TOXICOLOGICAL PERSPECTIVES ON PERFLUORINATED COMPOUNDS

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Introduction

Perfluorinated chemicals have been widely used in commerce for the last few decades. Until recently little was known about their environmental fate and even less was known about their potential environmental effects. Since Giesy and co-workers first demonstrated the widespread occurrence of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in wildlife there has been interest in determining the biological and possible ecological effects of these compounds. The assessment of possible effects of these chemicals has been hampered by a limited understanding of their mode of action. While certain of the chemicals are known to be peroxisome proliferators, the most abundant compound PFOS is not a classic or complete peroxisome proliferator. Here we summarize recent toxicological and mode of action studies available for perfluorinated compounds (PFCs) and use this information and environmental concentration data to determine the degree of environmental risk these compounds pose.

Previous Studies

Early studies on the biological effects of perfluorinated fatty acids focused on the carboxylic acids particularly PFOA. These studies demonstrated a variety of biochemical and physiological endpoints. In particular, interest was focused on the ability of PFCs to cause peroxisome proliferations through the peroxisome proliferators-activated receptor (PPAR) [1]. One well characterized cellular response to perfluorinated fatty acids is hypolipidemia however, the exact cause of this response was unclear [1]. A summary of all verifiable and available information on PFOS has recently been prepared by OECD (www.oecd.org/pdf/M00036000/M00036809.pdf).

Recent Mode of action studies

Recent studies on the mode of action have focused on PFOS since it has been found to accumulate in wildlife at measurable concentrations. The amphiphilic nature of these compounds suggests that they will be surface and membrane active. The ability of PFOS to non-specifically modulate membrane properties was assessed using cell culture bioassay procedures[2]. PFOS was able to alter physical membrane properties such as membrane permeability and fluidity. PFOS was also shown to inhibit gap junctional intercellular communication (GJIC) *in vitro* and *in vivo* [2].

The accumulation of PFOS in serum raised the prospect of this compound interfering with the binding of steroid hormones to their specific serum carrier proteins. Studies of the competitive binding of PFOS for steroid hormones indicated that in general threshold concentrations for these effects were greater than 100 mg/L [3]. The reason for the relatively great threshold for effects was determined to be the results of the binding of PFOS to serum albumin. The high affinity binding to albumin means that until all binding to albumin is saturated, PFOS is unable to bind to other active sites. The high affinity for albumin also explains the accumulation of this compound in

blood. Different analytical approaches were used to determine the binding capacity of albumin for PFOS. The binding of PFOS to albumin was determined by quadrapole time of flight mass spectrometry, a direct aqueous phase binding assay and equilibrium dialysis. All methods indicated binding of between one and two molecules of PFOS to each albumin molecule. Studies have also investigated the binding of PFOS to other proteins. These studies have demonstrated relatively weak binding of PFOS to proteins involved in fatty acid transport and metabolism [4].

Given the relatively wide range of observed effects PFOS appears to have on cellular lipid metabolism it still remains unclear which is the primary toxic effect of this compound. To elucidate the breadth of changes caused by PFOS we investigated alterations in gene expression caused by exposure of rat cell lines and rats in vivo to PFOS[5]. These studies revealed alterations in the expression of many genes. Genes involved in peroxisomal fatty acid oxidation were up-regulated while no changes were observed for the same metabolic pathway in mitochondria. PFOS also failed to induce all the genes associated with the 'normal' PPAR response. This observation is supported by work in monkeys suggesting that PFOS is not a peroxisome proliferator [6]. In contrast PFOA which appears to be a potent and archetypal peroxisome proliferator [7].

Recent Toxicological Studies

The most recent toxicological studies of PFCs investigated effects of PFOS on rats [8] and cynomolgous monkeys [6;9]. PFOS orally administered to monkeys at 0.75 mg/kg/day for 182 d resulted in mortality, decreased body weight and alterations in lipid and hormone concentrations [6]. Serum PFOS concentrations associated with no adverse effects were 82.6 mg/L in males and 66.8 mg/L in females. In the recovery phase of the experiment a clearance half-life of approximately 200 d was determined. In a similar experiment PFOA was administered up to 20 mg/kg/d. No adverse effects were observed at 3 and 10 mg/kg/day after 182 d. Liver concentrations associated with no adverse effects were approximately 15 mg/kg. PFOA appears to be more rapidly cleared than PFOS as a mean concentration of 14 mg/kg in the 10 mg/kg/d dose group had dropped to 0.12 mg/kg after a 90 d recovery period (half life of approximately 13 d). One key finding of these studies was that for effects thresholds total accumulated dose was a more robust measure of exposure than exposure concentration. Also, a very steep dose response curve was observed above the threshold dose. We suggest these dose response characteristics indicate binding of PFOS to albumin and once the albumin pool is saturated PFOS 'overflows' to targets.

Mammalian Risk Assessment

TRV Calculation

Since there are few studies that have examined the effects of PFOS on wildlife, TRVs were developed based on the results for standard laboratory species. TRVs were developed from these laboratory studies by applying uncertainty factors to toxicity data a derived by the method of Henningsen and Hoff (1997)[10]. This approach results in very conservative estimates of the threshold effect level and encourages the collection of additional information, especially for site-specific ERAs. If results were available from a definitive study with the species of concern, the TRV would likely be greater (less conservative) because safety factors would not be applied.

Several studies have characterized the acute and chronic toxicity of PFOS to mammals (OECD, 2002). Of these studies, a two-generation combined oral (gavage) fertility, developmental and prenatal/postnatal reproduction study of PFOS in rats provides the best data for characterizing

effects on terrestrial mammals (Table 1). No effects on mating, fertility, or estrous cycle occurred at any dose in the study in either F_0 or F_1 maternal or paternal animals. The F_0 NOEL for reproductive parameters was greater than 3.2 mg/kg/d, the greatest dose level in the study. The F_1 maternal and paternal animals also experienced no effects on mating or fertility at the greatest dose tested, 0.4 mg/kg/d. At concentrations of 1.6 mg PFOS/kg/d and greater, pre-implantation loss increased and litter size, pup viability, growth, and survival were less. The NOEL for F_1 and F_2 generation growth/survival was 0.4 mg/kg/d. No effects on pup survival/growth occurred at 0.4 mg/kg/d. 0.4 mg/kg/d is considered to be a more ecologically relevant NOEL than 0.1 mg/kg/d because the endpoints for the 0.4 mg/kg/d are more ecologically relevant.

Table 1. Treatment groups in the rat reproduction study[1]

Group[2]	Observed Effect
Control Fetus	None
Control Dam	None
0.1 mg/kg/d Dam PM	NOEL
0.1 mg/kg/d Fetus EG	NOEL
0.1 mg/kg/d Dam EG	NOEL
O.4 mg/kg/d Dam PM	NOEL
0.4 mg/kg/d Fetus EG	NOEL
0.4 mg/kg/d Dam EG	NOEL
1.6 mg/kg/d Dam PM	Slight body weight
1.6 mg/kg/d Fetus EG	Survival body weight
1.6 mg/kg/d Dam EG	Slight body weight
3.2 mg/kg/d Dam PM	Body weight
3.2 mg/kg/d Fetus BG	Stillbirth, survival
3.2 mg/kg/d Dam EG	Body weight

[1] Dosing for 6 weeks prior to mating and 21 days of gestation.
[2] PM=pre-mating, 42 d dosing; EG=end of gestation, 21 d gestation

Table 2 Hazard quotients (HQ) and Margins of Safety (MOS) for PFCs in mustellids. Analytical data from Kannan et al 2002[11]. TRV=12-72.5 mg/kg, ww, depending on uncertainty factors.

Species	**Location (State, USA)**	**PFOS (mg/kg, ww)**	**PFOA (mg/kg, ww)**	**HQ (PFOS)**	**MOS (PFOS)**
Mink	Illinois	1.4	0.02	0.02-0.12	8.3-50
	Massachusetts	0.2	0.008	0.003-0.02	50-333
	Sth. Carolina	1.7	<0.02	0.024-0.14	7-41
	Louisiana	0.14	<0.02	0.002-0.012	83-500
Otter	Washington	0.025-0.42	<0.008-0.019	0.003-0.02	63-333
	Oregon	0.034-1.0	<0.008-0.019	0.003-0.02	50-333

Depending on the relative sensitivity of mink to PFOS, the NOAEL could range from 12 to 72.5 mg PFOS/kg liver (ww). The dietary NOAEL could range from 0.4 to 0.038 mg PFOS/kg bw/d. To calculate HQs, the more conservative values (12mg PFOS/ kg liver, and 0.038 mg PFOS/kg/d) were used. Note that this is a very conservative approach, without a definitive study PFOS effects of PFOS on mink, this approach would be used in a screening-level risk assessment.

Summary

Clearly not all PFCs are created equal, even compounds which are closely related structurally (e.g. PFOS and PFOA) elicit different responses both *in vitro* and *in vivo*[2]. These differences are related to the different structures of the compounds and in particular the nature of the functional group. These differences result in both different potencies for the same mode of action and differences in actual modes of action for the compounds. It therefore seems unlikely that a TEF approach similar to that used for compounds such as dioxins will be applicable to PFCs. A potency equivalency model may be applicable to these compounds but any such model will have to take into account the different modes of action for the different PFC classes. While the ability of some PFCs to elicit PPAR responses is clear it remains uncertain whether these responses are relevant environmentally as species differ greatly in their sensitivity to PPAR active compounds [12] [13], for example humans appear to be very insensitive to PPAR related responses [12].

In general current concentrations of PFOS in mustellids do not seem to pose a significant threat. However, HQ values in some cases exceed 0.1 indicating that current exposure concentrations are within the same order of magnitude as adverse effect concentrations. Additional studies to better define the sensitivity of these species to PFCs would reduce the uncertainties in this assessment.

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