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OPINION

OF THE

SCIENTIFIC COMMITTEE ON FOOD

ON THE

RISK ASSESSMENT OF DIOXINS AND

DIOXIN-LIKE PCBS IN FOOD

UPDATE BASED ON NEW SCIENTIFIC INFORMATION AVAILABLE SINCE THE ADOPTION OF THE SCF OPINION OF 22ND NOVEMBER 2000

Adopted on 30 May 2001.

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Terms of Reference

The Committee is asked to consider whether there is a need to update its opinion on the risk assessment of dioxins and dioxin-like PCBs in food on the basis of new scientific information available since the release of the SCF opinion of 22nd November 2000.

Background

The Scientific Committee on Food (SCF) adopted its opinion on the risk assessment of dioxins and dioxin-like PCBs in food in November 2000 (SCF, 2000). In its derivation of the temporary tolerable weekly intake (t-TWI) of 7 WHO-TEQ/kg bw the Committee used a cluster of sensitive lowest observed adverse effect levels (LOAELs) for effects on the reproductive function and the immune system of the male offspring of rats administered a single gavage dose of 2,3,7,8-TCDD during gestation, a subtle effect on cognitive recognition in the offspring of rhesus monkeys fed a diet containing 2,3,7,8-TCDD for up to three years, and the development of endometriosis in the rhesus monkey dams from the same studies fed the diet for 42 months.

A key aspect of the assessment was the use of the "body burden approach" which the Committee used to scale doses across species. The Committee identified the limitations in the estimation of body burdens of the animals in the studies used, and consequently in the associated estimated human daily intakes (EHDI) of 2,3,7,8-TCDD derived from these studies. The Committee was unable to identify any single study as being sufficient, by itself, to provide a firm basis for the establishment of a tolerable intake. It therefore considered that these studies provided EHDIs in the range of 12.5 to 30 pg 2,3,7,8-TCDD/kg bw, and within the limits of precision of the estimates, all contributed to the derivation of a tolerable intake. Applying a 10-fold uncertainty factor to these EHDIs suggested a tolerable intake in the range 1 to 3 (rounded figures) pg 2,3,7,8-TCDD/kg bw per day. There were no scientific data to guide the Committee on selection of a single value from the range of 1 to 3 pg 2,3,7,8-TCDD/kg bw per day. However, because of the acknowledged uncertainties the Committee concluded that the lower end of the range, i.e. 1 pg/kg bw per day, should be considered as a temporary tolerable intake.

Since the adoption of the SCF opinion new scientific information on the toxicity of dioxins has been published, which might have removed some of the uncertainties in the previous opinion. In addition, the SCF took cognisance of comments received from the Swedish National Food Administration (2001), the Norwegian Food Control Authority (2001) and from some members of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) of the European Commission. These comments mainly addressed the use of the rat and monkey studies for the derivation of the t-TWI, particularly in the light of the new information published since the SCF expressed its opinion.

Introduction

In its opinion of 22nd November 2000, the Committee identified a number of shortcomings in the available database with respect to bolus dosing and repeated administration, the consequent foetal and maternal body burdens, and the availability of LOAELs instead of no observed adverse effect levels (NOAELs) for the most sensitive endpoints. Since some of the new studies might affect the evaluation of the pivotal studies used in its previous risk assessment the Committee found it appropriate to revisit and update its assessment. In its updated assessment the Committee has considered other relevant new studies, some older studies, and additional information that were found useful for the interpretation of the new findings. The Committee also considered further details supplied by the authors of some of the studies discussed in the previous opinion.

Updated evaluation of the pivotal studies

Studies of developmental toxicity in rats

Effects on the reproductive system of male offspring

In its risk assessment of 2,3,7,8-TCDD the Committee used results from studies of the offspring of pregnant rats given a single, oral dose by gavage on gestation day (GD) 15 (effects on reproductive organs) or GD 14 (effects on the immune system). The most sensitive effects reported were accelerated eye opening and a non-significant decrease (25%) in ejaculated sperm counts in the male offspring following a maternal bolus dose of 50 ng 2,3,7,8-TCDD/kg bw on GD 15 in Long Evans rats (Gray *et al*, 1997a). In another study in Holzman rats using a similar protocol, Mably *et al*. (1992) found statistically significant decreases in epididymis and cauda epididymis weights, daily sperm production, and cauda epididymal sperm number in the male offspring after a maternal single gavage dose of 64 ng/kg bw, the lowest dose tested in that study. In these and other single dose gavage studies, an additional number of reproductive and developmental parameters were affected in a dose-related manner in the male offspring at higher dose levels, i.e. from 160 ng 2,3,7,8-TCDD/kg bw onwards. At a maternal dose of 200 ng 2,3,7,8-TCDD/kg bw or higher Gray *et al*. (1997b) found external malformations of the genitalia in the female offspring.

In order to estimate the maternal body burden in the pregnant rats of these studies the Committee used results from a study by Hurst *et al*. (2000a). The Committee reviewed this study in which ^{3}H-2,3,7,8-TCDD concentrations were measured in the tissues of pregnant Long Evans dams at GD 16 following administration by gavage at GD 15 of 50, 200, 800 or 10000 ng/kg bw, and the average maternal body burdens were reported to be 30.6 (60%), 97.4 (48%), 522.8 (65%) or 585.2 (59%) ng 2,3,7,8-TCDD/kg bw (percentage of dose), respectively. The corresponding average foetal body burdens at GD 16 were 5.3, 13.2, 39.1 and 55.7 ng 2,3,7,8-TCDD/kg bw. This study led the Committee to use a figure of 60% for the amount of 2,3,7,8-TCDD retained in pregnant rats

following a single gavage dose. On the other hand, the Committee used a figure of only 50% for the absorption of 2,3,7,8-TCDD from a dietary matrix. In support of this latter figure, the net absorption was found to be 50-55% when 2,3,7,8-TCDD was contained in normal rat and cows diets (Fries and Marrow, 1975; Jones *et al*., 1989).

In its earlier discussion of the adequacy of using these single dose gavage studies for the risk assessment, the Committee had stressed that "The bioavailability of 2,3,7,8-TCDD to the foetus at a given maternal body burden may differ between a bolus dose (as in these rat studies) and dietary exposure at steady state. Intuitively, differences in foetal bioavailability would seem likely. Given that placental transfer will be mediated *via* the blood, it is serum rather than tissue levels that will be critical in determining the magnitude of foetal exposure. Following a bolus administration, serum 2,3,7,8-TCDD levels would be elevated before redistribution to the tissue compartments. In contrast, low-level chronic exposure will not significantly elevate serum levels. The time of dosing, GD 15, marks the onset of the endocrine-sensitive phase of sexual differentiation in rats and therefore represents a critical window for foetal exposure for these reproductive endpoints. (…) This would suggest that the critical determinant of these reproductive effects is the foetal concentration on GD 15, which, as noted above, is likely to be higher following a single bolus dose on this day than that resulting from lower level chronic exposure. This weakens the relevance to human dietary exposure." (SCF, 2000).

The issue of the difference in magnitude of the foetal body burden following an acute bolus dose compared to that resulting from a low level chronic exposure that leads to a similar maternal body burden now has been addressed in a new publication by Hurst *et al*. (2000b) who measured the radioactivity in both the maternal and foetal tissues of pregnant Long Evans dams at GD 9, 16, and 21 following subchronic administration of ^{3}H-2,3,7,8-TCDD. Female rats were dosed by gavage with 1, 10, or 30 ng of ^{3}H-2,3,7,8-TCDD/kg bw in corn oil, 5 days per week, for 13 weeks. At the end of this period, the rats were mated and dosing was continued every day throughout gestation (SCF 2000). The dosage regimen used produced a steady state of 2,3,7,8-TCDD in the dams. The average maternal and foetal body burdens at GD 16 are shown in Table 1 and compared with average maternal and foetal body burdens found at GD 16 following the single gavage administration of 2,3,7,8-TCDD on GD 15 in the previous study by Hurst *et al*. (2000a).

Table 1. Comparison of average maternal and foetal body burdens after single dose and subchronic 2,3,7,8-TCDD exposure to pregnant rats.

Single dose exposure at GD 15 [1]				Subchronic exposure [2]			
Single dose [3]	Body burden measured at GD 16			Adjusted daily dose [4]	Body burden measured at GD 16		
	Maternal [3]	Foetal [3]	Maternal/ Foetal		Maternal [3]	Foetal [3]	Maternal/ Foetal
50	30	5.3	5.7	0.71	20	1.4	14.3
200	97.4	13.2	7.4	7.1	120	7.5	16.0
800	523	39.1	13.4	21.3	300	15.2	20
1000	585	55.7	10.5				

[1] Data from Hurst *et al*. (2000a)
[2] Data from Hurst *et al*. (2000b)
[3] ng/kg bw
[4] ng/kg bw per day, adjusted to continuous exposure from 5 days/week

As expected, acute single gavage doses at GD 15 produced considerably higher foetal concentrations at GD 16 than subchronic administration of low daily doses leading to maternal steady state body burdens of similar magnitude. From Table 1 it appears that single gavage doses of 50 or 200 ng 2,3,7,8-TCDD/kg bw given at GD 15 produced foetal body burdens (5.3 or 13.2 ng/kg bw) at GD 16 that were 5.7 or 7.4 times lower than the corresponding maternal body burdens (30 or 97.4 ng/kg bw) whereas the foetal body burdens (1.4 or 7.5 ng/kg bw) obtained after subchronic administration of 0.71 or 7.1 ng 2,3,7,8-TCDD/kg bw per day were 14.3 or 16.0 times lower than the corresponding maternal steady state body burdens (20 or 120 ng/kg bw). The ratio of the maternal/foetal body burdens increased with increasing dose levels irrespective of the dosage regimen used.

The Committee noted that extrapolation of the relationship between the foetal and maternal body burdens using the data provided by Hurst *et al*. (2000a,b) did not intercept zero as would be expected since radiolabelled 2,3,7,8-TCDD had been used in both studies. The Committee therefore analysed the data and performed a best-fit analysis of each data set in the range of foetal body burdens from zero to 15.2 with the curves constrained to pass through the origin. It was found that both data sets could be fit to power equations (Annex I). The equations were used to calculate the corresponding acute and subchronic maternal body burdens for a number of foetal body burdens. From these calculations it was determined that the factor to convert maternal body burden following acute dosing into a corresponding steady state body burden is approximately 2.6 (Table 2).

It should be noted that these mathematical calculations of corresponding values for body burdens by no means provide assurance that the correct relationships have been found. Others could presumably be of equal validity. However, they were used solely to describe

the data as an aid to extrapolation between acute gavage dose studies and subchronic studies using daily doses for the purpose of estimating steady state body burdens.

Table 2. Calculated corresponding values of foetal, acute maternal and subchronic steady state maternal body burdens of 2,3,7,8-TCDD.

Foetal body burden (ng/kg bw)	Acute maternal body burden (ng/kg bw)	Subchronic (steady state) maternal body burden (ng/kg bw)	Ratio subchronic maternal/acute maternal body burden
1.2	5.0	12.3	2.5
1.4	5.9	14.6	2.5
1.7	7.5	18.6	2.5
1.8	8.0	20.0	2.5
1.9	8.5	21.0	2.5
2.1	10	25.0	2.5
3.0	15.5	39.0	2.5
5.3	31	78.6	2.5
6.3	38.5	99.0	2.6
7.5	47.5	122	2.6
8.0	52	134	2.6
9.0	60	156	2.6
13.2	95.7	251	2.6
15.2	113	299	2.7

Thus, a foetal body burden of 5.3 ng 2,3,7,8-TCDD/kg bw, which according to Hurst *et al*. (2000a) was associated with a maternal body burden of 31 ng/kg bw after a single bolus dose at the LOAEL of 50 ng/kg bw in the Long Evans rat in the study of Gray *et al*. (1997a), would correspond to a steady state maternal body burden of approximately 79 ng/kg bw. Similarly, the estimated maternal body burden of 38.5 ng/kg bw after the single gavage LOAEL dose of 64 ng/kg bw in the study by Mably *et al*. (1992) corresponds to a foetal body burden of 6.3 ng/kg bw which in turn would require a body burden of approximately 99 ng/kg bw at steady state (Table 2).

Hurst *et al*. (2000b) also discussed the study by Faqi *et al*. (1998) on the effects of low doses of 2,3,7,8-TCDD on the reproductive system of the male offspring of Wistar rats. In that study, the dams were treated subcutaneously prior to mating and throughout mating, pregnancy and lactation. They received an initial loading dose of 25, 60, or 300 ng ^{14}C-2,3,7,8-TCDD/kg bw 2 weeks prior to mating, followed by weekly maintenance doses of 5, 12, or 60 ng TCDD/kg bw. The size of the maintenance doses was based on a reported elimination half-life of 3 weeks for adult rats. For example, this means that at the low dose the initial loading dose would produce a maternal body burden of 25 ng 2,3,7,8-TCDD/kg bw which after one week had declined to 20 ng/kg bw but, following the weekly maintenance dose of 5 ng/kg bw, would again rise to 25 ng/kg bw. After birth, developmental landmarks in the male offspring were monitored. Effects on male

reproduction were studied on postnatal days (PND) 70 and 170. The number of sperm per cauda epididymis was reduced in all 2,3,7,8-TCDD treated groups at puberty and at adulthood. Daily sperm production was permanently decreased, as was the sperm transit rate in the 2,3,7,8-TCDD exposed male offspring, thus increasing the time required by the sperm to pass through the cauda epididymis. Moreover, the male offspring of the 2,3,7,8-TCDD groups showed an increased number of abnormal sperm when investigated at adulthood. Mounting and intromission latencies were significantly increased in the low and high dose groups, but not in the mid dose group. The Committee noted the lack of a clear dose-response relationship for most of these effects in the treated groups. In the high dose group, serum testosterone concentration was decreased at adulthood and permanent changes in the testicular tubuli included pyknotic nuclei and the occurrence of cell debris in the lumen. The fertility of the male offspring was not affected in any of the dosed groups. The intended (pseudo) steady state body burden at the LOAEL in this study using subcutaneous administrations was 25 ng 2,3,7,8-TCDD/kg bw which, according to Table 2, would correspond to a foetal body burden of 2.1 ng 2,3,7,8-TCDD/kg bw. However, the Committee noted that, following the dosage regimen of weekly maintenance doses that was used (see above), a maintenance dose of 5 ng/kg bw would have been given at GD 14 when the maternal body burden had declined to 20 ng/kg bw. According to Table 2, a maternal body burden of 20 ng/kg bw in equilibrium corresponds to a subchronic foetal body burden of 1.8 ng/kg bw. The additional acute dose of 5 ng/kg bw during this critical time period in gestation would produce an extra foetal body burden of 1.2 ng/kg bw, resulting in a total foetal body burden of 3.0 ng/kg bw. According to Table 2, a maternal body burden of 39 ng 2,3,7,8-TCDD/kg bw at steady state would be needed to produce this foetal body burden.

In a recent study by Ohsako *et al.* (2001) pregnant Holtzman rats were given a single oral dose of 0, 12.5, 50, 200 or 800 ng 2,3,7,8-TCDD/kg bw on GD 15, and the male offspring were examined on PND 49 or 120. In this study, there were no changes seen on testicular or epididymal weights nor in daily sperm production or sperm reserve at any of the doses used. However, the weight of the urogenital complex, including the ventral prostate, was significantly reduced at doses of 200 and 800 ng 2,3,7,8-TCDD/kg bw in rats sacrificed on PND 120. Moreover, the anogenital distance of male rats sacrificed on PND 120 showed a significant decrease in the groups receiving doses of 50 ng TCDD/kg or higher. TCDD administration resulted in no apparent dose-dependent changes in levels of either serum testosterone or luteinizing hormone. These results suggest that low-dose 2,3,7,8-TCDD administration had a greater effect on the development of the external genital organs and ventral prostate than on the development of the testis and other internal genital organs. Assuming that 60% of a single gavage dose was retained in the body at GD16 (Hurst *et al*., 2000a), the NOAEL of 12.5 ng 2,3,7,8-TCDD/kg bw would result in a maternal body burden of 7.5 ng/kg bw. This would translate into a maternal body burden of 19 ng/kg bw at steady state following subchronic daily 2,3,7,8-TCDD administration. The LOAEL level of 50 ng 2,3,7,8-TCDD/kg bw corresponds to a maternal body burden of 31 ng/kg bw which would equate to a steady state maternal body burden of 79 ng 2,3,7,8-TCDD/kg bw (Table 2).

The Committee noted that, in the study of Ohsako *et al*. (2001), reverse transcription-polymerase chain reaction analysis revealed that, in the ventral prostates of the PND 49 group, 2,3,7,8-TCDD administration at all dose levels resulted in a dose-dependent increase in 5α-reductase type 2 mRNA level and decrease in androgen receptor mRNA level. These changes were not observed at PND 120 and were not associated with any adverse sequelae at the lowest dose of 12.5 ng 2,3,7,8-TCDD/kg bw. The authors suggested that the decrease in the size of the ventral prostate observed after maternal 2,3,7,8-TCDD exposure at 200 and 800 ng/kg bw might be due to decreased responsiveness of the prostate to androgen caused by an insufficient level of expression of androgen receptor during puberty.

In an earlier 3-generation reproduction study using Sprague-Dawley rats Murray *et al*. (1979) found that chronic dietary administration of 10 ng 2,3,7,8-TCDD/kg bw per day was a clear LOAEL in producing significantly decreased fertility in the F_1 and F_2 generations, but not in the F_0 generation. Other effects seen at that dose level included decreases in litter size at birth, gestation survival (proportion of pups born alive), and neonatal survival and growth. A daily dose of 100 ng 2,3,7,8-TCDD/kg bw produced significant decreases in fertility and neonatal survival in the F_0 generation which precluded continuation of this high dose level in subsequent generations. The lowest dose level used was 1 ng 2,3,7,8-TCDD/kg bw/day, which produced no significant or consistent effects and was considered a NOAEL. Nisbet and Paxton (1982) have pointed out that mild renal morphological changes and reduced pup survival were also seen in the low dose group, however these effects did not occur consistently across all generations. Cross-mating studies using untreated males and females mated with males and females of the 100 ng 2,3,7,8-TCDD/kg bw/day F_0 generation indicated that 2,3,7,8-TCDD affected the fertility of the females but not the fertility of the males. When simple first-order kinetics is used, assuming 50% absorption of 2,3,7,8-TCDD from the diet and an elimination half-life of 21 days in the rat, it can be calculated that the daily doses of 1 or 10 ng 2,3,7,8-TCDD used by Murray *et al*. (1979) would correspond to maternal body burdens at steady state of approximately 15 or 150 ng 2,3,7,8-TCDD/kg bw, respectively. The estimated foetal body burdens would be 1.4 and 8.8 ng 2,3,7,8-TCDD, respectively (Table 2). As judged from the results of the pivotal acute, single dose studies mentioned above such body burdens would not be expected to affect the fertility of the male offspring. However, it should be noted that the F_0 generation males were not exposed *in utero* and Murray *et al*. (1979) performed no cross-mating studies with animals of the F_1 and F_2 generations. More importantly, this study did not address the sensitive end-points included in the more recent studies. In view of this, and the relatively large margin between the body burdens associated with the NOAEL and the LOAEL (15 and 150 ng 2,3,7,8-TCDD/kg bw, respectively) the Committee did not include this study among the pivotal studies used in its previous assessment, nor in the current update.

Taken together, these studies provide evidence of adverse effects on the reproductive system in the male (and female) offspring of pregnant rats exposed to 2,3,7,8-TCDD. The studies demonstrate reduction in daily sperm production, cauda epididymal sperm number and epididymis weight as well as accelerated eye opening, reduction in anogenital

distance and feminised sexual behaviour in the male offspring associated with maternal steady state body burdens in the range of 39 – 99 ng 2,3,7,8-TCDD/kg bw. Reduction in weights of testes and size of sex-accessory glands, such as the ventral prostate in the male offspring, and development of external malformations of genitalia in female offspring as well as reduced male and/or female fertility require higher maternal body burdens. The Committee noted that the most sensitive end-points identified differed between studies. This might reflect strain differences in sensitivity and/or even minor differences in the experimental conditions, e.g. the diet (Ashby *et al.* 2000). The Committee also noted that in the study of Ohsako *et al*. (2001) a single maternal gavage dose of 12.5 ng 2,3,7,8-TCDD/kg bw produced a decrease in the androgen receptor mRNA level in the ventral prostate at puberty (PND 49), indicative of reduced androgenic responsiveness. However, at this dose level none of the above mentioned adverse effects were seen in the male offspring. This dose corresponds to an estimated maternal steady state body burden of approximately 19 ng 2,3,7,8-TCDD/kg bw. As with enzyme induction, altered expression of growth factors and enhanced oxidative stress, the Committee considered this effect to be either an early marker of exposure to 2,3,7,8-TCDD or an event induced in animals that may or may not result in adverse effects at higher body burdens.

Table 3 gives a summary of the NOAEL and LOAELs (rounded figures) for the most sensitive adverse effects of 2,3,7,8-TCDD on developmental endpoints in experimental animals.

TABLE 3. Estimated animal steady state body burdens of 2,3,7,8-TCDD and associated estimated human daily intakes (EHDI) at NOAEL and LOAELs in the pivotal studies

Study	Endpoint	NOAEL	LOAEL	Estimated maternal steady state body burden [1] (ng/kg bw)	Associated EHDI (pg/kg bw)
Mably *et al.*, 1992	**Holzman rats:** Decreased sperm count in male offspring		64 ng/kg bw single bolus dose by gavage	100 [2]	50
Gray *et al.*, 1997a	**Long Evans rats:** Accelerated eye opening and decreased sperm count in male offspring		50 ng/kg bw single bolus dose by gavage	80 [2]	40
Faqi *et al*., 1998	**Wistar rats:** Decreased sperm production and altered sexual behavior in male offspring		Maintenance of 25 ng/kg bw by subcutaneous injections	40 [2]	20
Ohsako *et al.*, 2001	**Holzman rats:** Decreased anogenital distance in male offspring	12.5 ng/kg bw single bolus dose by gavage		20 [3]	10
			50 ng/kg bw single bolus dose by gavage	80 [3]	40

[1] Increment over background. Background body burden in rats is about 4 ng TEQ/kg bw (WHO, 2000).

[2] Composite value resulting from pseudo steady state body burden and acute body burden on GD 15.

[3] Maternal body burden at GD 16.

Effects on the immune system in the male offspring

In the study of Gehrs and Smialowicz (1999), used by the Committee in its previous assessment, a modest but significant suppression of delayed type hypersensitivity to bovine serum albumin was observed in the male offspring of pregnant F344 rats given a single oral gavage dose on GD 14 of 100 ng 2,3,7,8-TCDD/kg bw (the lowest dose tested). Higher doses (300, 1000 or 3000 ng 2,3,7,8-TCDD/kg bw) also produced changes

in the thymic T-cell phenotypes and thymus in the offspring. According to Table 2 an estimated maternal body burden of 60 ng 2,3,7,8-TCDD/kg bw following the acute exposure would result in a foetal body burden of 9.0 ng 2,3,7,8-TCDD/kg bw which in turn would require a steady state body burden of 156 ng 2,3,7,8-TCDD/kg bw after chronic exposure at a lower dose.

The Committee noted a new study by Nohara *et al*. (2000) in which pregnant Holtzman rats were given a single oral dose of 0, 12.5, 50, 200 or 800 ng 2,3,7,8-TCDD/kg bw on GD 15 and the thymus and spleen of male offspring were examined on PND 5, 21, 49 or 120. The weights of the thymus and spleen of the 2,3,7,8-TCDD exposed offspring did not differ from those of the control animals. In the thymus, dose dependent induction of CYP1A1 mRNA was observed on PND 5 following maternal exposure to 50 ng 2,3,7,8-TCDD/kg bw and higher. The induction gradually decreased on PND 21 and 49. There were no changes in cell number and cellular populations in the thymus at any time. In contrast, CYP1A1 mRNA induction in the spleen was very weak, but the numbers of splenocytes were decreased in a dose-dependent manner at puberty on PND 49, but not on PND 21 and 120. However, this decrease only reached significance at the 800 ng 2,3,7,8-TCDD/kg bw exposure level. No changes were detected in the mRNA levels for a number of cytokines in the spleen.

These studies demonstrate that the effects on the immune system of the male offspring of pregnant rat exposed to 2,3,7,8-TCDD occur only at higher doses than the effects seen on the reproductive organs and their function. Therefore, the Committee did not consider these studies pivotal to the updated assessment.

Studies in rhesus monkeys

The Committee, in its opinion of 22 November 2000, identified two studies of the effects of 2,3,7,8-TCDD administered to groups of female rhesus monkeys of one colony as providing a LOAEL of 0.15 ng/kg bw per day after prolonged dietary administration. These studies (Schantz and Bowman, 1989; Rier *et al*., 1993) were included in the group of studies that was used in the Committee's determination of a tolerable intake for 2,3,7,8-TCDD. However, the Committee noted that it was not able to determine the clinical significance for humans, if any, of the findings of a subtle, non-persistent, neurobehavioural change in the offspring of the 2,3,7,8-TCDD treated monkeys in the first of the two studies (Schantz and Bowman, 1989). With regard to the findings of the second study (Rier *et al*., 1993), the development of endometriosis in the rhesus monkeys some 10 years after the dietary treatment with 2,3,7,8-TCDD had been discontinued, the Committee identified some problems in the reporting and results. These were that it was not clear whether identical surgical procedures had been carried out on control and treated monkeys, that body weights had not been reported and that the colony had a very high incidence of endometriosis (SCF, 2000). The publication of two additional studies of these monkeys (Rier *et al*., 2001a; Rier *et al*., 2001b) supplemented by unpublished data (Rier, personal communication) has provided the opportunity for the Committee to review its opinion.

Estimates of intake of 2,3,7,8-TCDD

In its previous opinion (SCF, 2000) the Committee determined the body burden of 2,3,7,8-TCDD in rhesus monkeys resulting from intakes corresponding to the LOAEL using a published estimate of the daily intake of 2,3,7,8-TCDD by monkeys of the 5 ng/kg diet group (0.151 ng/kg bw per day; DeVito *et al*., 1995). However, it was not possible to verify this estimate as not all the relevant information had been published. Additional information provided in a recent paper (Rier *et al*., 2001a), supplemented by unpublished data (Rier, personal communication), has somewhat clarified the situation. It would thus appear that, in estimating the intake of 0.15 ng 2,3,7,8-TCDD/kg bw per day by the 5 ng/kg diet group, a value for the mean body weight of this group equivalent to the median body weight of the 25 ng/kg diet group had been used (DeVito *et al*., 1995).

The original dietary consumption records have been used to estimate the cumulative intake of 2,3,7,8-TCDD by surviving individual animals of the 5 ng/kg diet group (Rier *et al.*, 2001b) and this data was provided to the Committee (Rier, personal communciation). However, the individual body weights to which these consumption figures relate are those measured when the animals were finally killed, not to their body weights during the period of administration of the 2,3,7,8-TCDD containing diets. It would appear that the mean body weight of the animals in the 5 ng 2,3,7,8-TCDD/kg diet group may have been greater than that of the 25 ng 2,3,7,8-TCDD/kg diet group. Therefore, depending on the assumptions used, the Committee could calculate intakes between 0.13 and 0.15 ng 2,3,7,8-TCDD/kg bw per day for this group of monkeys.

Endometriosis and serum levels of 2,3,7,8-TCDD analogues

In the first of the new papers, Rier and her colleagues (Rier *et al*., 2001a) have recorded the serum concentrations of dioxin congeners measured in those rhesus monkeys that had been studied previously to determine the incidence and severity of endometriotic lesions. Of the original experimental groups of eight animals receiving 0, 5 or 25 ng of 2,3,7,8-TCDD/kg diet for periods of approximately 4 years, there were six survivors of each of the 0 and 5 ng/kg diet groups and three survivors of the 25 ng/kg group. Thirteen years after termination of the 2,3,7,8-TCDD exposure samples of blood were collected for determination of the concentrations of 2,3,7,8-TCDD congeners and the incidence and severity of endometriosis was reassessed. It is stated that the diagnostic severity of endometriosis in the animals was similar on both occasions.

Serum samples were analysed for six chlorinated dibenzo-*p*-dioxins, ten chlorinated dibenzofurans and four chlorinated biphenyls. The mean concentrations of four congeners (2,3,7,8-TCDD, 1,2,3,6,7,8-hexachlorodibenzofuran [1,2,3,6,7,8-HxCDF], 3,3',4,4'-tetrachlorobiphenyl [TCB] and 3,3',4,4',5-pentachlorobiphenyl [PeCB]) in the serum of monkeys that had been treated with 2,3,7,8-TCDD were found to be statistically significantly higher than those of the control group of monkeys. There was a significant correlation of the total administered dose of 2,3,7,8-TCDD (dosing completed some 13 years previously) with the serum concentration of 2,3,7,8-TCDD. In addition, both these

parameters correlated with the serum concentrations of 1,2,3,6,7,8-HxCDF and PeCB whereas only the cumulative dose of 2,3,7,8-TCDD correlated with the serum concentrations of TCB.

Increased serum concentrations of 2,3,7,8-TCDD and 1,2,3,6,7,8-HxCDF were not associated with the presence of endometriosis in the monkeys, whereas the concentrations of both the TCB and PeCB congeners were increased in animals with the disease that had been treated with 2,3,7,8-TCDD. This paper has provided additional information that addresses some of the problems that the Committee had identified with the original study of the endometriosis occurring in monkeys administered 2,3,7,8-TCDD in the diet. It is recorded that similar surgical procedures, biopsy or laparoscopy, had been carried out on animals of both the treated and control groups. In addition, it has been reported that having been subjected to one or more laparoscopies is not a risk factor for the development of endometriosis in the rhesus monkey (Hadfield *et al*., 1997). Therefore, the use of these procedures does not constitute a bias in the original study of Rier *et al*. (1993).

The new results indicating an association of endometriosis with increased concentrations of polychlorinated biphenyl compounds (PCBs) has, however, raised a number of new questions. Several hypotheses to explain the observations were considered by the Committee. These hypotheses were that:

- the association of increased serum concentrations of PCBs with endometriosis reflects a causal relationship independent of the prior treatment with 2,3,7,8-TCDD;
- the association is fortuitous and the administration of 2,3,7,8-TCDD has either initiated or promoted the development of endometriosis;
- the accumulation of dioxin congeners represents a biomarker of exposure to 2,3,7,8-TCDD;
- the accumulation of dioxin congeners represents a biomarker of an effect of 2,3,7,8-TCDD.

Since there is no evidence that the accumulation of the dioxin congeners and dioxin-like PCBs had occurred prior to the development of endometriosis the Committee considered that the available data were inadequate to determine whether any one of these hypotheses was more probable than any other. Particular points considered by the Committee and other relevant information are detailed in Annex 2.

Due to the uncertainties raised by the new findings, the Committee had less confidence in the quantitative relationship between exposure to 2,3,7,8-TCDD and the incidence of endometriosis in monkeys. It therefore decided not to include Rier *et al*. (1993) as a pivotal study in the updated assessment, though it recognized that effects were reported at body burdens similar to those calculated for other (rat) studies.

Neurobehavioural effects in the offspring of 2,3,7,8-TCDD treated rhesus monkeys

Neither of the papers of Rier and colleagues (2001a, 2001b) provides new information that would affect the opinion of the Committee with regard to the neurobehavioural development study of the offspring (Schantz and Bowman, 1989). In view of the doubts expressed earlier by the Committee on the significance of the neurobehavioural observations (SCF, 2000), and the firmer basis for extrapolation from the pivotal rodent studies that is now available, the Committee decided not to include the study of Schantz and Bowman (1989) as a pivotal study in the updated assessment.

Immune function and serum levels of 2,3,7,8-TCDD analogues

The second new study by Rier and her colleagues (Rier *et al.*, 2001b) investigated the effects of 2,3,7,8-TCDD exposure on the immune system of rhesus monkeys as manifested in the phenotype and function of peripheral blood mononuclear cells (PBMC). Clinical studies have indicated a relationship between endometriosis and deficiencies in humoral and cell-mediated immunity.

Samples of blood were taken from the surviving animals from the original study (Rier *et al.*, 1993) and from twelve additional, similarly-aged animals with no exposure to 2,3,7,8-TCDD or polyhalogenated aromatic hydrocarbons. The phenotype of the PBMC was measured by flow cytometric analysis after staining with monoclonal antibodies specific to various human (and rhesus monkey) surface cell antigens. The secretion of the cytokines, tumour necrosis factor-α (TNF-α), interferon-γ and interleukins 6 and 10, by PBMC in response to stimulation by phytohaemagglutinin (PHA) or polyinosinic acid-polycytidylic acid (PIC) was measured. The cytolytic activity of PBMC was measured by ^{51}Cr release from two target cell lines (Rier *et al.*, 2001b).

In the study of the phenotype of PBMC no significant differences between 2,3,7,8-TCDD exposed and unexposed animals are recorded, though all 18 animals that had not been exposed to 2,3,7,8-TCDD were included in the control group. However, it is noted that, when the results from only the animals of the original study (Rier *et al.*, 1993) were considered, there was a significant increase in the numbers of CD16+/CD56+ natural killer cells in the 2,3,7,8-TCDD-treated animals.

Cytokine production by the PBMC in response to PHA or PIC was observed to differ significantly between control monkeys and those exposed to 2,3,7,8-TCDD only for release of TNF-α in response to PHA. Within group differences in terms of the responses to PHA and PIC were recorded for two other cytokines. Varied numbers of animals from the combined control group were included in this study. Significant correlations between PHA-induced TNF-α production and serum concentrations of 2,3,7,8-TCDD, 1,2,3,6,7,8-HxCDD, and PeCB, but notably not TCB, and also with serum triglycerides (Rier *et al.*, 2001a), were recorded when the data only from animals of the original experiment (Rier *et al.*, 1993) were analysed.

The lytic activity of rhesus monkey PBMC against RAJI (but not K562) cells exhibited a non-significant trend to decreased activity with increased group dietary concentration of 2,3,7,8-TCDD (Rier *et al.*, 1993). If, for individual animals, the results of the assay were plotted against cumulative dose of 2,3,7,8-TCDD this trend became significant.

The associations of effects on immune parameters with serum concentrations of 2,3,7,8-TCDD and some dioxin-like analogues (Rier *et al.*, 2001b) are consistent with prior observations of the immunotoxicity of 2,3,7,8-TCDD in many animal species and also with the possible involvement of TNF-α in the toxicity of 2,3,7,8-TCDD. However, the inclusion of additional 'control' animals in parts of this study, prospective to the study itself but retrospective to the original dietary administration of 2,3,7,8-TCDD, renders the study of little value to toxicological evaluation. The Committee considered that it was not possible to establish causality of immune system changes in rhesus monkeys receiving 2,3,7,8-TCDD in the diet and therefore did not include this study as a pivotal study in the updated assessment.

Derivation of a tolerable intake of 2,3,7,8-TCDD and related compounds for humans

Based on previously existing and additional uncertainties regarding the monkey studies, the Committee decided to base its updated assessment on the rodent studies rather than on the rodent and monkey studies. The above discussion has identified the pivotal studies, which provide a NOAEL and LOAELs for the most sensitive effects of 2,3,7,8-TCDD exposure in experimental animals, i.e. developmental effects in rat male offspring. The Committee has calculated that sensitive responses (LOAELs) were associated with steady state body burdens between 40 and 100 ng 2,3,7,8-TCDD/kg bw with associated estimated human daily intakes (EHDI) in the range of 20 - 50 pg 2,3,7,8-TCDD/kg bw (see Table 3). For the NOAEL as observed in the study of Ohsako *et al*. (2001) a maternal steady state body burden of 20 ng/kg bw and an associated EHDI of 10 pg 2,3,7,8-TCDD/kg bw was calculated.

In deriving a tolerable intake for 2,3,7,8-TCDD the Committee considered the associated EHDIs based on both the NOAEL and the LOAELs.

Tolerable intake

In order to arrive at a tolerable intake of 2,3,7,8-TCDD for humans an uncertainty factor needs to be applied. In the case of using the EHDI of 10 pg/kg bw based on a NOAEL the uncertainty factor should account for the possible differences between experimental animals and humans in susceptibility (toxicokinetics and toxicodynamics) to 2,3,7,8-TCDD and the potential interindividual variation in susceptibility (toxicokinetics and toxicodynamics) to 2,3,7,8-TCDD within the human population.

- The use of an uncertainty factor to account for differences between experimental animals and humans in toxicokinetics was not required since the default toxicokinetic

factor was replaced by actual data in calculating the body burdens used to scale doses across species.

- To account for interindividual variations in humans in toxicokinetics (i.e. absorption, biotransformation, accumulation and elimination of 2,3,7,8-TCDD) an uncertainty factor has to be applied. In considering aspects of the variability in the toxicokinetic properties of 2,3,7,8-TCDD in humans, the Committee noted that reported mean half-lives of 2,3,7,8-TCDD in man vary from 5.1 to 11.3 years (IARC, 1997). The Committee noted that the human data were primarily derived from occupationally exposed men. The distribution of these mean half-lives shows a mean of 8 years and a standard deviation of 2.1 years. Using the mean plus two standard deviations (12.2 years) to describe the 95% upper interval for the half-life of 2,3,7,8-TCDD in humans, a data-derived uncertainty factor of 1.5 (12.1/8) would be predicted for interindividual variations in toxicokinetics. However, the variability in toxicokinetics among females may not be adequately covered and the most sensitive effects of 2,3,7,8-TCDD were seen after 2,3,7,8-TCDD exposure of female animals. Because the Committee had no structured and useful information on the potential variations among women as regards the most important determinants in toxicokinetics, which are size of body fat stores, CYP1A2 concentrations in liver, and rate of metabolism of 2,3,7,8-TCDD, the Committee found it most appropriate to use the default uncertainty factor of 3.2 as recommended by WHO (WHO, 1994) to account for interindividual variations with regard to absorption, biotransformation, accumulation and elimination of 2,3,7,8-TCDD within the human population.

- With regard to the potential differences in toxicodynamics between experimental animals and humans and within the human population, studies of Ah receptor binding affinity and adverse responses directly dependent on Ah receptor activation suggest that humans are less sensitive to 2,3,7,8-TCDD than responsive rodent strains. However, studies of some biochemical or cellular effects, such as CYP1A1 and CYP1A2 induction, suggest a comparable sensitivity. Therefore, for some endpoints it can not be excluded that the most sensitive humans might be as sensitive to the adverse effects of 2,3,7,8-TCDD as experimental animals. The Committee concluded that no uncertainty factor needs to be applied for differences in toxicodynamics between experimental animals and humans and for interindividual variation among humans.

Therefore, the Committee considered an uncertainty factor of 3.2 applied to a NOAEL adequate for the protection of human health from exposure to 2,3,7,8-TCDD.

Applying this 3.2-fold uncertainty factor to the EHDI of 10 pg 2,3,7,8-TCDD/kg bw, calculated from the NOAEL in the Ohsako study, a tolerable intake of 3 pg/kg bw per day can be derived.

In using the LOAEL instead of the NOAEL an additional uncertainty factor needs to be applied. As the LOAELs reported for the sensitive endpoints were considered to be close to the NOAELs and were representing marginal effects, the Committee found it appropriate to allow a factor of 3 to account for the use of LOAELs instead of NOAELs. In this case, this leads to an overall uncertainty factor of 9.6 (3 x 3.2).

Applying this 9.6-fold overall uncertainty factor to the EHDI of 20 pg/kg bw calculated from the LOAEL in the study of Faqi *et al*. (1998) a tolerable intake of 2 pg/kg bw per day can be derived. Using a similar approach to the LOAELs in the other studies in Table 3 would result in the figures of 4 and 5 pg/kg bw per day for the tolerable intake.

The Committee recognized that the Wistar rats as used in the study by Faqi *et al*. (1998) might be the most sensitive rat strain. The Committee therefore concluded that 2 pg/kg bw per day should be considered as a tolerable intake for 2,3,7,8-TCDD.

Recognizing that compounds like 2,3,7,8-TCDD and related substances have very long half-lives in the human body, the Committee considered that the tolerable intake should be expressed on a weekly rather than a daily basis. Therefore the Committee established a tolerable weekly intake (TWI) of 14 pg 2,3,7,8-TCDD/kg bw.

In recognising that the other 2,3,7,8-substituted PCDDs, PCDFs and the dioxin-like PCBs have a similar mode of action as 2,3,7,8-TCDD, the Committee, as in its previous opinion, concluded that the TWI for 2,3,7,8-TCDD should be extended to include all 2,3,7,8-substituted PCDDs and PCDFs, and the dioxin-like PCBs, expressed as WHO TEQ (van den Berg *et al*. 1998) and established a group TWI of 14 pg WHO TEQ/kg bw for these compounds.

Because the new studies provided a firm basis for the evaluation of the pivotal rat studies the Committee removed the designation “temporary” from the TWI.

Although the Committee has now established a TWI of 14 pg WHO-TEQ/kg bw, it wishes to stress that, given the average dietary intakes of dioxins and dioxin-like PCBs in the European countries of 1.2 – 3.0 pg/kg bw per day, a considerable proportion of the European population would still exceed the TWI derived by the Committee.

The Committee therefore concluded that the considerations set out in the chapters on risk characterisation, risk management strategies and recommendations of the previous assessment of November 2000 were still valid.

Annex I

Establishment of a relationship between foetal 2,3,7,8-TCDD body burdens and maternal body burdens in pregnant rats at GD16 following either a single gavage dose on GD15 or following preceding subchronic low dose administration leading to steady state.

The critical determinant of the reproductive effects seen in the male offspring of pregnant rats given a single gavage dose of 2,3,7,8-TCDD on GD 15 is the foetal concentration on GD 15/GD 16, which is likely to be higher following a single bolus dose on this day than that resulting from lower level chronic exposure. Therefore, information is needed to compare maternal body burdens from either acute or chronic exposure that produce similar foetal concentrations. Studies by Hurst *et al*. (2000 a,b) provide a basis for this comparison (Table 1).

In the first study by Hurst *et al*. (2000a) in which ^{3}H-2,3,7,8-TCDD concentrations were measured in the tissues of pregnant Long Evans dams at GD 16 following administration by gavage at GD 15 of 0.05, 0.2, 0.8 or 1.0 μg/kg bw, the average maternal body burdens were reported to be 30.6 (60%), 97.4 (48%), 522.8 (65%) or 585.2 (59%) ng 2,3,7,8-TCDD/kg bw (percentage of dose), respectively. The corresponding average foetal body burdens at GD16 were 5.3, 13.2, 39.1 and 55.7 ng 2,3,7,8-TCDD/kg bw.

In the second study by Hurst *et al*. (2000b) the radioactivity was measured in both the maternal and foetal tissues of pregnant Long Evans dams at GD 16 following subchronic administration of ^{3}H-2,3,7,8-TCDD. Female rats were dosed by gavage with 1, 10, or 30 ng of ^{3}H-2,3,7,8-TCDD/kg bw in corn oil, 5 days per week, for 13 weeks. At the end of this period, the rats were mated and dosing was continued every day throughout gestation. The dosage regimen used produced a steady state of 2,3,7,8-TCDD in the dams. The average maternal and foetal body burdens at GD 16 are shown in Table a and compared with the average maternal and foetal body burdens found at GD 16 following the single gavage administration of 2,3,7,8-TCDD on GD 15 in the previous study by Hurst *et al*. (2000a).

Table a. Comparison of average maternal and foetal body burdens after single dose and subchronic 2,3,7,8-TCDD exposure to pregnant rats.

Single dose exposure at GD15[1)]				Subchronic exposure [2)]			
Single dose [3)]	Body burden measured at GD 16			Adjusted daily dose [4)]	Body burden measured at GD 16		
	Maternal [3)]	Foetal [3)]	Maternal/ Foetal		Maternal [3)]	Foetal [3)]	Maternal/ Foetal
50	30	5.3	5.7	0.71	20	1.4	14.3
200	97.4	13.2	7.4	7.1	120	7.5	16.0
800	523	39.1	13.4	21.3	300	15.2	20
1000	585	55.7	10.5				

[1)] Data from Hurst *et al*. (2000a)
[2)] Data from Hurst *et al*. (2000b)
[3)] ng/kg bw
[4)] ng/kg bw per day, adjusted to continuous exposure from 5 days/week

The Committee noted that linear extrapolation of the relationship between the foetal and maternal body burdens using the data provided by Hurst *et al*. (2000a,b) did not intercept zero as would be expected since radiolabelled 2,3,7,8-TCDD was used in both studies. The Committee therefore performed a best-fit analysis of each data set within the dose ranges of interest for the risk assessment, constraining the curves to pass through the origin.

The data from the acute study (Hurst *et al.*, 2000a) were treated in the following way: The two highest values were considered to be outside the dose range of interest for the assessment. Initially, the highest figure was taken out, and the data were fitted to a number of possible functions using SigmaPlot. It was found that the data were best fit to a power equation. Using SigmaPlot, corresponding values between foetal body burdens and maternal body burdens were generated. The Committee found that an estimated body burden of 112.5 ng 2,3,7,8-TCDD/kg bw would correspond to a foetal body burden of 15.2 ng 2,3,7,8-TCDD/kg bw and used this figure in its final calculation (Table b).

The data from the study of maternal and foetal concentration of 2,3,7,8-TCDD following subchronic administration leading to steady state were used as derived by Hurst *et al.* (2000 b) (Table a).

Table b. Comparison of average maternal and foetal body burdens after single dose and subchronic 2,3,7,8-TCDD exposure to pregnant rats.

Single dose exposure at GD15 [1]				Subchronic exposure [2]			
Single dose [3]	BBBody burden measured at GD 16			Adjusted daily dose [4]	BBBody burden measured at GD 16		
	Maternal [3]	Foetal [3]			Maternal [3]	Foetal [3]	
0	0	0		0	0	0	
50	30	5.3		0.71	20	1.4	
200	97.4	13.2		7.1	120	7.5	
-	112.5[5]	15.2		21.3	300	15.2	

[1] Data from Hurst *et al*. (2000a)
[2] Data from Hurst *et al*. (2000b)
[3] ng/kg bw
[4] ng/kg bw per day, adjusted to continuous exposure from 5 days/week
[5] Estimated figure

These two data sets were fit to power equations with the following result.

I. Acute study: $Y = 3.8791 \times X^{1.2418}$ ($R^2 = 0.999$) (Hurst *et al*. 2000a)

II. Subchronic study: $Y = 9.4843 \times X^{1,2685}$ ($R^2 = 0.999$) (Hurst *et al*. 2000b)

Where Y is the maternal body burden (ng 2,3,7,8-TCDD/kg bw) and X is the foetal body burden (ng 2,3,7,8-TCDD/kg bw).

These two equations were used to calculate the corresponding acute and subchronic maternal body burdens for a number of foetal body burdens ranging from 0 to 15.2 ng 2,3,7,8-TCDD/kg bw (Table c).

Table c. Corresponding values of foetal, acute maternal and subchronic steady state maternal body burdens of 2,3,7,8-TCDD.

Foetal body burden (ng/kg bw)	Acute maternal body burden (ng/kg bw)	Subchronic (steady state) maternal body burden (ng/kg bw)	Ratio subchronic maternal/acute maternal body burden
1.2	5.0	12.3	2.5
1.4	5.9	14.6	2.5
1.7	7.5	18.6	2.5
1.8	8.0	20.0	2.5
1.9	8.5	21.0	2.5
2.1	10	25.0	2.5
3.0	15.5	39.0	2.5
5.3	31	78.6	2.5
6.3	38.5	99.0	2.6
7.5	47.5	122	2.6
8.0	52	134	2.6
9.0	60	156	2.6
13.2	95.7	251	2.6
15.2	113	299	2.7

Annex II

Studies of endometriosis in rhesus monkeys - additional considerations

The Committee noted that the additional information available to it had resolved some of the matters that had been noted in its opinion of November 2000 (SCF, 2000). The high incidence (33%) of endometriosis in the colony noted previously (Rier *et al*., 1993) was, in that paper, compared with an incidence of 27% noted in control animals of a study of radiation-induced endometriosis (Fanton and Golden, 1991). This information removed one of the reservations that the Committee had expressed previously. However, as mentioned above, the new results indicating an association of endometriosis with the increased concentrations of polychlorinated biphenyl compounds (PCBs) raised new questions. These are discussed in more detail here.

The original report of endometriosis in 2,3,7,8-TCDD-exposed rhesus monkeys was the result of adventitious observations that initiated a more detailed study (Rier *et al*., 1993). The additional results reported in the recent paper (Rier *et al*., 2001a) are also adventitious and the association of endometriosis with the increased concentrations of PCBs requires further evidence if it is to be accepted as causal.

The main problem with the recent study is that any possible exposure to PCBs is completely undefined. It has been recorded that seven samples of feed for these monkeys during the initial four years 2,3,7,8-TCDD feeding trial were analysed and found to contain 7.6 ± 2 µg/kg of total PCBs and 1.0 ± 0.2 µg/kg of DDE, means ± s.e., analytical technique not specified (Schantz and Bowman, 1989). Both control and 2,3,7,8-TCDD treated monkey chow were analysed (Rier *et al.*, 2001a). From the PCB levels found it can be calculated that the monkeys received approximately 0.2 µg total PCB/kg bw perday (assumptions: daily intake of chow 190 g; weight of monkeys 7.5 kg). Otherwise, only the results of the recent analyses provide evidence of exposure to PCBs.

An additional problem relates to the properties of one of the PCBs that was analysed. The TCB congener has been the subject of a comparative study of its clearance from the bodies of rhesus monkeys and rats (Abdel-Hamid *et al*., 1981). Three female rhesus monkeys were administered an intravenous dose of ^{14}C-labelled TCB and held in metabolism cages for a period of 42 days. It was noted that 50% of the radioactivity was excreted within 14 days. However, recovery of the radioactivity was only 73% and it was suggested that, if the unrecovered material had been in the faeces, 50% of the dose could have been excreted in the first 8-10 days of the study. The residual radioactivity in the animals was predominantly in the adipose tissue, containing 2.3% of dose, more than twice that of all other tissues combined. Metabolites of TCB comprised more than 97% of the radioactivity in the faeces and, after 1 or 2 days, more than 50% of the radioactivity circulating in the blood was in the form of TCB metabolites. Therefore, if the half-life ($t_{½}$) of TCB in the rhesus monkeys of Rier and colleagues (2001a) is in the range of 1-14 days it can be estimated that a steady state body burden of 230 ng TCB/kg bw (lipid base; mean value for 2,3,7,8-TCDD exposed monkeys in the study) would be achieved by a

daily dietary intake of 3.4 or 48 ng TCB/kg bw, assuming $t_{½}$ of 14 or 1 days, respectively. Assumptions made are that the fat content of the rhesus monkey bodies is 15% and that the absorption of TCB from the gastrointestinal tract is 50%.
This body burden could be achieved within a period of less than 3 months provided the monkeys had the above-mentioned daily intake of TCB. However, most of the TCB circulating in the blood would be in the form of metabolites and not the parent compound that was analysed in the study of Rier *et al*. (2001a). On the other hand, in the absence of any TCB exposure no measurable amounts of TCB would be expected within less than 3 months. It is therefore not possible to attribute the endometriosis observed three years previously to exposure to PCBs with any certainty.

Several hypotheses have been offered to explain the observations. These can be summarised as followed:

- the association of increased serum concentrations of PCBs with endometriosis reflects a causal relationship independent of the prior treatment with 2,3,7,8-TCDD;
- the association is fortuitous and the administration of 2,3,7,8-TCDD has either initiated or promoted the development of endometriosis;
- the accumulation of dioxin congeners represents a biomarker of exposure to 2,3,7,8-TCDD;
- the accumulation of dioxin congeners represents a biomarker of an effect of 2,3,7,8-TCDD.

Not all of these possible hypotheses are mutually exclusive and most of them have been explicitly suggested as being possible by Rier and her colleagues (Rier *et al.*, 2001a). It is necessary to consider alternative options in turn.

A comparison of the first two options reveals that there are distinct differences.

Firstly, the treatment of the monkeys with 2,3,7,8-TCDD was undertaken in circumstances in which the dose and its period of administration were clearly defined; there is nothing that defines any dose or duration of exposure to PCB isomers.

Secondly, both the incidence and severity of the endometriosis as assessed earlier (Rier *et al.*, 1993) exhibited a dose-response relationship with 2,3,7,8-TCDD within the limitations of the use of only two treated groups in the original study.

Thirdly, there is an association of serum TCB concentrations with development of endometriosis in the absence of known exposure to PCBs. Three out of a group of four Pb-treated monkeys had endometriosis and the mean concentration of TCB in the serum of the group was elevated (Rier *et al.*, 2001a). This indicates a lack of specificity of association of endometriosis with exposure to PCBs.

Fourthly, there is a temporal association of 2,3,7,8-TCDD exposure with development of endometriosis, which cannot be shown to exist for any association with PCB isomers.

Fifthly, the relationship between incidence and severity of endometriosis and PCB exposure was studied in female rhesus monkeys; despite initial observations suggesting an association (Arnold *et al.*, 1990), the results of the final study did not support any relationship (Arnold *et al*., 1996). In that study, groups of 20 female rhesus monkeys received 0, 5, 20, 40 or 80 µg Aroclor 1254/kg bw per day for 6 years in a toxicological-reproduction study. The incidence of endometriosis in the control group was 37% (6/16 animals) and 25% (16/64) in the exposed groups. The PCB mixture used contained 0.05% TCB (and 0.01% PeCB) (Arnold *et al*. 1990). Thus the monkeys were exposed to 0, 2.5, 10, 20, or 40 ng TCB/kg bw per day. Interestingly, Arnold *et al*. (1996) report that the PCB mixture used contained polychlorinated dibenzofurans and dioxin-like PCBs equivalent to 182 µg TCDD equivalents/g Aroclor 1254. Therefore the monkeys in this study received 0, 0.91, 3.64, 7.28 or 14.56 ng TCDD equivalents/kg bw per day for 6 years without any increase in incidence and severity of endometriosis being noted. The method used to calculate 2,3,7,8-TCDD equivalents was not stated.

Finally, there are studies of the promotion by 2,3,7,8-TCDD, in mice and rat, of the growth of surgically induced endometriotic cysts, although at higher doses than in these monkeys (SCF 2000). In addition, the promotion by 2,3,7,8-TCDD of the growth and survival of autotransplanted endometrial tissue in the abdomens of cynomolgus monkeys has been observed by Yang *et al*. (2000). Female cynomolgus monkeys (5-6 per group) were orally dosed with 2,3,7,8-TCDD-containing gelatin capsules, 5 days per week for 12 months following the surgical auto-implantation of endometrial strips into multiple abdominal sites. Average delivered TCDD doses were 0, 0.71, 3.57 or 17.86 ng/kg bw per day. Significantly greater numbers of the endometrial strips survived in the two highest TCDD dose groups at necropsy compared to the controls (26.7% and 33.3% vs. 16.0%, respectively). The size of the implants increased only in the high dose group. It is noteworthy that surviving endometrial strips actually regressed in size in the lowest TCDD dose group. Serum concentrations of the cytokine IL-6 were significantly decreased while levels of IL-6 sR (soluble receptor) were increased in the high dose monkeys at termination. These studies provide evidence supportive of a causal relationship between 2,3,7,8-TCDD exposure and the development of endometriosis.

These differences are consistent with there being an association of 2,3,7,8-TCDD, rather than PCBs, with development of endometriosis in rhesus monkeys.

The third hypothesis, that the presence of dioxin congeners represents a biomarker of exposure to 2,3,7,8-TCDD, assumes that contamination of the solution of 2,3,7,8-TCDD during preparation of diets resulted in the PCB congeners being incorporated in the diet and retained in the tissues of the rhesus monkeys (Rier *et al.*, 2001a). This is implausible given the short half-life of TCB in the rhesus monkey and the time that had elapsed since dosing was terminated.

The fourth hypothesis, that the accumulation of TCB is a consequence of exposure to 2,3,7,8-TCDD and therefore a biomarker of effect, has been discussed by Rier and

colleagues (2001a). However, they note that it might be expected that treatment with 2,3,7,8-TCDD would be expected to increase the metabolism and excretion of TCB.

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