, 19 produced malformations in rats (66%) and 18 produced malformations in rabbits (62%), but 24 (83%) produced malformations in one of the two laboratory species.

8. A more recent assessment found that all but one of 50 human developmental toxicants produced at least one type of developmental toxicity in one or more laboratory species (including rats or rabbits) (Schardein and Macina, 2007). One substance was identified (misoprostol, a synthethic prostaglandin E1 analogue), which produces multiple malformations in humans and has abortifacient properties by stimulating uterine contraction in humans, but had apparently produced no type of developmental toxicity in oral teratology studies in either rats or rabbits; however, elsewhere it was reported that it did increase resorptions at a high dose level in rabbits, and it decreased implantations at a high dose in a fertility study in rats administered it up to gestation day 7 (Therapeutic Goods Administration, 2012).

9. If the general hazard of developmental toxicity can be identified, the question then remains whether the results can be quantitatively extrapolated to humans, i.e. whether applying a 10-fold uncertainty factor to a point of

departure in the most sensitive species tested adequately accounts for any greater quantitative susceptibility of humans. There have been a small number of reviews of this. Early work by Brown and Fabro (1983) calculated the ratios of the lowest reported teratogenic doses in the most sensitive animal species tested for eight substances with the lowest reported teratogenic doses in humans. The ratios ranged from 1.8-50. The animal data taken into account were from a wide range of species, including cats and monkeys.

10. Newman *et al.* (1993) conducted a similar assessment for four pharmaceutical substances (valproic acid, isotretinoin, thalidomide and methotrexate). The authors divided the no observed adverse effect level (NOAEL) for the most sensitive laboratory species tested by 100 to estimate a "safe dose" for humans, and compared this to the lowest dose level reported to be teratogenic in humans. The lowest dose reported to be teratogenic in humans was in each case >10 times higher than the estimated safe dose, and the authors therefore concluded that the standard 100-fold uncertainty factor applied to laboratory animal data was adequate to protect against teratogenicity. However, this took into account data from monkeys, which were the most sensitive animal species for three of the four substances, and primate data are not required or usually available for food chemicals.

11. Schardein and Keller (1989) reviewed the developmental toxicity data for 28 substances identified as developmental toxicants in humans. They did not restrict the assessment to teratogenicity, but instead considered all the endpoints of growth retardation, death/abortion, malformation and functional alteration. They compared overall lowest observed adverse effect levels (LOAELs) for developmental toxicity in the most sensitive animal species tested with the estimated LOAEL in humans. The ratios ranged from 1.2 to 200. The authors concluded, "The human is remarkably sensitive to those agents characterized here as human developmental toxicants." If the analysis of the Schardein and Keller (1989) data were restricted to chemicals assessed for developmental toxicity in both the rat and rabbit, and to data for these species (excluding data from mice and monkeys for some substances), then there would be 7 substances, with ratios ranging from 1.5 to 50. Three of the seven substances would have ratios greater than 10. Including the data from mice would not affect the results.

12. This paper builds upon the previous reviews, with a focus on data in rats and rabbits, for human developmental toxicants. Data for non-human primates were also sought for comparison.

Approach taken

Identification of human developmental toxicants

13. A list of human developmental toxicants was generated. The intention was that this list should be broad and all-encompassing (i.e. not limited to pharmaceuticals if possible) but should also be limited to substances with

positive developmental toxicity data in humans. The list was compiled from a combination of reviews of human developmental toxicity (Newman et al., 1993; Schardein and Keller, 1989; Nau, 1986), guidance to medical professionals on pharmaceuticals (The Merck Manual Online), and chemical classifications according to the EU Regulation on the classification, labelling and packaging of substances (Regulation (EC) No. 1272/2008). Some possible additional pharmaceuticals and other substances which are human developmental toxicants were indicated by general internet searching. These were confirmed by consulting resources such as the British National Formulary where possible.

14. The substances identified from the Merck Manual Online were those listed as known or suspected human teratogens. The substances identified from Regulation (EC) No. 1272/2008 were category 1A reproductive toxicants (known human reproductive toxicants) which also had one of the following hazard statement codes assigned: H360D ("May damage the unborn child"), H360FD ("May damage fertility. May damage the unborn child") or H360Df ("May damage the unborn child. Suspected of damaging fertility"). Further details and the list of substances taken forward can be found in Annex A.

15. Since angiotensin-converting enzyme (ACE) inhibitors as a group were identified as human developmental toxicants, a list of ACE inhibitors was produced based on listings in the British National Formulary and added to the list of human developmental toxicants.

16. Subsequently, additional substances were identified from the review of human developmental toxicants by Schardein and Macina (2007). The additional substances were selected for inclusion if the human data were reasonably conclusive that the substance is a human developmental toxicant, if the human developmental toxicity related to oral exposure, and if there were comparable data in rats, rabbits and/or non-human primates.

Literature searching

17. For each substance, Pubmed (http://www.ncbi.nlm.nih.gov/sites/entrez) was searched using the following search terms:

[Substance] developmental toxicity [species]
[Substance] teratogenicity [species]

18. For the non-human primates, the searches were undertaken using both "monkey" and "primate" in place of [species]. For ACE inhibitors, searches were conducted both under the individual pharmaceutical names and the general term "ACE inhibitor".

19. From the article headings, abstracts of studies which appeared to be relevant were examined. Articles were ordered where the abstract indicated that the article included developmental toxicity data and may be of use in identifying dose-response data. In addition, reviews of developmental toxicity

were used to identify additional references, as were the reference lists of retrieved papers.

20. Only papers written in English were retrieved. In a small number of cases, reviews written in English summarised results from papers written in other languages, and these summarised data were used. The focus was on studies using oral dosing, but due to data limitations it was decided to retrieve studies in laboratory animals which did not use oral dosing where data were available in humans using oral dosing. The data sought related to exposure *in utero*, i.e. not postnatal development.

21. For a few substances (lead, mercury, PCBs, iodine) the literature search identified very large numbers of references, many of limited relevance or of unclear relevance. In these cases it was decided to make use of recent evaluations by scientific advisory committees (or in the case of the PCBs and iodine, International Programme on Chemical Safety Concise International Chemical Assessment Documents) to identify the key studies and LOAELs. For caffeine, use was made of the COT's 2008 statement on reproductive effects in identifying a human LOAEL.

22. The additional developmental toxicants identified from Schardein and Macina (2007) were identified at a late stage and this review was used to identify the key references, considering its recent publication.

Identification of LOAELs

23. The intention was to compare LOAELs for developmental toxicity in humans and the various laboratory animal species, the same approach as taken in earlier reviews in the literature. In practice, a number of judgements needed to be made in selecting the LOAELs, particularly from the human data, and these are listed here:

- For laboratory animal data, the lowest LOAEL from the retrieved papers was extracted. In one case only the results of benchmark dose modelling were presented; the LOAEL was taken to be greater than the BMD5.
- Where multiple strains of the same species were tested, the lowest LOAEL from all the retrieved papers was still extracted. This was primarily the case for thalidomide; this is discussed further below.
- For the human data relating to pharmaceuticals, where the data were from small numbers of case reports and the dose was not reported, the upper level of the normal therapeutic dose range was taken as representing the LOAEL. This was also the case where the use of a human pharmaceutical was associated in epidemiological studies with a small increase in relative risk or odds ratio for a developmental endpoint, but no dose-response data were available. However, in some cases it was not possible to make a judgement on the point within the dose range that should be taken to be the LOAEL and it was necessary to enter the full normal therapeutic dose range as the LOAEL.
- Exceptionally, where the human data indicated that a pharmaceutical was highly teratogenic or gave some other indication that it would be

teratogenic at all dose levels, the lower level of the normal therapeutic dose range was taken as being the LOAEL.
- For human case reports in which the dose taken was reported, the lowest dose taken in a reported case was taken to be the LOAEL.
- For human epidemiological data comparing different dose groups, the lowest dose at which a relative risk or odds ratio was statistically significantly increased was taken to be the LOAEL.

24. Data for thalidomide were available for different strains of rabbit. Consideration was given to whether there were substantial differences in the susceptibilities of different strains. However, when taking into account dose-spacing and differences in the dose levels tested in different studies, susceptibility to thalidomide appeared to be similar in the New Zealand White, Dutch belted, Himalayan and white Danish strains of rabbit. Newman et al. (1993) also concluded that strain differences did not appear to markedly alter any conclusions. Therefore the lowest LOAEL identified from all the rabbit studies was entered.

25. There may be differences in the susceptibility of different species of non-human primates; however, in most cases developmental toxicity was only studied in one non-human primate species and the LOAEL was identified from that species. Thalidomide was the exception as it had been studied in the greater galago / greater bushbaby (*Galago crassicaudatus*), rhesus monkey, cynomolgus monkey, bonnet monkey, African green monkey, baboon and marmoset. However, there were differences in the study designs and the dose levels tested. The data appeared to indicate similar sensitivity of all the species other than the greater galago, and the lowest dose tested (and producing limb abnormalities) of 0.625 mg/kg bw/day in the rhesus monkey was taken as the LOAEL for non-human primates. In contrast, no skeletal abnormalities occurred in the greater galago dosed at 20 mg/kg bw for 1- or 3-day periods between gestation days 16 and 30, which was believed to include stages of development comparable to the known sensitive period in the rhesus monkey (Wilson, 1972).

Consideration of alternative approaches

26. Another approach to comparing the susceptibilities of laboratory animal species and humans might be to compare dose levels causing similar, quantified incidences of malformations or other developmental consequences. This may be more accurate than simply comparing LOAELs. This is complicated by the occurrence of litters in rats and mice, thus there can be a difference if the incidence per litter or the proportion of litters affected is used. It has been considered in the case of thalidomide in paras 36-43, below. However, data limitations precluded this for most substances.

27. At the February 2013 meeting, Members queried the taking of the lowest dose at which a relative risk or odds ratio was statistically significantly increased in an epidemiological study as being a LOAEL and suggested that benchmark dose modelling would be preferable, if feasible. In the event, suitable dose-response data were not available for almost all substances. For

caffeine, some information on dose-response was available from an FSA-funded study of fetal growth restriction (FGR), which was considered by the COT in 2008 (COT, 2008). The COT concluded from this study, “It seems likely that risk is increased in association with intakes in the order of 200 mg per day and perhaps even lower. However, if the relation is indeed causal, then the absolute increase in incidence of FGR from intakes less than 200 mg per day is likely to be less than 2% of infants.” Caffeine intake of 200-299 mg/day was the lowest dose group with a statistically-significantly increased odds ratio for fetal growth restriction (see Table 1). Taking into account this result and the conclusions of the COT, 200 mg/day, equivalent to 3.33 mg/kg bw/day, was taken to be the human LOAEL for the developmental effects of caffeine in humans in this paper. The Committee may wish to consider the appropriateness of this approach. However, the difference between the LOAELs in rats and humans for caffeine is less than 10-fold.

Table 1: Odds ratios for FGR from a logistic regression analysis that adjusted for smoking status, amount smoked (cotinine concentration) and alcohol intake. Taken from COT (2008)

	Caffeine (mg/day)	*OR*	*95% CI*	*P(trend)*
Average intake over pregnancy	<100	1	-	
	100-199	1.2	0.9, 1.6	
	200-299	1.5	1.1, 2.1	
	300+	1.4	1.0, 2.0	P=0.02

Results

28. A detailed table of the results can be found in Annex B. A summary table is presented below (Table 2). This summary does not include substances where human data were not found or substances for which data were only available for non-oral routes, the nature of the effects observed at the LOAEL or the references. Readers are referred to the detailed table in Annex B for these details. Note that while chemicals for which no data for oral exposure were available in any species have been excluded, some data have been included in Table 2 for laboratory animals for subcutaneous (s.c.), intraperitoneal (i.p.), intramuscular (i.m.) or intravenous (i.v.) routes of administration where no data for oral exposure were identified for those species. These LOAELs may be lower than LOAELs would be for oral dosing, and are italicised.

Table 2: Summary table of the comparison on LOAELs for developmental toxicity in humans, rats, rabbits and non-human primates.

Chemical	**Chemical / pharmaceutical group**	**LOAEL in humans (mg/kg bw)**	**LOAEL in rats (mg/kg bw)**	**LOAEL in rabbits (mg/kg bw)**	**LOAEL in non-human primates (mg/kg bw)**
Aminopterin	Antifolate	0.03	0.0125	*15 (i.v.)*	0.1-0.2*
Aspirin	NSAID	20-67	100	200	300
Busulfan	Alkylating antineoplastic	0.008-0.07	*18 (i.p.)*	N/A	N/A
Caffeine	Natural food constituent	3.3	6	100	10-15
Captopril	ACE inhibiter	1.67	10	13	N/A
Carbamazepine	Anticonvulsant	20	200	N/A	N/A
Chlorambucil	Alkylating antineoplastic	0.07	*3 (i.p.)*	N/A	N/A
Cyclophosphamide	Alkylating antineoplastic	3.3	6.2	*2 (i.v.)*	*5 (i.m.)*
Danazol	Androgen	3.3	>250	60	N/A
Diethylstilboestrol	Oestrogen	0.08-2.5	≥0.045	*1.75 (s.c.)*	0.11-0.26
Enalapril	ACE inhibiter	0.67	3	3	N/A
Ergotamine	Mycotoxin	0.025	10	1	N/A
Ethanol	Recreational drug	<19-114	1200	>2400	260
Ethisterone	Progestogen	0.5	40	<4	N/A
Etretin	Retinoid	1	25	0.6	N/A
Etretinate	Retinoid	0.75	4	N/A	N/A
Fluconazole	Fungicide drug	6.7	25	75	N/A
Iodine	Essential trace element	2.2	250	7.5	N/A
Isotretinoin	Retinoid	0.17	30	3	2
Lithium	Mood stabiliser	1-26?	100	>40	>25
Medroxyprogesterone	Progestin	0.04	4	*1 (s.c.)*	*300 (i.m.)*
Methimazole	Antithyroid	0.08-0.25	1.5	N/A	N/A
Methotrexate	Antifolate	0.04	0.2	*0.3 (i.v.)*	3
Methylmercury	Environmental contaminant	≥0.0018	0.268	N/A	0.05
Methyltestosterone	Androgen	0.17	2	N/A	N/A
Misoprostol	Prostaglandin E1 analogue	0.0067	1	1.6	N/A
Norethisterone	Progestogen	0.17	20	1	3.6
Paramethadione	Anticonvulsant	25	264	N/A	170
Penicillamine	Chelating agent and immunosuppressant	17	540	N/A	N/A
Phenobarbital	Anticonvulsant	1.5	80	50	N/A
Phenytoin	Anticonvulsant	1.67	100	75	10
Propranolol	Beta-blocker	0.5	50	N/A	N/A
Propylthiouracil	Antithyroid drug	2.5	N/A	22	N/A

Primidone	Barbiturate-type anticonvulsant	2.1	120	N/A	N/A
Tetracycline	Antibiotic	17	540	*>10 (i.v.)*	N/A
Thalidomide	Sedative drug	0.42	50	25	0.625
Trimethadione	Anticonvulsant	15-40	200	N/A	60
Valproic acid	Anticonvulsant	13-17	100	150	20
Valsartan	Angiotensin II receptor antagonist	1.1	600	5	N/A
Vitamin A	Essential nutrient	>0.05	7.5	2.5	6
Warfarin	Anticoagulant	0.04-0.08	0.16	*1 (i.m.)*	N/A

*Some of the LOAELs are ranges. For the human data, this was due to limitations meaning that it was not possible to identify more precisely a LOAEL. For the animal data (non-human primates for two substances) this was due to the dose tested being reported as a range (aminopterin) or a dose level per animal being used (diethylstilboestrol), which resulted in different intakes per kg bodyweight in the animals as their bodyweights varied.

29. Table 3 presents the ratios of the lowest LOAELs from studies in rats, rabbits and non-human primates to the LOAELs in humans, and also the ratio of lowest LOAELs for the most sensitive of rats and rabbits to the LOAELs in humans.

Table 3: Ratios of LOAELs in laboratory animals to humans

Chemical	**Chemical / pharmaceutical group**	**Ratio of LOAEL in species to humans**			**Ratio of LOAEL in most sensitive of rats or rabbits to humans**
		Rats	**Rabbits**	**Non-human primates**	
Aminopterin	Antifolate	0.4	N/A	3.3-6.7	0.4
Aspirin	NSAID	1.5-5	3-10	4.5-15	1.5-5
Busulfan	Alkylating agent	257-2250 (i.p.)	N/A	N/A	*257-2250 (i.p.)*
Caffeine	Natural food constituent	1.8	30	3-4.5	1.8
Captopril	ACE inhibiter	6	7.8	0.6	6
Carbamazepine	Anticonvulsant	10	N/A	N/A	10
Chlorambucil	Alkylating antineoplastic	*43 (i.p.)*	N/A	N/A	*43*
Cyclophosphamide	Alkylating antineoplastic	1.9	*0.6 (i.v.)*	*1.5 (i.m.)*	*0.6 (i.v.)* or 1.9
Danazol	Androgen	>76	18	N/A	18
Diethylstilboestrol	Oestrogen	0.02-0.6	*0.7-22 (s.c.)*	0.04-3.3	0.02-0.6
Enalapril	ACE inhibiter	4.5	4.5	N/A	4.5
Ergotamine	Mycotoxin	400	40	N/A	40
Ethanol	Recreational drug	10.5->63	>21->126	2.3->14	10.5->63

Ethisterone	Progestogen	80	<8	N/A	<8
Etretin	Retinoid	25	0.6	N/A	0.6
Etretinate	Retinoid	5.3	N/A	N/A	5.3
Fluconazole	Fungicide drug	3.7	11	N/A	3.7
Iodine	Essential trace element	114	3.4	N/A	3.4
Isotretinoin	Retinoid	176	17.6	1.8	17.6
Lithium	Mood stabiliser	3.8-100	>1.5->40	>0.96->25	>1.5->40
Medroxyprogesterone	Progestin	100	*25 (s.c.)*	*7500 (i.m.)*	*25 (s.c.)* or 100
Methimazole	Antithyroid	6-19	N/A	N/A	6-19
Methotrexate	Antifolate	5	*7.5 (i.v.)*	75	5
Methylmercury	Environmental contaminant	≤149	N/A	≤28	≤149
Methyltestosterone	Androgen	12	N/A	N/A	12
Misoprostol	Prostaglandin E1 analogue	149	239	N/A	149
Norethisterone	Progestogen	118	5.9	21	5.9
Paramethadione	Anticonvulsant	10.6	N/A	6.8	10.6
Penicillamine	Chelating agent and immunosuppressant	32	N/A	N/A	32
Phenobarbital	Anticonvulsant	53	33	N/A	33
Phenytoin	Anticonvulsant	60	45	6	45
Primidone	Barbiturate-type anticonvulsant	57	N/A	N/A	57
Propranolol	Beta-blocker	100	N/A	N/A	100
Propylthiouracil	Antithyroid drug	N/A	8.8	N/A	8.8
Tetracycline	Antibiotic	32	*>0.6 (i.v.)*	N/A	*>0.6 (i.v.)* or 32
Thalidomide	Sedative drug	120	60	1.5	60
Trimethadione	Anticonvulsant	5-13	N/A	1.5-4	5-13
Valproic acid	Anticonvulsant	5.9-7.7	8.8-11.5	1.2-1.5	5.9-7.7
Valsartan	Angiotensin II receptor antagonist	545	4.5	N/A	4.5
Vitamin A	Essential nutrient	<150	<50	<120	<50
Warfarin	Anticoagulant	2-4	*12.5-25 (i.v.)*	N/A	2-4

30. Considering data resulting from oral exposure only, a 10-fold uncertainty factor applied to the most sensitive of the rat or rabbit would be clearly adequate for 19 substances, which is 46.3% of the total. This would rise to up to 56.1% of substances if including the data for lithium, methimazole, trimethadione and vitamin A, for which the ratio of the LOAEL in the most sensitive of either rats or rabbits to humans is estimated as a defined range or a "<" range which could include 10, as being consistent with a factor of 10.

31. If only considering the substances tested in both rats and rabbits, using oral dosing in both species, and excluding unclear results expressed as ranges or "<" a factor greater than 10, a 10-fold uncertainty factor would

clearly be adequate for 11 out of 19 substances, which is 58%. This would increase to 60% (12 out of 20) if the data for lithium were considered consistent with a 10-fold factor being adequate, and potentially to 62% (13 out of 21) if the data for vitamin A were considered consistent with a 10-fold uncertainty factor being adequate.

32. In order to estimate a mean ratio of LOAELs for the most sensitive of either rats or rabbits to humans, again limiting the assessment to substances with oral dosing studies in both rats and rabbits, it is necessary to exclude all ratios that are expressed as greater than or less than a value, which further restricts the total number of substances to 17. The calculated mean difference is 23.6-23.9 (the range is due to either using the lower values of ratios which were expressed as ranges or the upper).

Consideration of the results

33. Given the limitations of the dose-response data, the fact that the results indicate that a 10-fold uncertainty factor is adequate to allow for interspecies variation for up to 58-62% of the substances tested in both rats and rabbits could be interpreted as providing some support for this uncertainty factor. However, it appears that an uncertainty factor much higher than 10 would be required for some substances even when they have been tested in both rats and rabbits. As a result the mean ratio of the LOAEL for the most sensitive of either rats or rabbits to humans is more than twice the value of the uncertainty factor of 10.

34. Possible reasons for apparently high interspecies variation could include that the human LOAELs additionally reflect interindividual variation, in contrast to the data in laboratory animals; limitations in the sensitivity of laboratory animal studies for some endpoints; or experimental limitations such as some studies in laboratory animals only testing high dose levels. Many of the developmental toxicity studies were old and/or did not entirely conform to modern guidelines, though most tested a range of doses through the major periods of organogenesis; it is unclear what impact this would have had. Alternatively, differences may genuinely be large due, for example, to large species differences in toxicokinetics, perhaps including differences in placental transfer.

35. Table 4 lists the seven substances tested in both rats and rabbits for which a 10-fold factor would appear not to be adequate (excluding substances for which the ratio is a range which could include 10), and notes apparent from evaluating the data which may aid the interpretation of these differences. The ratios of the LOAELs in non-human primates to humans are also included for comparison. The subsequent sections consider further some of these possible reasons for apparent large interspecies differences.

Table 4: List of substances tested in both rats and rabbits for which the ratios of LOAELs in the most sensitive of these species to those in humans were more than 10-fold higher and comments

Chemical	**Chemical / pharmaceutical group**	**Ratio of lowest LOAEL in rats or rabbits to humans**	**Comments**	**Ratio of LOAEL in non-human primates to humans**
Danazol	Androgen	18	No obvious reason for difference. Modern developmental toxicity studies in rats and rabbits, but the critical effects in humans (virilisation of female fetus) does not appear to have been observed in rats or rabbits	No data
Ergotamine	Mycotoxin	40	No obvious reason for difference. Human LOAEL was taken to be the mean dose taken, for which a case-control surveillance study indicated that taking ergotamine caused adverse effects.	No data
Ethanol	Recreational drug	10.5->63	The estimate of human LOAEL was based on a prospective study which identified an association between four variants in alcohol dehydrogenase genes and IQ at 8 years of age in the children of mothers who consumed small-moderate amounts of alcohol during pregnancy (<1-6 UK units/week) but not in the children of mothers who abstained from alcohol during pregnancy. This may, therefore, reflect effects in sensitive individuals, not individuals of average susceptibility.	2.3->14
Isotretinoin	Retinoid	17.6	No clear reason for difference. As the	1.8

			human LOAEL was based on the lowest dose taken in case reports (ca. 0.2 mg/kg bw) and many of the case reports related to doses of ca. 0.5-1.5 mg/kg bw it is possible there is some contribution of inter-individual variation to it.	
Medroxyprogest-erone	Progestin	At least 25*	No clear reason for difference, but lower doses do not appear to have been tested in rats and the oral LOAEL in rats (100 times the human LOAEL) was described as causing a large effect on urogenital development.	*7500 (i.m.)*
Misoprostol	Prostaglandin E1 analogue	149	No obvious reason for difference. Modern developmental toxicity studies.	No data
Phenobarbital	Anticonvulsant	33	No clear reason for difference. Taking the lowest dose from case reports as the human LOAEL may affect the results slightly but not likely to have been to a large extent.	No data
Phenytoin	Anticonvulsant	45	No obvious reason for difference. Combination therapy was taken in many human cases, but the human LOAEL was taken from a case report where only phenytoin was taken.	6
Thalidomide	Sedative drug	60	No obvious reason for difference. Thalidomide is considered further below.	1.5

*25 based on using s.c. dosing data from rabbits, 100 based on oral dosing data from rats

Are the apparent differences between LOAELs in laboratory animals and humans partly due to interindividual variability in the human population?

36. One possibility for differences in the susceptibility compared to the most sensitive of either rats and rabbits being apparently greater than 10-fold could be that the LOAELs in rats and rabbits are derived from studies involving small numbers of relatively homogenous animals whereas the LOAELs in humans often derive from case reports, and the lowest positive doses identified from case reports might reflect particularly susceptible individuals in the population rather than humans of average susceptibility. This might have affected the results for some substances (e.g. see comments in Table 4). If true, the implication of this would be that the differences in the LOAELs should be compared to a margin of up to 100 (the total default uncertainty factor used to allow for both interspecies and intraspecies variation) rather than 10. On the other hand, however, for the pharmaceuticals, the estimated human LOAELs were sometimes the lowest doses that have been taken therapeutically, e.g. for thalidomide, and therefore it is also possible that lower dose levels would also cause developmental toxicity in humans.

37. Ideally, the variation between humans and laboratory animals would be addressed by comparing dose levels in laboratory animals and humans that cause similar levels of response, e.g. frequencies of malformations, especially if this was 50%. Unfortunately, the available data are generally insufficient to be able to determine dose-response relationships in humans. This is because the data are often from case reports, and because even when there are reports of pregnancies in which there was exposure to the substance without developmental toxicity occurring, it is often unclear whether the exposure occurred during the critical period of susceptibility. The available data for thalidomide are considered closely here as thalidomide was subject to substantial investigation in attempts to identify critical dose periods, doses associated with teratogenicity and frequencies of malformed human infants, and the frequency of malformations was high.

38. Dose levels in humans reported to be associated in case reports with teratogenic effects clearly due to thalidomide exposure ranged 25-200 mg/day (equivalent to 0.42-3.33 mg/kg bw/day, assuming a body weight of 60 kg). This appears to have been the full range of the dose levels that were taken during pregnancy. Estimates of the total incidence of malformations related to the taking of thalidomide during pregnancy have varied and have been highly uncertain because although many women took thalidomide during pregnancy without apparent effects on the fetus, there are relatively few identified definite cases of exposure during the critical period without effects on the fetus. However, it has been estimated that the overall incidence was likely to have been in the range 10-50% (Newman, 1985; Newman et al., 1993).

39. A study of clinical records from a maternity clinic in Japan provides some useful data. One hundred and thirteen women were prescribed thalidomide during pregnancy. The daily dose taken was 50 mg “in most

instances", although it varied from 30-100 mg. Only seven of the women were known to have taken it during at least some part of the identified critical window for induction of malformations since all were in hospital because of nausea and vomiting at the time thalidomide was taken. Three of these seven pregnancies resulted in malformations (one of these also resulted in a spontaneous abortion) and four did not (Kajii et al., 1973). All four of the mothers with unaffected pregnancies started to take thalidomide only in the last few days of the identified critical exposure window for teratogenicity. However, assuming that these findings indicate the true incidence of malformations, this is 43%. The exact doses taken by the seven women are unknown. Assuming a typical body weight for an Asian adult of 55 kg, and that the usual dose of 50 mg/day was taken, this equates to an intake of 0.9 mg/kg bw/day.

40. A dose level of 50 mg/kg bw/day in the New Zealand rabbit produced malformations in 68% of live fetuses, or 59% of total fetuses, an incidence a little higher than in the offspring of the Japanese women (Fratta et al., 1965). The number of litters affected by malformations was not reported. In another study in New Zealand rabbits, the dose level of 50 mg/kg bw/day produced malformations in an average of 17.8% of fetuses per litter, with 6/10 litters being affected (Schumacher et al., 1968). In a study in Himalayan rabbits at the same dose, the total incidence of malformations was not reported, though based on reported frequencies of different types of malformations it must have been ≤27% of fetuses, but 57% of litters were affected by malformations (Sterz et al., 1987). In another study in Himalayan rabbits at the same dose, the total incidence of malformations was 11%, and four out of 10 litters were affected (Lehmann and Niggeschultze, 1971). The difference between this dose level of 50 mg/kg bw/day and that taken by the Japanese women is 56.

41. At the next dose up, 100 mg/g bw/day, the average percentage of the litter malformed was 29.5, but 100% of litters were affected (Schumacher et al., 1968), and in another study the total incidence of malformations was not reported but was ≤57%, and 94% of litters were affected (Sterz et al., 1987). This dose level is 111 times that taken by the Japanese women.

42. The LOAEL for malformations in rabbits is 25 mg/kg bw/day. The average percentage of malformed fetuses per litter was only 3.8%, but malformations occurred in four of ten litters (Schumacher et al., 1968). The difference between this LOAEL and the dose level of 0.9 mg/kg bw/day in the Japanese women is 28.

43. It appears that the difference in dose levels causing broadly similar incidences of malformations in rabbits and humans is greater than 28 and up to 56 or more. This estimate is complicated by uncertainties in the human data, variability in the results between different studies in rabbits, and by whether the incidence in rabbits is taken to be the total incidence of fetuses affected or the incidence of litters containing at least one malformed fetus. The difference is greater than the uncertainty factor of 10 used to allow for interspecies variation. In contrast there is little interspecies variation between non-human primates (except the Greater Gallago, see para 24) and humans.

Consideration of variation in toxicokinetics as an explanation for high species variation

44. Nau (1986) considered species differences in pharmacokinetics as a possible explanation for differences in drug teratogenesis between species. The authors noted that a number of pharmacokinetic parameters were important determinants of the exposure of the embryo and that all of those factors could exhibit substantial interspecies variation, thus it was not surprising that there would be large interspecies differences in teratogenicity. Nau concluded that species differences were particularly pronounced in regard to drug elimination rates, maternal plasma protein binding and placental transfer. Table 5, below, lists half-lives of elimination for some of the pharmaceuticals considered in this paper, as reported by Nau (1986).

Table 5: Half-lives of elimination for substances considered in this paper, as reported by Nau (1986)

	Predominantly eliminated by		Half-lives, hr			
Drug	Liver	Kidney	Human	Rat	Rabbit	Monkey
Caffeine	X		4.2	0.8	1.6	3.2
Isotretinoin	X		10-30	1		
Phenytoin	X		10-60	3-5		10-15
Trimethadione	X		20-24	1.5-2.6	1-2	
Valproic acid	X		12	0.3		0.7-3

45. It is possible that the large differences in half-life for isotretinoin between humans and rats may contribute to the large difference in LOAELs identified between these two species (ratio of 176). Otherwise there is no clear relationship between differences in half-lives and differences in LOAELs for developmental toxicity except to observe that half-lives of elimination are longer in humans than rats or rabbits and that the differences are large for some substances.

46. Nau (1986) presented examples of differences in placental transfer between species - e.g. digoxin readily crosses the rodent and human placenta but the ovine placenta is relatively impermeable to it, gentamycin efficiently crosses the human placenta but not the goat placenta – and of differences in plasma protein binding which were presumed to result in differences in placental transfer - e.g. free fractions of valproic acid were much higher in the serum of mice than in rabbits or primates. However, comparative data between species were available for relatively few substances. It may be possible to conduct a more detailed review of the literature on these areas of species variation in toxicokinetics if the Committee considers that would be worthwhile.

47. However, the inherent susceptibility of the embryo also appears to vary. Thus, Nau (1986) noted that relatively low maternal doses of methotrexate and hydroxyurea produced a teratogenic effect in rats despite

low embryonic concentrations, whereas even high embryonic levels in monkeys produced only a limited teratogenic effect (Nau, 1986).

48. The half-life of elimination of thalidomide has been reported to be 2.2 hours in rabbits and 7.3 hours in humans (multiple myeloma patients) (Chung et al., 2004), which is not a large difference. However, thalidomide appears to show large interspecies variation not only in its teratogenicity/developmental toxicity but also for other effects such as its sedative effect and ability to cause peripheral neuropathy. For example, while doses of thalidomide of 3-6 mg/kg bw/day in humans have resulted in peripheral neuropathy, sedation, lethargy, skin rashes, light-headedness and constipation, thalidomide was well tolerated in dogs at doses up to 1000 mg/kg bw/day for 53 weeks (Teo et al., 2001). Sedation was also not observed in rabbits at 100 mg/kg bw/day or mice or rats at 2000-3000 mg/kg bw/day, and peripheral neuropathy has not been observed in rats or dogs (Teo et al., 2001). These differences may be due to species differences in hydrolysis products or other factors (Teo et al., 2001).

Discussion

49. Published work has indicated that testing for developmental toxicity in both rat and rabbits adequately allows for the identification of human developmental toxicants, although the types of developmental toxicity do not always correlate. The question addressed in this paper is whether a quantitative extrapolation can be made, i.e. whether applying a 10-fold uncertainty factor to the dose-response data for the most sensitive of either rats or rabbits allows for extrapolation to humans.

50. For 58-62% of the human developmental toxicants which had been tested in both rats and rabbits in studies using oral dosing, applying a 10-fold factor to the LOAEL in the most sensitive of either rats or rabbits would be adequate to allow for extrapolation to the LOAEL in humans. In some of the cases where the species differences were greater, the estimate of the human LOAEL may have partly taken into account human inter-individual variation, exaggerating to some extent the apparent difference in mean susceptibilities between the species.

51. However, it appears that a 10-fold factor would not be adequate for some substances. This is most clearly shown for thalidomide and misoprostol, but other substances include phenytoin, ergotamine, medroxyprogesterone, and possibly phenobarbital and danazol. Data from non-human primates indicate that these are a good model for thalidomide and phenytoin. Data from non-human primates were limited to a study which administered a single high intramuscular dose for medroxyprogesterone and are not available for the other substances highlighted here.

52. For thalidomide, the data indicate that the high susceptibility of humans is not limited to developmental toxicity. The reason for this level of species variation is not fully understood.

53. In general, data reported elsewhere for other toxicological endpoints have been more supportive of the adequacy of a 10-fold uncertainty factor for interspecies variation. In particular, mean differences between laboratory animals and humans have tended to be smaller, even when considering data for only one laboratory species (not the most sensitive of two). Key studies include:

- Hayes (1967) published data on the acute and chronic toxicity of pesticides in rats and humans. An analysis of these data found that the difference in acute toxicity between rats and humans ranged 1.9 to 100, with a geometric mean of 11 (Zeilmaker et al., 1995). The difference in chronic toxicity ranged 0.58-9.4, with a geometric mean of 2.9.
- Price et al. (2008) compared maximum tolerated doses in humans for 64 antineoplastic drugs following subacute exposure (5 days) with similar toxicological data for up to four laboratory species. Limitations included that the dosing was parenteral in both humans and the laboratory animals, and most of the substances were directly toxic rather than metabolically activated, but the mean difference between rats and humans was 6.5 and an uncertainty factor of 10 would be adequate for extrapolating from rats to humans for 81% of chemicals. For mice to humans the figures were a mean difference of 20 and a 10-fold uncertainty factor would be adequate for 63% of chemicals, and for dogs to humans 3.5 and 97%. Testing in multiple species tended to increase the adequacy of the 10-fold uncertainty factor. For example, while the 10-fold uncertainty factor using data for mice alone would only be adequate for 63% of chemicals, and using data for rats alone would be adequate for 81%, this increased to 85% of chemicals when combining data for rats and mice and using the most sensitive of these species for each chemical.
- In contrast, an assessment of EPA chronic oral reference doses (RfDs) for a range of chemicals indicated that for 7 out of 18 chemicals (39%), RfDs set using human data were lower than what they would have been if based on animal data, hence the use of the animal data would apparently have been adequately protective for 61% of chemicals, which is similar to the estimate for developmental toxicity in this paper (Dourson et al., 2001). This was due to the critical effect in humans not being identified in laboratory animals or humans being more than 10-times more sensitive to the same effect. RfDs set using human data were more than 3-fold lower than they would have been using animal data for four chemicals (22%).

54. A possible next step is to consider further the reasons for the apparent large differences between rats and rabbits and humans for thalidomide, misoprostol, phenytoin, ergotamine, medroxyprogesterone, phenobarbital and danazol. However, the purpose of this exercise was to examine the adequacy of the default uncertainty factor of 10 for interspecies variation for the purposes of setting a health-based guidance value for substances for which there will usually be no data on, for example, comparative toxicokinetics between laboratory animals and humans.

55. When setting health-based guidance values within some regulatory schemes, an extra uncertainty/safety factor of up to 10 has often been applied when the critical NOAEL used to set the guidance value has based on increased incidence of malformations (but not typically other types of developmental toxicity) or increased incidence of tumours for non-genotoxic carcinogens. For example, the EU rules for establishing maximum residue limits (MRLs) for residues of veterinary medicines in food state, "Where the results of animal studies indicate teratogenic effects at doses which do not cause maternal toxicity, an uncertainty factor of up to 1000 will be applied to the NOEL for teratogenicity. For nongenotoxic threshold carcinogens an uncertainty factor of up to 1000 may be used depending on the mechanism involved." (European Commission, 2005). Similar extra uncertainty factors have been applied for pesticides in the EU, depending on the judgement of the Member State experts involved (e.g. EFSA, 2006).

56. In the 2007 COT report on variability and uncertainty in toxicology, it was noted that "an extra factor might be required in certain situations that might include non-genotoxic carcinogenicity (especially for rare tumours where the pathogenic mechanism is unknown), teratogenicity, fetotoxicity and developmental neurotoxicity in the absence of observed maternal adverse effects. The extra uncertainty factor might give additional assurance that people exposed at doses less than the health-based guidance value will not subsequently suffer serious irreversible adverse effects." (COT, 2007). However, such extra uncertainty/safety factors have been suggested to be due to the risk management philosophy used, rather than science-based (IGHRC, 2003). They can be inconsistently applied and are not typically applied when setting health-based guidance values for unavoidable contaminants in the food chain. If a total uncertainty factor of 600 or greater were applied to the developmental toxicity data in rabbits for thalidomide, this would adequately allow for extrapolation to humans while also allowing a margin of at least 10 for interindividual variation; however, even this magnitude of uncertainty factor would apparently not be adequate for misoprostol.

Questions on which the views of the Committee are sought

57. Members are invited to consider the following questions and to raise any other matters that arise from the data presented:

i). Can any conclusions be drawn about the adequacy of the 10-fold uncertainty factor for interspecies variation in relation to developmental toxicity when studied in rats and rabbits from the data presented; if so, what can be concluded?

ii). If conclusions cannot be drawn at present, what further information or analysis of data would the Committee wish to see?

iii). Does the Committee have any comments on the scientific value of applying an extra uncertainty factor for the severity of the effect when basing a health-based guidance value on a NOAEL for teratogenicity?

Secretariat
October 2013

References

Brown NA, Fabro S (1983). The value of animal teratogenicity testing for predicting human risk. *Clin Obstet Gynecol* 26: 467-477

Chung F, Lu J, Palmer BD, Kestell P, Browett P, Baguley BC, Tingle M, Ching L-M (2004). Thalidomide pharmacokinetics and metabolite formation in mice, rabbits, and multiple myeloma patients. *Clin Cancer Res* 10: 5949-5956

COT (2007). Variability and Uncertainty in Toxicology of Chemicals in Food, Consumer Products and the Environment. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. Available at http://cot.food.gov.uk/pdfs/vutreportmarch2007.pdf

COT (2008). Statement on the reproductive effects of caffeine. COT Statement 2008/04. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

Dourson ML, Andersen ME, Erdreich LS, MacGregor JA (2001). Using human data to protect the public's health. *Regul Toxicol Pharmacol* 33: 234-256

EFSA (2006). Conclusion regarding the peer review of the active substance metconazole. EFSA Scientific Report 64: 1-71 Available at http://www.efsa.europa.eu/en/efsajournal/doc/64r.pdf

European Commission (2005). The rules governing medicinal products in the European Union. Volume 8: Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin. October 2005. Available at http://ec.europa.eu/health/files/eudralex/vol-8/pdf/vol8_10-2005_en.pdf

Fratta ID, Sigg EB, Matorana K (1965). Teratogenic effects of thalidomide in rabbits, rats, hamsters, and mice. *Toxicol Appl Pharmacol* 7: 268-286

Hayes WJ (1967). Toxicity of pesticides to man: risks from present levels. *Proc R Soc Lond B* 167: 101-127

Hurtt ME, capon GD, Browning A (2003). Proposal for a tiered approach to developmental toxicity testing for veterinary pharmaceutical products for food-producing animals. *Food Chem Toxicol* 41: 611-619

IGHRC (2003). Uncertainty factors: their use in human health risk assessment by UK Government.

IPCS (2009). Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food. International Programme on Chemical Safety. World Health Organization, Geneva, Switzerland

Kajii T, Kida M, Takahashi K (1973). The effect of thalidomide intake during 113 human pregnancies. *Teratology* 8: 163-166

Lehmann H, Niggeschulze A (1971). The teratologic effects of thalidomide in Himalayan rabbits. *Toxicol Appl Pharmacol* 18: 208-219

Nau H (1986). Species differences in pharmacokinetics and drug teratogenesis. *Environ Health Perspect* 70: 113-129

Newman CGH (1985). Teratogen update: clinical aspects of thalidomide embyropathy – a continuing preoccupation. *Teratology* 32: 133-134

Newman LM, Johnson EM, Staples RE (1993). Assessment of the effectiveness of animal developmental toxicity testing for human safety. *Reprod Toxicol* 7: 359-390

Price PS, Keenan RE, Swartout JC (2008). Characterizing interspecies uncertainty using data from studies of anti-neoplastic agents in animals and humans. *Toxicol Appl Pharmacol* 233: 64-70

Schardein JL, Keller KA (1989). Potential human developmental toxicants and the role of animal testing in their identification and characterization. *Crit Rev Toxicol* 19: 251-339

Schardein JM, Macina OT (2007). Human developmental toxicants: aspects of toxicology and chemistry. CRC Taylor and Francis.

Schumacher H, Blake DA, Gurian JM, Gillette JR (1968). A comparison of the teratogenic activity of thalidomide in rabbits and rats. *J Pharm Exp Ther* 160: 189-200

Sterz H, Nothdurft H, Lexa P, Ockenfels O (1987). Teratologic studies on the Himalayan rabbit: new aspects of thalidomide-induced teratogenesis. *Arch Toxicol* 60: 376-381

Teo SK, Evans MG, Brockman MJ, Ehrhart J, Morgan JM, Stirling DI, Thomas SD (2001). Safety profile of thalidomide after 53 weeks of oral administration in beagle dogs. *Toxicol Sci* 59: 160-168

Therapeutic Goods Administration (2012). Australian Public Assessment Report for misoprostol. Proprietary product name: GyMiso. Department of Health and Ageing, Australian Government. October 2012. Accessed at http://www.tga.gov.au/pdf/auspar/auspar-misoprostol-121002.pdf

Wilson JG (1972). Abnormalities of intrauterine development in non-human primates. *Acta Endocrinol* 71: 261-292

Wilson JG (1973). Present status of drugs as teratogens in man. *Teratology* 7: 3-16

Zeilmaker MJ, Slob W, Jensen EHJM, van Leeuwen FXR (1995). Evaluation of quantitative methods for the determination of the Acceptable Daily Intake. Report nr. 659101003. National Institute for Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands