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May 5, 2009

Thomas M. Mack, M.D., M.P.H., Chairperson
Committee Members
Carcinogen Identification Committee

RE: *DUPONT COMMENTS REGARDING PRIORITIZATION OF PFOA*

Dear Chairman Mack and Committee Members:

On behalf of E.I. du Pont de Nemours and Company ("DuPont"), we are writing to recommend that PFOA (perfluorooctanoic acid and its salts and transformation and degradation precursors[1]) should not be advanced to the next stage of the listing process under Proposition 65, or that it be assigned the lowest priority among the thirty-eight chemicals nominated for review.

EXECUTIVE SUMMARY

We have summarized below five reasons why PFOA should not be advanced or should be assigned the lowest priority. We call attention to ***new studies and reports reinforcing the conclusion that PFOA is not carcinogenic and that exposure to PFOA is declining***, which apparently were not considered in screening PFOA and thus were not provided to the Committee.

1. *OEHHA's Summary Table Shows Little Meaningful Evidence of Carcinogenic Potential for PFOA Relative to Most of the Candidate Chemicals.* The Summary Table that accompanies OEHHA's March 5 request for public comments[2]" shows that:

- ***Thirty-three*** chemicals other than PFOA show carcinogenic activity in two or more animal studies. By contrast, the animal data for PFOA show only "one study and [a] second study with benign tumors only," according to the

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[1] We understand that OEHHA has designated PFOA by this terminology to encourage the consideration of all data relevant to PFOA, including data showing that certain precursors may transform to or degrade to PFOA. We see no reason to consider such data for the purpose of determining whether PFOA meets the criteria for listing, and point out that such data would not be a basis for listing transformation or degradation precursors unless those chemicals in their own right are "clearly shown . . . to cause cancer" Cal. Code Regs., *tit.* 27, § 23505(a)(1).

[2] "Prioritization of Chemicals for Carcinogen Identification Committee Review: Proposed Chemicals for Committee Consideration and Consultation March 2009 - Reproductive and Cancer Hazard Assessment Branch - Office of Environmental Health Hazard Assessment - California Environmental Protection Agency," hereinafter, "Summary Table." (Attachment A).

Summary Table, and even this description overstates the case. In fact, the animal evidence of carcinogenicity is limited to an increase in benign tumors in only one sex (males) of one species (rats), by a mechanism (or mechanisms) of action of questionable relevance to humans. No increase in malignant tumors has been reported in any carcinogenicity studies of PFOA in animals.

- ***Thirty*** of the compounds other than PFOA are genotoxic. By contrast, PFOA is not genotoxic.
- ***Twenty-seven*** chemicals other than PFOA "overlap" in these two important characteristics. PFOA does not register in either.

Higher priority should be assigned to compounds that cause malignant tumors, produce tumors in more than one sex of one species, produce tumors by a mechanism (or mechanisms) of action of questionable relevance to humans, are genotoxic, and for which epidemiological data show cause for greater concern, than to PFOA, which fails for listing under all of these criteria.

2. *The Data Compiled for the Committee's Review Show that PFOA Should Not Be Advanced or Warrants the Lowest Priority.* The document referring PFOA to the Committee for consultation includes a "compilation of the relevant studies."[3] Of these data, the animal evidence on PFOA shows only an increase in benign tumors in one sex (males) in one species (rats) by a mechanism (or mechanisms) of action of questionable relevance to humans. The human data show no convincing evidence of carcinogenicity in epidemiological studies in workers or the general population. The chemical is not genotoxic. Thus, the data listed in the compilation would not support a determination that PFOA is "clearly shown through scientifically valid testing according to generally accepted principles to cause cancer."[4] In the absence of data that would support listing, it is not a good use of resources to send the chemical to the next stage of the listing process.

3. *New Epidemiological Studies Reinforce the Conclusion that PFOA is Not Carcinogenic.* A new epidemiological study on PFOA and a new evaluation of the epidemiological (and animal) data were published within the past year.

- A study of a large Danish cohort, published in April 2009 in the Journal of the National Cancer Institute, concluded that "plasma concentrations [of PFOA] in the general Danish population are not associated with increased risk of prostate, bladder, pancreatic, or liver cancer."[5]

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[3] "Perfluoroctanoic acid (PFOA) and Its Salts and Transformation and Degradation Precursors," OEHHA (March 2009). (Attachment B).

[4] Cal. Code Regs., *tit.* 27, § 23505(a)(1).

[5] Eriksen KT, Sorensen, M, McLaughlin JK, Lipworth L, Tjonneland A, Overvad K, Raaschou-Nielsen O (2009) Perfluorooctanoate and Perfluorooctanesulfonate Plasma Levels and Risk of Cancer in the General Danish Population, *J Natl Cancer Inst*; 101: 605-609, at 608. (Attachment C).

- An evaluation published in 2008 by the European Food Safety Authority concluded that "[e]pidemiological studies in PFOA-exposed workers do not indicate an increased cancer risk."[6] This is significant because occupational exposure is far greater than exposure in the general population.

Thus, the human data would not support listing PFOA or reviewing the chemical.

4. ***New Exposure Data Show That Levels of PFOA in Human Blood and the Environment Are Declining.*** In the absence of data showing carcinogenicity, exposure alone is not a concern under Proposition 65, and certainly is not a basis for listing. To the extent that exposure remains a concern for prioritization, the Committee should be informed of new studies showing that exposure is declining:

- The Center for Disease Control issued a report in 2007, concluding from two NHANES studies that ***exposure to PFOA in the general population decreased approximately 25%*** in the period beginning in 1999 and ending in 2004.[7]

- The New York State Department of Health reported in 2008 a ***"sharp decrease" in exposure to PFOA in infants and, by extension, their mothers***. The New York investigators measured PFOA and other perfluorinated substances in blood spot composite samples collected between 1997 and 2007, representing a total of 2640 infants.[8]

- The most recent progress reports regarding the US EPA 2010/15 PFOA Stewardship Program indicate that ***manufacturers are "on target to meet the 95 percent reduction goal in PFOA emissions and product content by 2010,"*** and that "[f]urther reductions are anticipated by 2015."[9]

Thus, any reason to review PFOA that arises from exposure is less compelling than it may have been before.

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[6] Perfluorooctane sulfonate (PFOS), perflurooctanoic acid (PFOA) and their salts Scientific Opinion of the Panel on Contaminants in the Food chain, *The EFSA Journal* (2008) 653, 4-131, available on-line at www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902012410.htm.

[7] Calafat AM, Wong L-Y, Kuklenyik Z, Reidy JA, Needhan LL (2007) Polyfluoroalkyl Chemicals in the U.S. Population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and Comparisons with NHANES 1999-2000, *Environmental Health Perspectives* Vol. 115 - No. 11: 1596-1602. (Attachment D).

[8] Spliethoff HM, Tao L, Shaver SM, Aldous KM, Pass KA, Kurunthachalam K, and Eadon GA (2008) Use of Newborn Screening Program Blood Spots for Exposure Assessment: Declining Levels of Perfluorinated Compounds in New York State Infants, *Environ Sci Technol* 42(14):5361-7. (Attachment E)

[9] US EPA OPPT Accomplishments Report January 2007 - 2009, available on-line at www.epa.gov/oppt/ar/2007-2009/managing/potential_risks.htm.

5. ***An Authoritative Body and Another Respected Governmental Agency Reviewed PFOA and Neither Concluded that PFOA is a Carcinogen.*** We reported in November 2006, when the Committee last considered PFOA for "expedited consideration" for listing, that US EPA prepared a Draft Risk Assessment on PFOA in 2005, and did not designate PFOA as carcinogenic. As noted above, the European Food Safety Authority now has reached the same conclusion. No agency has classified PFOA as carcinogenic.

DETAILED ANALYSIS

PFOA SHOULD NOT BE ADVANCED TO THE NEXT STAGE OF THE LISTING PROCESS OR SHOULD BE ASSIGNED THE LOWEST PRIORITY FOR REVIEW

We discuss fully below the reasons that PFOA should not be considered for listing, or should be assigned the lowest priority for review. In doing so, we have summarized all of the data listed in OEHHA's "compilation of the relevant studies." We also have summarized a new study and a new evaluation that support previous conclusions that PFOA is not carcinogenic, and other new reports showing that exposure to PFOA is declining. Because these new studies, evaluations and reports were not included in the OEHHA compilation, we are submitting them as attachments to this letter for the Committee's consideration in determining whether PFOA should be advanced or what priority should be assigned for its review.[10]

1. THE SUMMARY TABLE SHOWS LITTLE MEANINGFUL EVIDENCE OF CARCINOGENIC POTENTIAL FOR PFOA RELATIVE TO MOST OF THE CANDIDATE CHEMICALS

The Summary Table provides a convenient vehicle for comparing the database for PFOA with the databases for other candidate chemicals, and for examining the quantity and quality of data that would support listing or the assignment of a priority to any of the candidate chemicals. A systematic analysis, following the sequence presented in the Summary Table, follows.

EXPOSURE

- Although the Summary Table is correct in indicating that exposure to PFOA is "widespread," exposure in the blood of the general population also is extremely low (approximately 5 parts per billion) and is declining. Moreover, exposure alone is not a basis for listing unless a chemical is carcinogenic, and therefore is not a good reason for assigning a chemical a high priority for

[10] The EFSA report [Perfluorooctane sulfonate (PFOS), perflurooctanoic acid (PFOA) and their salts Scientific Opinion of the Panel on Contaminants in the Food chain, *The EFSA Journal* (2008)] is not attached due to its length (131 pages), but is available on-line at www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902012410.htm.

consideration, either. Furthermore, as discussed below, extensive data indicate an absence of any harmful effects whatsoever to humans.

- According to US EPA, there is "no information linking current levels of PFOA in the blood of the general public to any adverse effects in people."

- Biomonitoring data collected by the Center for Disease Control and the State of New York indicate that exposure to PFOA has declined significantly in recent years.

HUMAN DATA

- Although the Summary Table indicates that there are "analytical data" providing evidence of carcinogenicity, this characterization can be accurate only if it means that there are some human data available for review. It should not be taken to mean that the human data are positive.

- For the reasons below, the epidemiological data identified in the compilation would not support listing or even advancement for review:

  - The 3M "Cottage Grove" study showed no significant increase in the incidence of cancer among approximately 4,000 workers exposed to PFOA for approximately 108,000 person years.

  - The DuPont "Washington Works" study found no increase in overall mortality from cancer among 6027 workers exposed to PFOA from 1947 through 2002 than among other DuPont employees, West Virginia residents, and the U.S. population at large.

- Moreover, the recently published Danish cohort study (discussed in more detail below) concluded that there was no association between plasma PFOA concentration and an increased risk of any of four cancers evaluated. [11]

ANIMAL DATA

- The Summary Table indicates that evidence of carcinogenicity for PFOA is limited to "one study and [a] second study with benign tumors only."

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[11] Eriksen KT, Sorensen M, McLaughlin JK, Lipworth L, Tjonneland A, Overvad K, Raaschou-Nielsen O (2009) Perfluorooctanoate and Perfluorooctanesulfonate Plasma Levels and Risk of Cancer in the General Danish Population, *J Natl Cancer Inst*; 101:605-609. (Attachment C).

- Even the description of "one study and a second study with benign tumors only," as it appears in the Summary Table, is misleading for PFOA. In fact, the animal evidence of carcinogenicity is limited to an increase in benign tumors in only one sex (males) of one species (rats) by a mechanism (or mechanisms) of action of questionable relevance to humans. No increase in malignant tumors has been reported in any carcinogenicity studies of PFOA in animals.

- By contrast, the Summary Table indicates that 33 of the remaining 37 chemicals (89%) show evidence of carcinogenesis in "Two or more studies."

GENOTOXICITY (OTHER RELEVANT DATA)

- The Summary Table indicates that PFOA is not genotoxic

- By contrast, the Summary Table indicates that 30 of the remaining 37 chemicals (81%) are genotoxic.

MECHANISTIC DATA (OTHER RELEVANT DATA)

- The Summary Table indicates that there are "other mechanistic studies." As in the case of the human data, this characterization can be accurate only if it means that there are some data that OEHHA and the Committee could review them; the mechanistic data do not support listing.

- The mechanistic data indicate that the primary biological activity of PFOA is through PPARα activation. The hypolipidimic effects of PFOA in rodents are limited to activation of PPARα, and are consistent with those produced by a wide range of chemicals that cause peroxisome proliferation. A recent study, Rosen et al. 2008[12], concluded that 85% of the genes altered by PFOA were dependent on the activity of PPARα. It is clear that other mechanism(s) of action exist, because PPARα knockout mice continue to show some liver hypertrophy, an effect in rodents that is a hallmark of PPARα activation. However, the overriding mechanism of the biological activity remains dependent on PPARα activation. In recent years, it has been shown that activation of the human form of PPARα does not result in proliferation of hepatic cells and that the mechanism for the proliferation response from activation of rodent PPARα is not operational with

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[12] Rosen MB, Lee JS, Ren H, Vallanat B, Liu J, Waalkes MP, Abbott BD, Lau C, Corton JC (2008a) Toxicogenomic dissection of the perfluorooctanoic acid transcript profile in mouse liver: evidence for the involvement of nuclear receptors PPAR alpha and CAR, *Toxicol Sci* 103:46-56.

human PPARα[13] Therefore, the predominant liver effects in rodents and the tumorigenicity of PFOA in rats are of questionable relevance to humans.

In summary, of the thirty-eight candidate chemicals that OEHHA has selected for prioritization, thirty are genotoxic, and thirty-three show carcinogenic activity in two or more animal studies. Twenty-seven chemicals "overlap" in these two critical characteristics. In contrast to these compounds, PFOA is remarkable because of the absence of data to suggest carcinogenicity: the chemical did not cause an increase in malignant tumors in any study; produced an increase in benign tumors only — and, even then, only in one sex (males) in one species (rats) by mechanism(s) of action of questionable relevance to humans; is not genotoxic; and does not have any human data to indicate a causal relationship between the chemical and cancer. Among the thirty-eight candidate chemicals, higher priority should be assigned to compounds that cause malignant tumors, that produce tumors in more than one sex of one species, that have a mechanism of action considered to be relevant to humans, are genotoxic, or that have evidence of carcinogenicity in epidemiological studies than to a chemical that does not meet any of these criteria, especially where levels of exposure are decreasing.

2. *THE DATA COMPILED FOR THE COMMITTEE'S REVIEW SHOW THAT PFOA SHOULD NOT BE ADVANCED OR WARRANTS THE LOWEST LOW PRIORITY*

Under Proposition 65, its implementing regulations and listing criteria, PFOA is not "clearly shown" to cause cancer[14] because the human data do not show a "causal relationship between the chemical and cancer"[15] and because there is not "sufficient evidence of carcinogenicity . . . from studies in experimental animals."[16]

It is entirely appropriate for the Committee to consider the results of the epidemiological and animal studies, including both the quantity and quality of these data, in making its recommendation for prioritization. Indeed this is expressly contemplated under the Prioritization

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[13] Cheung C, Akiyama TE, Ward JM, Nicol CJ, Feigenbaum L, Vinson C, Gonzalez FJ (2004) Diminished Hepatocellular Proliferation in Mice Humanized for the Nuclear Receptor Peroxisome Proliferator-Activated Receptor alpha, *Cancer Research* 64, (11), 3849-3854.

Morimura K, Cheung C, Ward JM, Reddy JK, Gonzalez FJ (2006) Differential susceptibility of mice humanized for peroxisome proliferator-activated receptor alpha to Wy-14,643-induced liver tumorigenesis, *Carcinogenesis* 27, (5), 1074-1080.

Shah YM, Morimura K, Yang Q, Tanabe T, Takagi M, Gonzalez FJ (2007) Peroxisome proliferator-activated receptor alpha regulates a microRNA-mediated signaling cascade responsible for hepatocellular proliferation, *Mol Cell Biol* 27, 4238-4247.

[14] Cal. Health & Safety Code § 25249.8(b); Cal. Code Regs., *tit.* 22, § 25305(a)(1).

[15] Cal. Code Regs., *tit.* 22, § 25305(e)(1).

[16] Cal. Code regs., *tit.* 22, § 25305(e)(2).

Process,[17] and is necessary if the Process is to work as intended. The Process charges the OEHHA staff with responsibility to screen chemicals for "epidemiological evidence suggesting [whether] they cause cancer," and to conduct a "preliminary toxicological evaluation" for "overall evidence of carcinogenicity .... including epidemiologic, animal bioassay and other relevant information ... based on original research articles and literature compilations or reviews." The public is encouraged to "comment on the scientific evidence pertaining to the selection of the chemical for prioritization," and ***it is the role of the Committee to provide "[a]ppraisals of the evidence."***[18]

The data that OEHHA has cited in its compilation are largely the same studies that the agency and the Committee considered in November 2006, in response to a petition to expedite consideration of PFOA, with the addition of ***some*** of the studies that have been completed and published since that time. For the convenience of the Committee, we have summarized all of these studies below. Because the Committee should consider "[b]oth positive and negative" data,[19] we have included negative data in this summary. As shown below, the weight of the evidence is clearly negative.

HUMAN DATA

All of the epidemiological data cited in the OEHHA compilation[20] were considered by the US EPA in its Draft Risk Assessment, in which US EPA concluded that "[n]o remarkable health effects that can be directly attributed to PFOA exposure have been reported in fluorochemical production workers described in the studies ...."[21] (As noted above and discussed in more detail below, the European Food Safety Authority also reviewed the same data recently, and concluded that "[e]pidemiological studies in PFOA-exposed worker do not indicate and increased cancer risk."[22]).

The studies that were the basis for the US EPA conclusion (as well as the conclusion of the European Food Safety Authority, discussed below) are referred to as the "3M Cottage Grove" and "DuPont Washington Works" studies. In addition, to those studies, the OEHHA

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17 "Process for Prioritizing Chemicals for Consideration Under Proposition 65 by the 'State's Qualified Experts,'" Office of Health Hazard Assessment, California Environmental Protection Agency (December 2004), hereinafter "Prioritization Process," at 4. (Attachment F).

18 *Id.* at 3-5. (Attachment F).

19 *Id* at 4. (Attachment F).

20 "Perfluoroctanoic acid (PFOA) and Its Salts and Transformation and Degradation Precursors," OEHHA (March 2009). (Attachment B).

21 U.S. EPA (2005) at 13.

22 Perfluorooctane sulfonate (PFOS), perflurooctanoic acid (PFOA) and their salts Scientific Opinion of the Panel on Contaminants in the Food chain, *The EFSA Journal* (2008) 653, 4-131.

compilation also identifies two epidemiological studies conducted by Alexander *et al.*[23] and Alexander and Olsen,[24] which we refer to as the "3M Decatur PFOS Study." For the Committee's convenience, we have summarized the results of those studies here.

3M Cottage Grove Study. This was a mortality study of approximately 4000 workers at the 3M Cottage Grove, Minnesota manufacturing facility who were exposed to PFOA for approximately 108,000 person-years, in which no significant increase in cancer risk was observed. In total, 3183 male and 809 female workers were followed for vital status from 1947 through 1997. The all-cause mortality (SMR = 0.9, 95% CI 0.8-0.9) and all-cancer mortality (SMR = 0.8, 95% CI 0.7-1.0) ratios for the entire study population regardless of classification, as well as for the exposure sub-cohorts, were less than expected in the general population. Specifically, there was no association between cohort members (employees with a minimum of 1 year employment in a job with definite or probable PFOA exposure) and all-cancer mortality (SMR = 0.9, 95% CI 0.7-1.1), liver cancer (SMR = 0.6, 95% CI 0.0-3.3), pancreatic cancer (SMR = 1.4, 95% CI -0.5-3.1), or prostate cancer (SMR = 1.2, 95% CI 0.4-2.5).

DuPont Washington Works Study. In a 2006 epidemiological study of workers at the DuPont Washington Works, West Virginia facility, about 6000 employees were followed for more than 50 years. No increased mortality risk was found in workers exposed to PFOA. The results showed lower mortality rates than those found in both West Virginia and the U.S. general population. They were also consistent with mortality rates in comparable workers from other DuPont plants, a worker population generally more healthy than the population at large. The study, which was reviewed by an external board of scientists, examined the causes of death of 6,027 people who had worked at the Washington Works facility between 1948 until the end of 2002, and compared the causes of death for those who had died over the 54-year period and their mortality rates to those found in three groups: other DuPont workers, West Virginia residents, and members of the U.S. general population. The study found no increase in overall mortality from cancer. Prostate cancer rates among the cases studied were found to be lower than rates in all three reference populations.[25]

3M Decatur PFOS Studies. These studies similarly do not support listing or even a high priority for reviewing PFOA. Neither are studies of PFOA. Rather, they are follow-up studies of workers at a PFOS manufacturing facility in Decatur, Alabama. In a study of 2083 Decatur workers, Alexander *et al.* showed that workers in jobs involving high exposure to PFOS-based

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[23] Alexander BH, Olsen GW, Burris JM, Mandel JH, Mandel JS (2003) Mortality of employees of a pefuorooctanesulphonyl fluoride manufacturing facility, *Occup Environ Med* 60:722-9.

[24] Alexander BH, Olsen GW (2007) Bladder cancer in perfluorooctoanesulfonyl fluouride manufacturing workers, *Ann Epidemiol* 17:471-478.

[25] This contrasts with a previous non-DuPont study where an increase in prostate cancer initially was reported, but subsequently was discounted when the study was updated. Across the entire study population, there was a slight, but not statistically significant, site-wide increase in the rate of kidney cancer mortality. Only a third of the employees at the facility worked with PFOA, and most of the cases showed little exposure to PFOA; the numbers were too small to allow any conclusions.

materials had a 13-fold increased risk for bladder cancer mortality compared with the general population of Alabama (SMR = 12.77, 95% confidence limit 2.63-37.35). However, this observation was based on only three cases of bladder cancer, and the workers were exposed to several compounds. Hence, it is difficult to draw conclusions from this study. More importantly, a follow-up study (Alexander and Olsen, 2007) reported no statistically significant associations between PFOS exposure and an increased risk of bladder cancer.

ANIMAL DATA

As context for considering the animal data, it is important to review the Proposition 65 regulations. In explaining when "sufficient evidence of carcinogenicity [for listing] exists from studies in experimental animals," the regulations provide that:

> "'sufficient evidence' means studies in experimental animals indicate that there is an increased incidence of ***malignant tumors or*** combined ***malignant and benign tumors in multiple species or strains***, ***in multiple experiments*** (e.g., with different routes of administration or using different does levels), or, to an unusual degree, in a single experiment with regard to high incidence, site or type of tumor, or age at onset."[26]

The critical studies in determining whether PFOA meets this standard are the Sibinski[27] and Biegel[28] studies, the findings from which are summarized below. It is clear from those studies that PFOA does not meet these criteria for listing.

In the first study (Sibinski, 1987), PFOA was given to male and female rats at dietary levels of 0, 30, and 300 parts per million ("ppm"). In the second study (Biegel, *et al.*, 2001), PFOA was administered to male rats only at a single dietary level of 300 ppm, and the study included both *ad libitum* and pair-fed controls.

In the Sibinski study, there was an increase in the incidence of benign testicular Leydig cell tumors exposed to PFOA at a dietary dose of 300 ppm, but not at 30 ppm.[29] In the Biegel

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[26] Cal. Code Regs., *tit.* 22, § 25305(e)(2) (emphasis added).

[27] Sibinski LJ, (1987) Final report of a two-year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctanane ammonium carboxylate) in rats, Vol. 1-4, *3M Company/RIKER Exp.* No. 0281CR0012; 8 EHQ-1087-0394, October 16, 1987.

[28] Biegel LB, Hurtt ME, Frame SR, O'Connor JC, Cook JC (2001) Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats, *Toxicol. Sci.* 60:44-55.

[29] There was some initial evidence of an increase in the incidence of mammary fibroadenomas in the female rats; the incidence was originally reported as 22%, 42%, and 48% at 0, 30, and 300 ppm in the diet, respectively. But there was no apparent difference in the incidence, despite dietary levels that were an order of magnitude apart. The study authors concluded that the mammary tumor data did not reflect an effect of PFOA. Unfortunately, the laboratory did not have an adequate historical control database for comparison. However, the historical control data of the supplier and of DuPont's Haskell Laboratories showed an average incidence of mammary fibroadenomas of 41% and 37%, respectively. A subsequent reevaluation of the mammary tissues by an independent Pathology Working Group showed no significant increase in mammary tumors in the Sibinski (1987) study.

et al. study, there was an increase in benign hepatocellular, Leydig cell, and pancreatic acinar-cell tumors in rats exposed to PFOA at 300 ppm. A similar finding on hepatocellular and pancreatic acinar-cell tumors was not observed in the Sibinski study, although a subsequent (2001) peer review of pancreatic tissues from both studies revealed evidence of acinar-cell hyperplasia in the Sibinski study, but no increase in adenoma.[30] Moreover, neither of the rat carcinogenicity studies shows a statistically significant increase in ***malignant*** tumors of any type.

These findings are important because the Committee's "Guidance Criteria for Identifying Chemicals for Listing as 'Known to the State to Cause Cancer'" states that "if the weight of evidence ***clearly shows*** that a certain chemical causes ***invasive cancer*** in humans, or that it causes ***invasive cancer*** in animals (unless the mechanism of action has been shown not to be relevant to humans), the committee will normally identify that chemical for listing." (Emphasis added.) The term "invasive cancer" refers to malignant tumors. No study of PFOA has shown any statistically significant increase in "invasive cancer." The animal data, therefore, do not support listing PFOA as a chemical that is "known to the State" to cause cancer.

SUMMARY OF HYPERPLASIA AND NEOPLASIA IN LIVER, TESTES, AND PANCREAS OF RATS FED PFOA (DATA FROM BIEGEL *et al.*, 2001)

ORGAN/LESION	CONTROL (FED AD LIB), INCIDENCE (%)	PAIR-FED CONTROL, INCIDENCE (%)	PFOA (300 PPM), INCIDENCE (%)
Liver			
Adenoma	2/80 (3)	1/79 (1)	10/76 (13)b
Carcinoma	0/80 (0)	2/79 (3)	0/76 (0)
Testes, Leydig-cell			
Hyperplasia	11/80 (14)	26/78 (33)	35/76 (46)a
Adenoma	0/80 (0)	2/78 (3)	8/76 (11)b
Pancreas, Acinar-cell			
Hyperplasia	14/80 (18)	8/79 (10)	30/76 (39)b
Adenoma	0/80 (0)	1/79 (1)	7/76 (9)b
Carcinoma	0/80 (0)	0/79 (0)	1/76 (1)

[a] Significantly different from *ad libitum* control group.
[b] Significantly different from pair-fed control group.

[30] Kennedy GL Jr, Butenhoff JL, Olsen GW, O'Connor JC, Seacat AM, Perkins RG, Biegel LB, Murphy SR, Farrar DG (2004) The toxicology of perfluorooctanoate, *Crit. Rev. Toxicol.* 34(4):351-384.

TUMOR PROMOTION STUDIES

OEHHA's compilation also included two studies focusing on tumor promotion characteristics. These studies provide little evidence, if any, to support a conclusion that PFOA is carcinogenic. PFOA has been shown to be a tumor promoter, but not a carcinogen, in initiation-promotion bioassays in rats[31] and in rainbow trout.[32] For example, the experimental design for rats included dosing the animals with three potent liver carcinogens (diethylnitrosamine, 2-AAF and carbon tetrachloride) prior to exposure to PFOA. It is not surprising that a chemically-damaged liver can subsequently respond to PFOA, which produces peroxisome proliferation in rodents.

MECHANISTIC DATA

The Summary Table indicates that there are "Other Mechanistic Studies" to consider. As in the case of the human and animal data above, however, it is clear that these studies are ***not*** a basis for listing PFOA. Most of these data also were reviewed in the US EPA Draft Risk Assessment, which concluded that the benign tumors observed in rats in the animal studies discussed above resulted from peroxisome proliferation, to which rats are uniquely susceptible, indicating that the results of those studies probably are not relevant to humans:

> "There is sufficient evidence to indicate that PFOA is a PPARα-agonist and that the liver carcinogenicity (and toxicity) of PFOA is mediated by binding to the PPARα in the liver. A mode of action analysis has demonstrated that the hepatic effects are due to a PPARα-agonism, and that this mode of action is unlikely to occur in humans. The modes of action for the Leydig cell and pancreatic acinar cell tumors have been investigated, but there is insufficient evidence to link these modes of action to PPARα. The [Leydig cell tumors] and [pancreatic acinar cell tumors] inducted in the rat by PFOA probably do not represent a significant cancer hazard for humans because of quantitative differences in the expressions of [CCKa] [luteinizing hormone] and [cholecystokinin-33 (human); cholecystokinin (rat)] receptors and of other toxicodynamic differences between the rat and the human."[33]

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31 Abdellatif AG, Préat V, Vamecq J, Nilsson R, Roberfroid M (1990) Peroxisome proliferation and modulation of rat liver carcinogenesis by 2,4-dichlorophenoxyacetic acid, 2,4,5,-trichlorophenoxyacetic acid, perfluorooctanoic acid and nafenopin, *Carcinogenesis* **11**:1899-1902. Nilsson R, Beije B, Préat V, Erixon K, Ramel C (1991) On the mechanism of the hepatocarcinogenicity of peroxisome proliferators, *Chem Biol Interact* 78:235-50.

32 Tilton SC, Orner GA, Benninghoff AD, Carpenter HM, Hendricks JD, Pereira CB, Williams DE (2008). Genomic profiling reveals an alternative mechanism for hepatic tumor promotion by perfluorooctanoic acid in rainbow trout, *Environ Health Perspect* 116:1047-1055.

33 US EPA (2005) at 8.

The additional mechanistic studies published since the CIC last considered PFOA would not change any previous conclusions. Ito *et al.* (2007), Takashima *et al.* (2008), and Yang *et al.* (2007), provide additional perspectives on the role of PPARα activation and tumorigenesis, but none of these studies tested PFOA. Wolf, *et al.* (2008) tested PFOA and the results of the study support the PPARα theory.

GENOTOXICITY

The Summary Table, as noted above, indicates that there are no genotoxicity data to support listing. For that reason, the analysis could end there. Nevertheless, the following summary of the genotoxicity data from the US EPA Draft Risk Assessment should inform the Committee's judgment.

> "APFO [the ammonium salt of PFOA] is not mutagenic. APFO did not induce mutation in either S. typhimurium or E. coli when tested either with or without mammalian activation. APFO did not induce gene mutation when tested with or without metabolic activation in the K-1 line of Chinese hamster ovary (CHO) cells in culture. APFO did not induce chromosomal aberrations in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations. APFO was tested twice for its ability to induce chromosomal aberrations in CHO cells. In the first assay, APFO induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, APFO induced significant increases in chromosomal aberrations and in polyploidy. APFO was negative in a cell transformation assay in C3H 10T1/2 mouse embryo fibroblasts and in the mouse micronucleus assay."[34]

3. *NEW EPIDEMIOLOGICAL STUDIES REINFORCE THE CONCLUSION THAT PFOA IS NOT CARCINOGENIC*

In February 2008, the European Food Safety Authority ("EFSA") published a "Scientific Opinion" on "Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts." EFSA reviewed the same data considered by US EPA.[35] Describing the human data, EFSA found:

> "In summary, a retrospective cohort mortality study showed a statistically significant association between prostate cancer mortality and employment duration in the chemical facility of a plant that manufactures APFO. However, in

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[34] US EPA (2005) at 7.

[35] Perfluorooctane sulfonate (PFOS), perflurooctanoic acid (PFOA) and their salts Scientific Opinion of the Panel on Contaminants in the Food chain, *The EFSA Journal* (2008) 653, 93-131.

> an update of this study, in which more specific exposure measures were used, a significant association for prostate cancer was not observed."[36]

Evaluating the weight of the epidemiological evidence, EFSA concluded that ***"[e]pidemiological studies in PFOA-exposed workers do not indicate an increased cancer risk."***[37]

The Committee also should consider the Danish Cohort Prospective Cancer Study that was published in 2008 but was not included in the compilation. This study, published in the Journal of the National Cancer Institute, reports that ***"[p]lasma concentrations of [PFOA] in the general Danish population appear not to be associated with risk of prostate, bladder, pancreatic or liver cancer."***[38]

A brief summary of the study follows:

> "From December 1, 1993, through May 31, 1997, a total of 57,053 individuals who were aged 50-65, born in Denmark, and had no previous cancer diagnosis were enrolled in a prospective cohort. After enrollment, [the authors] identif[ied] 713, 332, 128 and 67 patients with prostate, bladder, pancreatic and liver cancer, respectively, who were diagnosed between December 1, 1993, and July 1, 2006 . . . [within] 0-12 years . . . after enrollment . . . ."[39]

From the same cohort, a random subcohort of over 750 individuals without cancer were identified for comparison, and plasma PFOA concentration was measured in both subcohorts. The authors concluded: ***"plasma concentrations [of PFOA] in the general Danish population are not associated with increased risk of prostate, bladder, pancreatic, or liver cancer."***[40]

4. NEW EXPOSURE DATA SHOW THAT LEVELS OF PFOA IN HUMAN BLOOD AND THE ENVIRONMENT ARE DECLINING

The Summary Table indicates that exposure to PFOA is "Widespread." Although this is correct, it is important that the level of exposure in the general population is extremely low (approximately five parts per billion in blood) and is declining. Moreover, as we have pointed out in previous submissions, exposure alone, in the absence of carcinogenicity (or reproductive toxicity), does not make PFOA a candidate for listing under Proposition 65.

Indeed, exposure alone is not an indicator of any toxic effect, much less a carcinogenic effect. In this regard, it is important to recall that agencies other than OEHHA have been

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36 *Id.* at 653. 93-131.

37 *Id.* at 4-131 (emphasis added).

38 Eriksen KT, Sorensen M, McLaughlin JK, Lipworth L, Tjonneland A, Overvad K, Raaschou-Nielsen O (2009) Perfluorooctanoate and Perfluorooctanesulfonate Plasma Levels and Risk of Cancer in the General Danish Population, *J Natl Cancer Inst* 2009; 101: 605. (Attachment C).

39 *Id.* (emphasis added).

40 *Id.* at 608 (emphasis added).

monitoring actively for exposure to PFOA for many years. In particular, US EPA monitors production levels, groundwater levels and other markers for exposure to PFOA constantly, as well as the most current epidemiological and toxicological data, in order to determine whether exposure causes ***any*** adverse effects in humans.

As recently as 2006, the US EPA had detected no adverse health effects from exposure to PFOA. In December 2005, Susan Hazen, Acting Administrator of US EPA's Office of Prevention, Pesticides and Toxic Substances, stated: "The agency has information based on animal and toxic effects in animals, [but] we have ***no information at this point that would lead us to believe there is a significant human health impact***." On January 25, 2006, US EPA Administrator Johnson announced that "[a]lthough our risk assessment activities are not complete and new data may change the current picture, to date ***EPA is not aware of any studies specifically relating current levels of PFOA exposure to human health effects***." In its June 8, 2006 PFOA Information Forum, US EPA reiterated: "***EPA has no information linking current levels of PFOA in the blood of the general public to any adverse health effects in people***." (Emphasis added in all quotations.)

This is not new information, however. To the extent that exposure is an issue for the Committee to consider, in the absence of data that would support a finding of carcinogenicity, the Committee should be informed that US EPA reports that emissions of PFOA have declined significantly since 2006, and will be virtually eliminated by 2015. Similarly, the level of exposure to humans has decreased, both in the United States and worldwide.

The US EPA 2010/15 PFOA Stewardship Program requires participants (which include the eight major manufacturers of PFOA) to reduce product content and facility emissions by 95% no later than 2010, and to work toward elimination of PFOA emissions and product content altogether by 2015, and to submit annual reports to the federal agency concerning their emissions, product content and progress toward those goals. The US EPA Annual Progress Reports on achieving those goals are available on-line at www.epa.gov/opptintr/pfoa/pubs/sewardship/preports2.html. The most recent reports indicate that the manufacturers are "on target to meet the 95 percent reduction goal in PFOA emissions and product content by 2010." The Agency also indicates that "[f]urther reductions are anticipated by 2015."[41]

Reductions in manufacture, product content and emissions have had a correlative effect in reducing exposure to the human population. As noted previously, the CDC recently published a study comparing exposure levels of PFOA in human blood serum from comparable populations in 1999 - 2000 and 2003-2004. US EPA described the results of the study as follows:

> "In August, 2007, a U.S. Centers for Disease Control report showed significant reductions in human blood levels of [PFOS] and PFOA from 1999-2000 compared to the most recent data in 2003-2004 in a representative sample of the

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[41] US EPA OPPT Accomplishments Report January 2007 - 2009, available on-line at www.epa.gov/oppt/ar/2007-2009/managing/potential_risks.htm.

> U.S. population. The geometric mean for PFOA in human blood was reduced by 25 percent over this period and PFOS was reduced by 32 percent. The report concluded that these reductions were most likely related to changes brought about by EPA efforts on these chemicals and other related efforts by government and industry."[42]

In light of these data, any priority for PFOA that arose from exposure, in the absence of data showing carcinogenicity, is diminishing.

5. *AN AUTHORITATIVE BODY AND ANOTHER RESPECTED GOVERNMENTAL AGENCY REVIEWED PFOA AND NEITHER CONCLUDED THAT PFOA IS A CARCINOGEN*

The Prioritization Process recognizes that determinations by other agencies, particularly those that qualify as "authoritative bodies," are important and should be considered in determining which chemicals OEHHA and the Committee should review. Specifically, the Prioritization Process states:

> ***"It is unlikely that chemicals will be proposed for CIC . . . review that have been recently reviewed by an authoritative body and found to have insufficient evidence of carcinogenicity*** . . . . Exceptions to this generalization may occur, for example, if an authoritative body has evaluated a chemical but failed to review all relevant data, or ***compelling new data*** have become available since the evaluation."[43]

While this policy does not forbid the agency from "re-reviewing" chemicals recently reviewed by other agencies, it advises that OEHHA's resources should not be expended on doing so, unless there is compelling new evidence that might warrant listing.

This aspect of the Prioritization Process thus provides another good reason that PFOA does not merit consideration, or should be assigned a very low priority. As noted above, the US EPA Draft Risk Assessment did not conclude that PFOA causes cancer.[44] More recently, EFSA published its "Scientific Opinion" on "Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid

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42 *Id.*

43 Prioritization Process at 4 (emphasis added). (Attachment F)

44 Our November 2006 submission acknowledged a disagreement between the US EPA Office of Prevention, Pesticides and Toxic Substances ("OPPTS") and the Agency's Scientific Advisory Board ("SAB") as to the appropriate hazard descriptor for PFOA under US EPA's then-new Guidelines for Carcinogen Risk Assessment. The OPPTS authors of the Draft Risk Assessment characterized PFOA as "suggestive" of carcinogenicity. With some new data remaining to be considered, one quarter of the SAB panel members voted for the descriptor "suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential," and three quarters chose "likely to be carcinogenic." As of this writing, US EPA did not adopt the Draft Risk Assessment or the SAB findings, and announced its intention to incorporate the new data into the Draft Risk Assessment and to convene a new SAB Review.

(PFOA) and their salts,", as discussed above.[45] EFSA reviewed the animal data and the human data and did not conclude that PFOA is carcinogenic. Evaluating the weight of the epidemiological evidence, EFSA specifically concluded that "***[e]pidemiological studies in PFOA-exposed workers do not indicate an increased cancer risk.***"[46]

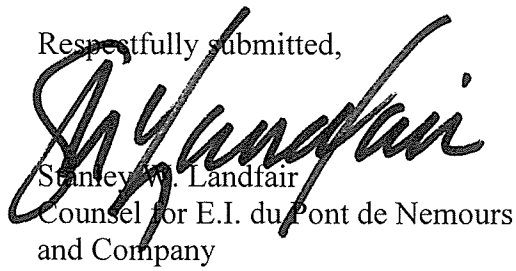
Thus to summarize, an authoritative body, US EPA, reviewed PFOA recently on the basis of the data available at the time, and did not find that PFOA causes cancer. The more recent review of the same data by EFSA, another respected (though not "authoritative") agency, reinforces the US EPA conclusion. The only significant "new" data are the findings from the Danish cohort study, summarized above. These data do not "compel" a new review; rather, they reinforce the previous conclusion. Therefore, under the very terms of the Prioritization Process, PFOA should not advance to the next stage of the listing process and therefore would warrant the lowest priority for review, as well.

CONCLUSION

PFOA is not a good candidate for listing. The human data would not support listing under Section 25305(e)(1).[47] The animal data would not support listing under Section 25305(e)(2).[48] In the final analysis, this is the best reason the PFOA should not be advanced to the next stage of the listing process.

Focusing on the issue of prioritization alone, PFOA warrants less attention than most of the thirty-eight chemicals nominated for review. Higher priority should be assigned to compounds that cause malignant tumors, produce tumors in more than one sex of one species, produce tumors by a mechanism of action relevant to humans, are genotoxic, and for which epidemiological data show cause for greater concern than to PFOA, which does not meet any of these criteria.

Respectfully submitted,



Stanley W. Landfair
Counsel for E.I. du Pont de Nemours
and Company

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45 Perfluorooctane sulfonate (PFOS), perflurooctanoic acid (PFOA) and their salts Scientific Opinion of the Panel on Contaminants in the Food chain, *The EFSA Journal* (2008) 653, 1-131.

46 *Id.* at 4-131 (emphasis added).

47 Cal. Code Regs., *tit.* 22, § 25305(e)(1).

48 Cal. Code regs., *tit.* 22, § 25305(e)(2).

cc: Joan Denton, Ph.D., Director, OEHHA
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SF:27361515.1

Prioritization of Chemicals for Carcinogen Identification Committee Review:

Proposed Chemicals for Committee Consideration and Consultation March 2009

Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is proposing 38 chemicals for review by the Carcinogen Identification Committee (CIC) under Proposition 65, using the process endorsed by the CIC and adopted by OEHHA in 2004. These chemicals (see Table 1 below) are not proposed for listing at this time. OEHHA is seeking public comment and the CIC's consultation regarding which of these chemicals should proceed to the next stage of the listing process. The next stage would be the development of hazard identification materials by OEHHA and the consideration of a chemical for listing by the CIC at a future meeting.

Introduction

OEHHA has applied two data screens to roughly half the chemicals in a tracking database of chemicals to which Californians are potentially exposed. The two screens are a human data screen and an animal data screen. This screening approach was discussed at the November 17, 2007 and November 5, 2008 meetings of the CIC. The screening follows the procedure established in December 2004 and described in the OEHHA document "Process for Prioritizing Chemicals for Consideration under Proposition 65 by the "State's Qualified Experts."

Evidence of hazard was assessed by application of an epidemiologic data screen and an animal data screen, both of which are described below. Chemicals that passed either of these hazard screens were then subjected to preliminary toxicological evaluation, to determine whether they should be proposed for CIC consideration for possible preparation of hazard identification materials. The preliminary toxicological evaluation entails consideration of the available overall evidence of carcinogenicity (i.e., epidemiology, animal bioassay, other relevant information), but is of necessity an initial, abbreviated appraisal of the information identified through screening level literature searches.

OEHHA has applied two data screens to roughly half the candidate chemicals, and identified 38 chemicals through preliminary toxicological evaluations of the chemicals that passed either of the data screens. This document, which is the subject of a 60-day public comment period, presents these 38 chemicals. For each of the chemicals, a separate listing has been compiled of the relevant studies that were identified during the

preliminary toxicological evaluation. Listings for each of the 38 chemicals are found in the Appendix.

At its next meeting, the CIC will provide advice and consultation regarding possible development of hazard identification materials on these chemicals, as described in "Next Steps" below. The following is a description of the process OEHHA conducted in applying these data screens.

Chemicals Screened

Under this process, only candidate chemicals are screened. These are chemicals in the tracking database with data suggesting that they cause cancer and have exposure potential in California. The evaluation of exposure potential is qualitative, based primarily on production, use or monitoring data.

Candidate chemicals that are candidates for listing via an administrative listing mechanism were not screened. OEHHA has applied two data screens to roughly half the candidate chemicals, and identified 38 chemicals (see Table 1) for CIC review and consultation. Screening continues on the remaining candidate chemicals. The additional chemicals identified for CIC review and consultation will be presented in future documents.

Applying the Epidemiology Data Screen

The epidemiology data screen was applied to 80 candidate chemicals (or chemical groups). The screen entails the identification of chemicals with epidemiological studies suggesting evidence of carcinogenicity. The screen involved finding relevant epidemiology studies through a literature search and evaluating them. Applying the screen required identification of epidemiology studies of the chemical reporting an association between exposure to the chemical and increased cancer risk. More weight was given to analytical studies, and less weight to descriptive studies and case reports. Single case reports were not sufficient to satisfy the screen. For those chemicals with studies available, the studies were examined in some detail. Studies were reviewed to determine whether there was a positive finding of cancer associated with exposure to the chemical. The studies were further reviewed to determine whether the effect might be attributed to exposure to the chemical of concern with some confidence.

For each chemical, the steps used in applying the epidemiology data screen were as follows:

1. The chemical's Chemical Abstracts Service (CAS) registry number and synonyms were identified using TOXLINE (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE).
2. The chemical identifiers were used in a search of the literature, using PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez). The search included a standardized search term (cancer [sb]) in the PubMed lexicon. Further refinement of the search was performed if necessary (e.g., enormous volume of articles returned).
3. Epidemiological studies were identified from the titles retrieved in the online search.

4. Abstracts of epidemiological articles were reviewed for relevance to the possible finding of cancer in humans exposed to the chemical. The full article was retrieved if the study appeared relevant upon review of the abstract. For articles lacking abstracts, copies of those with titles suggesting possible relevance were requested for review.
5. All articles identified as potentially relevant were considered in assessing whether evidence existed of human cancer related to exposure to the chemical.

Applying the Animal Data Screen

Subsequent to the epidemiology data screen, the animal data screen was applied to those candidate chemicals or chemical groups not identified through the human data screen. The animal data screen is based on "positive" bioassays and involved finding relevant animal cancer bioassays through a literature search and evaluating them with regard to the screening criteria. A positive animal cancer bioassay is a study in which a treatment-related increase in the incidence of malignant or combined malignant and benign tumors is observed in a given tissue or organ, or for a given type of tumor (e.g., hemangiosarcoma). An increased incidence is either statistically significant ($p < 0.05$) by pairwise comparison with controls, or biologically significant (e.g., an increased incidence of a rare tumor type).

The animal screen identified chemicals with:

- Two or more positive animal cancer bioassays;
- One positive animal cancer bioassay with findings of tumors at multiple sites or with malignant (or combined malignant and benign) tumors occurring to an unusual degree with regard to incidence, site, type of tumor or age at onset;
- One positive animal cancer bioassay and evidence from a second animal cancer bioassay of benign tumors of a type known to progress to malignancy.

For each chemical, the steps used in applying the animal data screen were as follows:

1. The chemical identifiers were used in a search of the literature, using PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez). The search included a standardized search term (cancer [sb]) in the PubMed lexicon. Further refinement of the search was performed if necessary (e.g., enormous volume of articles returned).
2. Animal cancer bioassays were identified from the titles retrieved in the online search.
3. Abstracts of the identified articles were reviewed. The full article was retrieved if the abstract indicated that animal cancer bioassay findings were presented or discussed in the article. For articles lacking abstracts, copies of those with titles suggesting possible relevance were requested for review.
4. All articles identified as potentially relevant were considered in assessing whether the animal data screen employed in this round of prioritization had been met for the chemical (or chemical group) in question.

Preliminary Toxicological Evaluation

A preliminary toxicological evaluation was made of chemicals identified through application of the epidemiology and animal data screens. Further search of the literature was performed to identify additional information relevant to carcinogenicity, such as studies on genotoxicity, mechanism of action, metabolism and pharmacokinetics (and animal cancer bioassays for those chemicals identified through the epidemiology data screen). This additional information was used to conduct a preliminary evaluation of the overall evidence of carcinogenicity for each of the chemicals identified by the data screens. Chemicals for which a preliminary evaluation of the overall evidence indicated that carcinogenicity may be a concern have been proposed here for CIC consideration.

Chemicals Proposed for CIC Consideration

OEHHA identified the 38 chemicals listed below for possible preparation of hazard identification materials. The CIC will provide OEHHA with advice on the prioritization of these chemicals for possible preparation of hazard identification materials at its next meeting on Friday, May 29, 2009.

Table 1. Chemicals Identified through Prioritization and Proposed for Consideration by the Carcinogen Identification Committee.

- 2-Amino-5-nitrothiazole
- 11-Aminoundecanoic acid
- Amphetamine and its salts
- Anthanthrene
- Aspartame
- Benoxacor
- 2-Chloro-1,1,1-trifluoroethane
- D & C Yellow #11
- 1,3-Dichloro-2-propanol
- Dicofol
- Diethanolamine (DEA)
- N,N-Diethylthiourea
- Diisononyl phthalate (DINP)
- 2,6-Dimethyl-n-nitrosomorpholine
- 1,3-Dinitropyrene
- Ethynodiol diacetate
- Fluoride and its salts
- Haloperidol
- Hydroquinone
- Methoxychlor
- Methyl ethyl ketoxime
- Molybdenum trioxide
- 3-Monochloropropane-1,2-diol (3-MCPD)
- 3-Nitrofluoranthene
- Nitrofurantoin
- N-Nitrosoanabasine
- N-Nitrosohexamethyleneimine
- 5-Nitro-*o*-toluidine
- Perfluorooctanoic acid and its salts and transformation and degradation precursors
- Permethrin
- Rock wool
- Tetrachlorvinphos
- Thiamethoxam
- Triamterene
- Triclosan
- Tris(1,3-dichloro-2-propyl) phosphate
- Triethanolamine (TEA)
- Vinylidene chloride

For each of the chemicals, a separate listing has been compiled of the relevant studies that were identified during the preliminary toxicological evaluation. Listings for each of the 38 chemicals are found in the Appendix.

Chemicals for CIC Consultation May 29, 2009
Exposure Characteristics and Types of Studies Providing Evidence of Carcinogenicity

Chemical Name	Exposure				Human Data				Animal Data					Other Relevant Data				
	Widespread	High in frequent consumers	Limited / occupational	High in infrequent consumers	Analytical	Descriptive	Case series / reports	Analytical: mixed / poorly defined exposures	Two or more studies	One study w/ unusual incidence, site/type, age at onset	One study and second study with benign tumors only	One study	Tumor initiation / promotion or co-carcinogenicity studies	Genotoxicity	Carcinogenic metabolites	Structural similarity with tumorigens or P65 carcinogens	Hormonal activity / disruption	Other mechanistic studies
2-Amino-5-nitrothiazole			X						X					X				
11-Aminoundecanoic acid		X							X					X				
Amphetamine and its salts		X			X								X	X				X
Anthanthrene	X								X				X	X		X		
Aspartame	X					X			X					X	X			
Benoxacor	X								X									
2-Chloro-1,1,1-trifluoroethane			X							X				X		X		
D & C Yellow #11	X								X					X				
1,3-Dichloro-2-propanol	X								X					X	X	X		
Dicofol	X				X	X						X				X	X	X
Diethanolamine (DEA)	X							X	X					X		X		X
N,N-Diethylthiourea			X						X				X	X		X	X	
Diisononyl phthalate (DINP)	X								X							X	X	X
2,6-Dimethyl-n-nitroso-morpholine			X						X				X	X		X		
1,3-Dinitropyrene	X								X					X		X		
Ethynodiol diacetate		X						X	X				X		X		X	
Fluoride and its salts	X				X	X				X				X				
Haloperidol		X							X					X				

Chemical Name	Exposure				Human Data				Animal Data					Other Relevant Data				
	Widespread	High in frequent consumers	Limited / occupational	High in infrequent consumers	Analytical	Descriptive	Case series / reports	Analytical: mixed / poorly defined exposures	Two or more studies	One study w/ unusual incidence, site/type, age at onset	One study and second study with benign tumors only	One study	Tumor initiation / promotion or co-carcinogenicity studies	Genotoxicity	Carcinogenic metabolites	Structural similarity with tumorigens or P65 carcinogens	Hormonal activity / disruption	Other mechanistic studies
Hydroquinone	X							X	X				X	X		X		
Methoxychlor	X				X				X							X	X	
Methyl ethyl ketoxime			X						X					X				
Molybdenum trioxide			X		X				X					X				
3-Monochloropropane-1,2-diol (3-MCPD)	X								X					X		X		
3-Nitrofluoranthene	X								X					X		X		
Nitrofurantoin				X	X		X		X					X		X		
N-Nitrosoanabasine	X								X					X		X		
N-Nitrosohexamethylene-imine			X						X					X		X		
5-Nitro-*o*-toluidine			X						X					X				
Perfluorooctanoic acid and its salts and transformation and degradation precursors	X				X						X		X				X	X
Permethrin	X								X					X		X		
Rock wool			X		X				X					X		X		X
Tetrachlorvinphos	X								X					X				
Thiamethoxam	X								X									X
Triamterene		X							X					X				
Triclosan	X								X					X		X	X	X
Tris(1,3-dichloro-2-propyl) phosphate	X								X					X		X		
Triethanolamine (TEA)	X							X	X							X		X
Vinylidene chloride			X						X				X	X		X		

Next Steps

With the release on March 6, 2009 of the 38 chemicals proposed for CIC consideration, OEHHA opened a public comment period that closes on May 5, 2009.

The CIC will consider the chemicals in Table 1 at their May 29, 2009 meeting, providing advice and consultation regarding possible development of hazard identification materials by OEHHA. Written public comments received by OEHHA by May 5, 2009, will be provided to the CIC for consideration. The public is also given the opportunity to comment on the chemicals being proposed for hazard identification materials preparation at the CIC meeting.

The CIC may also suggest other chemicals for which hazard identification materials should be prepared. The CIC can vote on recommendations or provide less formal advice to OEHHA concerning which chemicals should be brought back for their consideration for listing following preparation of hazard identification materials.

Hazard identification materials summarizing the available scientific evidence on the carcinogenic potential of the selected chemicals would be prepared following an exhaustive search and evaluation of the scientific literature. These materials will be provided to the CIC, and released for public comment, prior to the public meeting at which the CIC deliberates on a listing decision.

Further details on prioritization, the development of hazard identification materials and committee consideration of the listing of chemicals under Proposition 65 are given in OEHHA (2004).

Reference

Office of Environmental Health Hazard Assessment (OEHHA, 2004). *Process for Prioritizing Chemicals for Consideration under Proposition 65 by the "State's Qualified Experts."* California Environmental Protection Agency, OEHHA, Sacramento, CA, December. Available online at: www.oehha.ca.gov/prop65/CRNR_notices/state_listing/pdf/finalPriordoc.pdf

Perfluorooctanoic acid (PFOA) and Its Salts and Transformation and Degradation Precursors

Perfluorooctanoic acid (PFOA, C8) and its salts are perfluorinated organic compounds with surfactant properties. PFOA can be released from several fluorochemicals, such as fluorinated telomere alcohols and other precursor compounds of fluorinated polymers by biotic and /or metabolic decomposition. PFOA and its salts are used in a variety of industrial applications, such as plasticizers, lubricants, wetting agents, and emulsifiers. They are used in the manufacture of fluoroelastomers and fluoropolymers, such as polytetrafluoroethylene (Teflon) and polyvinylidine fluoride. These are used as protective finishes to make non-stick cookware and water and stain repelling treatments for carpets, furniture upholstery and textiles. Fluoroelastomers and fluoropolymers are also used in the automotive, mechanical, aerospace, chemicals, electrical, medical, and building/construction industries. Uses in consumer products include coatings on paper, textiles, and carpet, personal care products, and nonstick coatings on cookware.

Biomonitoring studies indicate widespread exposure of the population to PFOA. PFOA can cross the placenta and accumulate in amniotic fluid. It has also been measured in cord blood from newborns. PFOA is persistent in the environment. The general population is exposed through the use of products containing PFOA and its salts and transformation and degradation precursors, and through environmental exposures, and occupational exposures occur in workplaces were these chemicals are manufactured and used.

PFOA and its salts and transformation and degradation precursors passed the animal data screen, underwent a preliminary toxicological evaluation, and are being brought to the Carcinogen Identification Committee for consultation. This is a compilation of the relevant studies identified during the preliminary toxicological evaluation.

Epidemiological data

- Retrospective occupational cohort mortality studies
  - Workers at 3M Cottage Grove, Minnesota plant where APFO production occurred: Gilliland and Mandel (1993); U.S. EPA (2005, pp. 13-16).
  - Workers at a perfluorooctanesulphonyl fluoride-based fluorochemicals production facility, with likely exposure to multiple fluorochemicals, including PFOA: Alexander *et al.* (2003), Alexander and Olsen (2007)
  - Workers Dupont's Washington Works plant in Parkersburg, West Virginia: U.S. EPA (2005, p. 16)

Animal carcinogenicity data

- Long-term diet studies in rats
  - Two-year studies in male and female Sprague-Dawley rats: Sibinski (1987), as described in U.S. EPA (2005, pp. 55-58)
  - Two year study in male Sprague-Dawley rats: Cook *et al*. (1994) as fully described in Biegel *et al*. (2001); described in U.S. EPA (2005, p. 58)

- Tumor promotion studies
  - Male Wistar rats (initiation-selection-PFOA promotion protocol, seven month study duration): Abdellatif *et al*. (1990); Nilsson *et al*. (1991)
  - Rainbow trout: Tilton *et al*. (2008)

Other relevant data

- Genotoxicity
  - Review: U.S. EPA (2005, p. 47)
  - Micronuclei in the human hepatoma cell line HepG2: Yao and Zhong (2005)
  - DNA strand breaks in the human hepatoma cell line HepG2: Yao and Zhong (2005)
  - 8-hydroxydeoxyguanosine in the human hepatoma cell line HepG2: Yao and Zhong (2005)

- Hormonal effects
  - Studies in Saccharomyces cervisiae: Ishibashi *et al*. (2007)
  - Studies in freshwater male tilapia hepatocyte cultures: Liu *et al*. (2007)
  - Studies in MCF-7 breast cancer cells: Maras *et al*. (2006)
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BRIEF COMMUNICATION

Perfluorooctanoate and Perfluorooctanesulfonate Plasma Levels and Risk of Cancer in the General Danish Population

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Perfluorooctanoate and perfluorooctanesulfonate are used in many industrial products and have been widely detected in human blood. Both chemicals are associated with tumor development in animal studies, but data on carcinogenic potential in humans are sparse. We investigated the association between plasma levels of perfluorooctanoate and perfluorooctanesulfonate and cancer risk within a prospective Danish cohort of participants with no previous cancer diagnosis at enrollment. From enrollment, between December 1, 1993, and May 31, 1997, and through July 1, 2006, we identified 713 participants with prostate cancer, 332 with bladder cancer, 128 with pancreatic cancer, and 67 with liver cancer in the entire cohort and we selected a comparison subcohort of 772. Plasma concentrations of perfluorooctanoate and perfluorooctanesulfonate were measured in each participant by use of high-pressure liquid chromatography coupled to tandem mass spectrometry. We found no clear differences in incidence rate ratios for these cancers in relation to plasma concentrations of perfluorooctanoate or perfluorooctanesulfonate. A 30%–40% increase in risk estimates for prostate cancer was observed for the three upper quartiles of perfluorooctanesulfonate concentration compared with the lowest quartile (eg, for the lowest vs the fourth quartile, incidence rate ratio = 1.38, 95% confidence interval = 0.99 to 1.93). Plasma concentrations of perfluorooctanoate and perfluorooctanesulfonate in the general Danish population appear not to be associated with risk of prostate, bladder, pancreatic, or liver cancer.

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Perfluorooctanoate and perfluorooctanesulfonate are used in many industrial products for their antiwetting and surfactant properties (1,2). These synthetically produced perfluorinated chemicals are extensively used as processing aids in the manufacture of fluoropolymers to produce items such as nonstick surfaces on cookware and oil- and stain-resistant treatments for carpets, furniture, shoes, fabrics, and convenience food packaging (1,2). They are resistant to metabolic and environmental degradation, are bioaccumulative, and have been detected in wildlife (3) and in blood of occupationally exposed workers and of the general population (4–7). The entry routes of these chemicals are by inhalation, mouth, and dermal absorption. Elimination half-lives in humans have been estimated at 4 years for perfluorooctanoate and at 5 years for perfluorooctanesulfonate (8).

Perfluorooctanoate and perfluorooctanesulfonate exposures at high levels have been associated with adverse health effects in animals, including delayed physical development; endocrine disruption; neonatal mortality; and liver, pancreatic, and testicular tumors (9–11). Only three epidemiological studies of cancer risk among workers exposed to perfluorooctanoate and perfluorooctanesulfonate have been conducted (12–14), with suggestive but inconsistent associations between exposure and bladder and prostate cancers. No studies have been published on exposure to perfluorinated chemicals and cancer risk in nonoccupational groups.

From December 1, 1993, through May 31, 1997, a total of 57053 individuals who were aged 50–65 years, born in Denmark, and had no previous cancer diagnosis were enrolled in a prospective cohort (15). After enrollment, we used the Danish Cancer Registry (16) and the Danish Pathology Data Bank to identify 713, 332, 128, and 67 patients with prostate, bladder, pancreatic, and liver cancer, respectively, who were diagnosed between December 1, 1993, and July 1, 2006. All patients were diagnosed 0–12 years (median = 7 years) after enrollment in the cohort. From the same cohort, we randomly selected a subcohort of 680 men and 92 women, approximating the male-to-female ratio among the patients, as a comparison group. Data on known confounders were obtained from detailed questionnaires administered at enrollment. Potential confounders used in this study were as follows: For prostate cancer, potential confounders were years of school attendance (<8, 8–10, or >10 years), body mass index (continuous variable), dietary fat intake (continuous variable), and fruit and vegetable intake (continuous variable). For bladder cancer, potential confounders were smoking status (present, former, or never smoker), smoking intensity (continuous variable), smoking duration (continuous variable), years of school attendance (<8, 8–10, or >10 years), and occupation associated with risk for bladder cancer (rubber industry; textile industry [dyeing]; metal processing [painting]; glass industry; driver of truck, bus, or taxi; painter; hairdresser; waiter; and cook). For pancreatic cancer, potential confounders were smoking status (present, former, or never smoker), smoking intensity (continuous variable), smoking duration (continuous variable), dietary fat intake (continuous variable), and fruit and vegetable intake (continuous variable).

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CONTEXT AND CAVEATS

Prior knowledge

Perfluorooctanoate and perfluorooctanesulfonate have been associated with tumor development in animal studies, but data on their carcinogenic potential in humans are sparse.

Study design

The associations between plasma levels of perfluorooctanoate and perfluorooctanesulfonate and cancer risk were investigated in a prospective cohort with no previous cancer diagnosis at enrollment. Concentrations of both chemicals were determined in plasma that was collected from all participants at recruitment.

Contribution

No clear differences in risk for these cancers in relation to plasma concentrations of perfluorooctanoate or perfluorooctanesulfonate were detected.

Implications

Plasma concentrations of perfluorooctanoate and perfluorooctanesulfonate among people in the general population appear not to be associated with risk of prostate, bladder, pancreatic, or liver cancer.

Limitations

There was only a single measure of the plasma concentration of perfluorooctanoate and perfluorooctanesulfonate for each individual. Consequently, misclassification may have occurred because the concentration at one moment in time may not reliably reflect the relevant plasma concentrations decades ago or at other times.

From the Editors

For liver cancer, potential confounders were smoking status (present, former, or never smoker), years of school attendance (<8, 8–10, or >10 years), alcohol intake (continuous variable), and occupation associated with risk for liver cancer (waiter or cook). Cut points for years of school attendance were given by the questionnaire used to collect the data. Plasma samples were taken at recruitment and stored at −150°C. Relevant Danish ethical committees approved the study, and written informed consent was obtained from all participants.

Plasma concentrations of perfluorinated chemicals were measured by use of high-pressure liquid chromatography coupled to tandem mass spectrometry in the 3M Toxicology Laboratory, as described in detail elsewhere (17). The laboratory was blinded to disease status. Three perfluorooctanoate values were below the lower limit of quantification (L) of 1 ng/mL and were assigned the value $1/\sqrt{2}$, according to the formula $L/\sqrt{2}$ for replacement of nondetectable values (18).

Double determinations of perfluorooctanoate and perfluorooctanesulfonate concentrations of 50 random plasma samples were done to test the replication uncertainty of the laboratory measurements. The 3M Toxicology Laboratory was blinded to this series of parallel measurements. Mean coefficients of variation, indicating the replication uncertainty, were very low (5.9% for perfluorooctanoate and 1.8% for perfluorooctanesulfonate).

Data were sampled according to the case–cohort design, and the unweighted case–cohort approach was used for analyses (19). Incidence rate ratios for the cancers were estimated by Cox proportional hazards model, stratified by sex. The proportional hazards assumptions were tested by use of Schoenfeld's residuals (20) and accepted. Age was the time axis, and analyses were corrected for delayed entry. We calculated two-sided 95% confidence intervals (CIs) and P values on the basis of robust estimates of the variance–covariance matrix (21) and Wald test statistic for regression parameters in Cox regression models. All statistical tests were two-sided.

We estimated crude and adjusted incidence rate ratios (IRRs) for quartiles of perfluorooctanoate or perfluorooctanesulfonate concentration, which were based on the distribution of patients specific to the analysis of each cancer. We chose to use the distribution of the patients because there were fewer patients than control subjects for three of the four cancers. Therefore, the number of patients in each quartile was the limiting factor with respect to statistical power, and an equal distribution of patients provides a similar power for each test (quartile 2 vs 1, quartile 3 vs 1, and quartile 4 vs 1). We evaluated a linear association between plasma concentrations of perfluorooctanoate and perfluorooctanesulfonate and cancer risk by use of a linear spline—first with three boundaries placed at the quartiles (ie, 25th, 50th, and 75th percentiles) and then with nine boundaries placed at the 10th, 20th, 30th, 40th, 50th, 60th, 70th, 80th, and 90th percentiles—among patients, as covariates in Cox models for perfluorooctanoate and perfluorooctanesulfonate (22). We found no statistically significant deviations from linearity (data not shown) and, therefore, we estimated linear trends as both crude and adjusted incidence rate ratios per plasma concentration increase in perfluorooctanoate of 1 ng/mL or per plasma concentration increase in perfluorooctanesulfonate of 10 ng/mL. The trends were analyzed overall and by sex, and we tested whether the associations for plasma concentration of perfluorooctanoate and perfluorooctanesulfonate were the same among men and women.

Distributions of age, sex, and plasma concentration of perfluorooctanoate and perfluorooctanesulfonate are presented in Table 1 and Figure 1. Mean plasma concentrations of perfluorooctanoate and perfluorooctanesulfonate were higher for men than for women. Concentrations of both perfluorooctanoate and perfluorooctanesulfonate were highly correlated (Spearman correlation coefficient, $r = .70$ and $P < .001$).

No linear trend in risk in relation to perfluorooctanoate and perfluorooctanesulfonate concentrations was observed for any cancers examined (Table 2). Sex did not modify the associations between plasma concentrations of perfluorooctanoate or perfluorooctanesulfonate and cancer risk in adjusted trend analyses (data not shown). The quartile analyses indicated no differences in incidence rate ratios in relation to plasma concentrations of perfluorooctanoate or perfluorooctanesulfonate (Table 2). Crude and adjusted incidence rate ratios were similar in both trend and quartile analyses (results not shown).

We observed a 30%–40% increase in incidence rate ratios for prostate cancer for the three upper quartiles of perfluorooctanesulfonate compared with the lowest quartile (eg, for the lowest vs the fourth quartile, IRR = 1.38, 95% CI = 0.99 to 1.93). However, incidence rate ratios were almost identical for each of the three upper quartiles of exposure, indicating a threshold or that the different risk for the lower quartile was due to chance. The latter

Table 1. Age, sex, and plasma concentrations of perfluorooctanoate and perfluorooctanesulfonate in the study population that was nested in the prospective Danish cohort Diet, Cancer, and Health*

	Cancer patients (n = 1240)			Subcohort comparison group (n = 772)		
Group	No.	PFOA,† ng/mL (5%–95% percentiles)	PFOS,† ng/mL (5%–95% percentiles)	No.	PFOA,† ng/mL (5%–95% percentiles)	PFOS,† ng/mL (5%–95% percentiles)
Men	1111	6.8 (3.1–14.0)	35.1 (17.4–60.9)	680	6.9 (3.2–13.3)	35.0 (16.8–62.4)
Women	129	6.0 (2.6–11.0)	32.1 (14.0–58.1)	92	5.4 (2.2–11.6)	29.3 (14.2–55.6)
Disease status						
Prostate cancer	713	6.9 (3.4–14.1)	36.8 (18.2–62.5)			
Bladder cancer	332	6.5 (2.7–13.4)	32.3 (15.2–58.0)			
Pancreatic cancer	128	6.7 (3.0–12.8)	32.7 (15.2–56.4)			
Liver cancer	67	5.4 (2.5–13.7)	31.0 (15.8–62.9)			
Subcohort				772	6.6 (3.0–13.0)	34.3 (16.2–61.8)

* The median age of cancer patients was 59 years (5%–95% percentiles = 51–65 years) and that of the comparison subcohort was 56 years (5%–95% percentiles = 51–64 years). PFOA = perfluorooctanoate; PFOS = perfluorooctanesulfonate.

† Data are the median plasma concentrations of either PFOA or PFOS.

interpretation appears more likely because of the modest increase in risk and because we found no deviations from linearity when evaluating plasma concentrations of perfluorooctanoate and perfluorooctanesulfonate in relation to cancer risk. In general, the results indicated modest inverse risk associations for bladder and liver cancer (eg, for the lowest vs the fourth quartile of perfluorooctanoate, IRR for bladder cancer = 0.81, 95% CI = 0.53 to 1.24; for the lowest vs the fourth quartile of perfluorooctanesulfonate, IRR for bladder cancer = 0.70, 95% CI = 0.46 to 1.07; for the lowest vs the fourth quartile of perfluorooctanoate, IRR for liver cancer = 0.60, 95% CI = 0.26 to 1.37; and for the lowest vs the fourth quartile of perfluorooctanesulfonate, IRR for liver cancer = 0.59, 95% CI = 0.27 to 1.27) (Table 2). We also observed a positive association between plasma concentrations of perfluorooctanoate and risk for pancreatic cancer (for the lowest vs the fourth quartiles, IRR for pancreatic cancer = 1.55, 95% CI = 0.85 to 2.80) (Table 2).

Plasma concentrations measured in this study were much lower than those in occupational cohort studies (12–14,23,24), which could explain why we did not replicate previous findings of associations between plasma concentration of perfluorooctanesulfonate and bladder cancer (12,13) and plasma concentration of perfluorooctanoate and prostate cancer (14) among occupationally exposed workers. However, the exposure ranges in this study were wide, from 1.0 to 76.4 ng/mL for perfluorooctanoate and from 1 to 130.5 ng/mL for perfluorooctanesulfonate, so that if a carcinogenic effect is associated with exposure at these relatively low concentrations, it could be detected.

The strengths of this study include the prospective design that was based on a well-defined cohort with data on

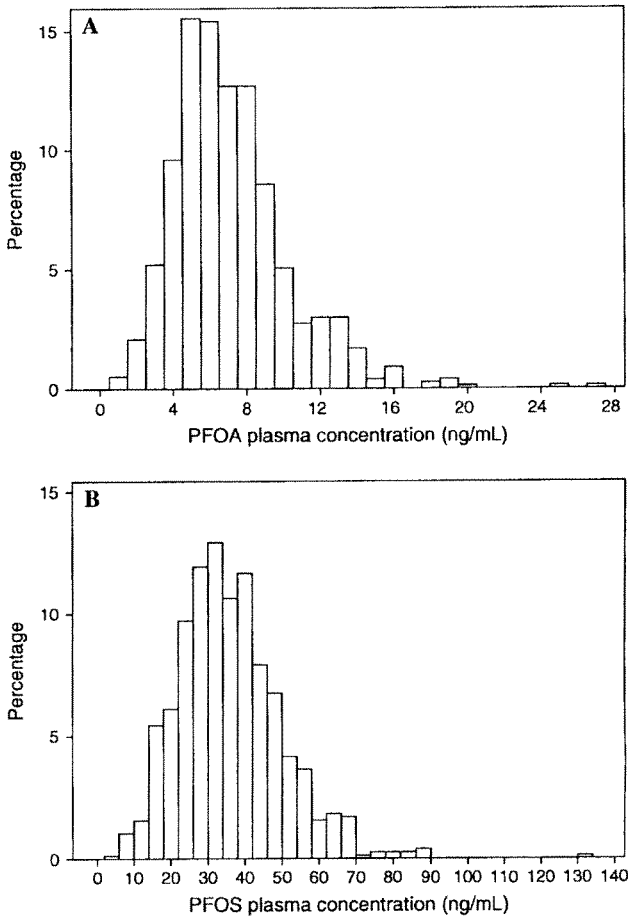

Figure 1. Distributions of plasma concentrations of perfluorinated chemicals in a randomly selected comparison subcohort of 680 men and 92 women from the prospective Danish cohort Diet, Cancer, and Health. **A)** Perfluorooctanoate. **B)** Perfluorooctanesulfonate. The distributions are based on one measurement per individual. Because an overview is presented, estimates of the variation in each group are not presented. (PFOA = perfluorooctanoate; PFOS = perfluorooctanesulfonate).

Table 2. Adjusted incidence rate ratios for prostate, bladder, pancreatic, and liver cancer for the three upper quartiles of plasma concentration of perfluorooctanoate and perfluorooctanesulfonate, compared with the lowest quartile*

		Perfluorooctanoate		Perfluorooctanesulfonate	
Cancer site	Quartile	No. of patients/No. subcohort participants	IRR (95% CI)	No. of patients/No. subcohort participants	IRR (95% CI)
Prostate†	Q1	179/175	1.00 (ref.)	179/208	1.00 (ref.)
	Q2	178/165	1.09 (0.78 to 1.53)	178/161	1.35 (0.97 to 1.87)
	Q3	178/182	0.94 (0.67 to 1.32)	180/160	1.31 (0.94 to 1.82)
	Q4	178/158	1.18 (0.84 to 1.65)	176/151	1.38 (0.99 to 1.93)
	Trend‡	713/680	1.03 (0.99 to 1.07)	713/680	1.05 (0.97 to 1.14)
Bladder§	Q1	84/151	1.00 (ref.)	83/145	1.00 (ref.)
	Q2	82/215	0.71 (0.46 to 1.07)	84/198	0.76 (0.50 to 1.16)
	Q3	83/184	0.92 (0.61 to 1.39)	83/195	0.93 (0.61 to 1.41)
	Q4	83/222	0.81 (0.53 to 1.24)	82/234	0.70 (0.46 to 1.07)
	Trend‡	332/772	1.00 (0.95 to 1.05)	332/772	0.93 (0.83 to 1.03)
Pancreas‖	Q1	32/179	1.00 (ref.)	32/161	1.00 (ref.)
	Q2	32/216	0.88 (0.49 to 1.57)	32/183	1.02 (0.57 to 1.84)
	Q3	32/178	1.33 (0.74 to 2.38)	32/184	1.24 (0.67 to 2.31)
	Q4	32/199	1.55 (0.85 to 2.80)	32/244	0.91 (0.51 to 1.65)
	Trend‡	128/772	1.03 (0.98 to 1.10)	128/772	0.99 (0.86 to 1.14)
Liver¶	Q1	17/108	1.00 (ref.)	17/108	1.00 (ref.)
	Q2	17/141	1.00 (0.44 to 2.23)	17/193	0.62 (0.29 to 1.33)
	Q3	17/281	0.49 (0.22 to 1.09)	17/217	0.72 (0.33 to 1.56)
	Q4	16/242	0.60 (0.26 to 1.37)	16/254	0.59 (0.27 to 1.27)
	Trend‡	67/772	0.95 (0.86 to 1.06)	67/772	0.97 (0.79 to 1.19)

* IRR = incidence rate ratio; CI = confidence interval; ref. = referent.

† Adjusted for years of school attendance (<8, 8–10, or >10 years), body mass index (kg/m²), dietary fat intake (g/d), and fruit and vegetable intake (g/d).

‡ In the trend analyses, plasma concentrations of perfluorooctanoate and perfluorooctanesulfonate were entered as continuous variables; IRRs were estimated per increase in perfluorooctanoate plasma concentration of 1 ng/mL and per increase in perfluorooctanesulfonate concentration of 10 ng/mL.

§ Adjusted for smoking status (present, former, or never smoker), smoking intensity (tobacco as g/d), smoking duration (years), years of school attendance (<8, 8–10, or >10 years), and occupation associated with risk for bladder cancer (rubber industry; textile industry [dyeing]; metal processing [painting]; glass industry; drivers of trucks, buses, or taxis; painter; hairdresser; waiter; or cook).

‖ Adjusted for smoking status (present, former, or never smoker), smoking intensity (tobacco as g/d), smoking duration (years), dietary fat intake (g/d), and fruit and vegetable intake (g/d).

¶ Adjusted for smoking status (present, former, or never smoker), years of school attendance (<8, 8–10, or >10 years), alcohol intake (g/d), and occupation associated with risk for liver cancer (waiter or cook).

potential confounders. Furthermore, cancer patients were identified through the nationwide Danish Cancer Registry, which has virtually complete ascertainment (16).

A limitation of this study is that nondifferential misclassification (25) may occur when using a single measure of the plasma concentration of perfluorooctanoate and perfluorooctanesulfonate for each individual, because the concentration at one moment in time may not reliably reflect the relevant plasma concentrations decades ago or at other times. However, half-lives of perfluorooctanoate and perfluorooctanesulfonate have been reported to be 4 years and 5 years, respectively (8), indicating that one prospective measure may represent a relatively stable internal dose.

In conclusion, our results suggest that perfluorooctanoate and perfluorooctanesulfonate plasma concentrations in the general Danish population are not associated with increased risk of prostate, bladder, pancreatic, or liver cancer. Additional research is warranted to investigate this relationship further in other cohorts, because this is, to our knowledge, the first study on perfluorinated chemicals and risk for cancer in a general population.

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Polyfluoroalkyl Chemicals in the U.S. Population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 and Comparisons with NHANES 1999–2000

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BACKGROUND: Polyfluoroalkyl chemicals (PFCs) have been used since the 1950s in numerous commercial applications. Exposure of the general U.S. population to PFCs is widespread. Since 2002, the manufacturing practices for PFCs in the United States have changed considerably.

OBJECTIVES: We aimed to assess exposure to perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and eight other PFCs in a representative 2003–2004 sample of the general U.S. population ≥ 12 years of age and to determine whether serum concentrations have changed since the 1999–2000 National Health and Nutrition Examination Survey (NHANES).

METHODS: By using automated solid-phase extraction coupled to isotope dilution–high-performance liquid chromatography–tandem mass spectrometry, we analyzed 2,094 serum samples collected from NHANES 2003–2004 participants.

RESULTS: We detected PFOS, PFOA, PFHxS, and PFNA in > 98% of the samples. Concentrations differed by race/ethnicity and sex. Geometric mean concentrations were significantly lower (approximately 32% for PFOS, 25% for PFOA, 10% for PFHxS) and higher (100%, PFNA) than the concentrations reported in NHANES 1999–2000 ($p < 0.001$).

CONCLUSIONS: In the general U.S. population in 2003–2004, PFOS, PFOA, PFHxS, and PFNA serum concentrations were measurable in each demographic population group studied. Geometric mean concentrations of PFOS, PFOA, and PFHxS in 2003–2004 were lower than in 1999–2000. The apparent reductions in concentrations of PFOS, PFOA, and PFHxS most likely are related to discontinuation in 2002 of industrial production by electrochemical fluorination of PFOS and related perfluorooctanesulfonyl fluoride compounds.

KEY WORDS: biomonitoring, C8, exposure, PFCs, PFOA, PFOS, prevalence, serum. *Environ Health Perspect* 115:1596–1602 (2007). doi:10.1289/ehp.10598 available via *http://dx.doi.org/* [Online 29 August 2007]

Concern about exposure of the ecosystem, including humans, to halogenated persistent organic pollutants (POPs) has existed for several decades. Many of these chemicals are persistent and toxic, tend to bioaccumulate, and can undergo long range atmospheric transport; for these reasons, their production has been banned or reduced worldwide, leading to their decreased concentrations in the ecosystem. In addition, adherence to provisions set forth in the Stockholm Convention on POPs for 12 organochlorine chemicals (United Nations Environment Programme 2004) probably will result in continued decreasing environmental concentrations. More recently, the focus of environmental and public health concern has shifted from chlorinated chemicals to brominated and fluorinated chemicals.

Among the fluorinated chemicals, the polyfluoroalkyl chemicals (PFCs) have been used extensively since the 1950s in commercial applications, including surfactants, lubricants, paper and textile coatings, polishes, food packaging, and fire-retarding foams. Some of these PFCs, including perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), persist in humans and the environment and have been detected worldwide in wildlife (Houde et al. 2006 and references therein). Exposure to PFOS and PFOA in the general population also is widespread, although demographic, geographic, and temporal differences may exist (Calafat et al. 2006b, 2007; Fromme et al. 2007; Guruge et al. 2005; Hansen et al. 2001; Harada et al. 2007; Kannan et al. 2004; Karrman et al. 2006; Olsen et al. 2005; Taniyasu et al. 2003; Yeung et al. 2006).

No definite association has been established between exposure to PFOS and PFOA and adverse health effects in several occupational studies (Alexander et al. 2003; Gilliland and Mandel 1993; Grice et al. 2007; Olsen et al. 2004a) and in one population exposed to PFOA through contaminated drinking water (Emmett et al. 2006). Negative associations between cord serum concentrations of both PFOS and PFOA and birth weight and ponderal index, but not newborn length or gestational age, have been reported in a nonoccupational population (Apelberg et al. 2007). By contrast, no association has been reported between employment in jobs with high exposure to PFOS before the end of pregnancy and maternally reported birth weight (Grice et al. 2007). In animals, exposure to PFOS and PFOA is associated with adverse health effects (Kennedy et al. 2004; Lau et al. 2004; Organisation for Economic Co-operation and Development 2002) albeit at serum concentrations orders of magnitude higher than the concentrations observed in the general population (Butenhoff et al. 2004; Luebker et al. 2005). Because of these compounds' known toxicity to animals, their ubiquitous presence, and their persistence in humans, wildlife, and the environment, PFCs research is of interest to toxicologists, epidemiologists, and environmental and public health scientists.

Biomonitoring data for these PFCs in the general population are needed to assess current exposures and to determine whether technologic changes affect human exposures to these compounds. As part of the continuous U.S. National Health and Nutrition Examination Survey (NHANES), urine and serum samples are collected and analyzed for selected environmental chemicals [Centers for Disease Control and Prevention (CDC) 2005]. NHANES participants also provide sociodemographic information and medical history and undergo standardized physical examinations (CDC 2003). We recently reported the concentrations of PFOS, PFOA, and nine other PFCs in 1,562 participants from NHANES 1999–2000 (Calafat et al. 2007). The high frequency of detection of PFOS and PFOA suggested highly prevalent exposures to these compounds at a time when both were being manufactured in the United States. In 2002, the 3M Company (St. Paul, MN), the sole U.S. producer of PFOS, discontinued its production of PFOS and related perfluorooctanesulfonyl fluoride

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(POSF)–based chemistries by electrochemical fluorination. Although PFOA and its salts and precursors still are manufactured by others by a different process, reductions in their manufacturing emissions have been proposed [Prevedouros et al. 2006; U.S. Environmental Protection Agency (EPA) 2006]. We now report the serum concentrations of 12 PFCs, including PFOS and PFOA, in 2,094 participants from NHANES 2003–2004 and compare these data with data from NHANES 1999–2000 (Calafat et al. 2007). The 2003–2004 data provide the first estimates of serum PFC concentrations in a representative U.S. population since implementation of the changes in manufacturing practices for some PFCs in the United States.

Materials and Methods

We obtained serum samples analyzed for PFCs from 2,094 participants ≥ 12 years of age from NHANES 2003–2004. The National Centers for Health Statistics Institutional Review Board reviewed and approved the study protocol. All participants provided informed written consent; parents or guardians provided consent for participants < 18 years of age (CDC 2006a).

We measured perfluorooctane sulfonamide (PFOSA), 2-(*N*-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH), 2-(*N*-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), perfluorobutane sulfonic acid (PFBuS), perfluorohexane sulfonic acid (PFHxS), PFOS, PFOA, perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDeA), perfluoroundecanoic acid (PFUA), and perfluorododecanoic acid (PFDoA) in 1 mL of serum, using a modification of the method of Kuklenyik et al. (2004), which involved automated solid-phase extraction coupled to reversed-phase high-performance liquid chromatography–tandem mass spectrometry. We used $^{18}O_2$-PFOS (for all sulfonic acids and all amides) and $^{13}C_2$-PFOA (for all carboxylic acids) for quantification. To compensate for the lack of isotope-labeled internal standards for the other analytes and to partially account for matrix effects, the calibration standards were spiked into calf serum. The limits of detection (LODs) ranged from 0.1 to 1.0 µg/L; the accuracy ranged from 84 to 135% at three concentrations (Kuklenyik et al. 2004); and the precision ranged from around 10 to 26% at two different levels (Table 1). Low-concentration (~ 3 µg/L to ~ 9 µg/L) and high-concentration (~ 10 µg/L to ~ 30 µg/L) quality-control (QC) materials, prepared from a base calf serum pool, were analyzed with reagent blank, serum blank, and NHANES samples (Kuklenyik et al. 2004). Standard, blank, QC, and NHANES samples were analyzed by the procedure described above.

We analyzed the data using SAS (version 9.1.3; SAS Institute Inc., Cary, NC) and SUDAAN (version 9.0.1; Research Triangle Institute, Research Triangle Park, NC). SUDAAN calculates variance estimates after incorporating the sample population weights, designed for the one-third subset of the full survey, which account for unequal selection probabilities and planned oversampling of certain subgroups resulting from the complex multistage area probability design of NHANES. Race/ethnicity was defined on the basis of self-reported data as non-Hispanic black, non-Hispanic white, and Mexican American. Persons not defined by these groups were included only in the total population estimate. Age was reported in years at the most recent birthday. We estimated the weighted percentage of detection and calculated weighted geometric means and percentiles for the serum concentrations (in micrograms per liter) of the various PFCs. For concentrations below the LOD, as recommended for the analysis of NHANES data (CDC 2006b), we used a value equal to the LOD divided by the square root of 2 (Hornung and Reed 1990). Parametric statistics were computed only for analytes for which the frequency of detection was ≥ 60%. Because PFC concentrations were not normally distributed, we used the natural log transformation. Weighted Pearson correlation coefficients and related *p*-values were calculated in SAS. Statistical significance was set at $p < 0.05$.

We used analysis of covariance to examine the influence of demographic and socioeconomic variables on the log-transformed serum concentrations of PFOS, PFOA, PFHxS, and PFNA. For multiple regression, we calculated the least square geometric means (LSGM) and compared them for each categorical variable. The variables included in the initial model were as follows: age as a continuous variable, sex, race/ethnicity, smoking status (yes/no), and education (less than high school, high school diploma, more than high school). Participants were categorized as smokers if their serum cotinine concentrations were > 10 µg/L. We chose to include education in the model without household income to minimize the possibility of collinearity because *a*) income and education are strongly associated (chi-square $p = 0.001$) and *b*) the final model yielded comparable results with either variable separately (except for PFOS, which included one additional significant term between income and smoking status). We assessed all possible two-way interaction terms in the model.

To reach the final reduced model, we used backward elimination with a threshold of $p < 0.05$ for retaining the variable in the model, using Satterwaite adjusted *F* statistics. We evaluated for potential confounding by adding each of the excluded variables back into the final model one by one and examining changes in the β coefficients of the statistically significant main effect. If addition of one of these excluded variables caused a change in a β coefficient by ≥ 10%, the variable was re-added to the model.

Results

The distribution of PFC serum concentrations is reported stratified by age, sex, and race/ethnicity (Tables 2–5). Four analytes were detected in > 98% of the samples (PFOS, 99.9%; PFOA, 99.7%; PFHxS, 98.3%; PFNA, 98.8%). Concentrations of these four PFCs ranged from < 0.4 µg/L to 435 µg/L (PFOS), < 0.1 µg/L to 77.2 µg/L (PFOA), < 0.3 µg/L to 82.0 µg/L (PFHxS), and < 0.1 µg/L to 11.5 µg/L (PFNA). Six other analytes were detected at lower frequencies: PFDeA (31.3%), Me-PFOSA-AcOH (27.5%), PFOSA (22.2%), PFUA (9.7%),

Table 1. LOD and precision data for the 12 polyfluoroalkyl compounds included in this study and a comparison of these parameters to the previously reported data for NHANES 1999-2000.

	LOD (µg/L)[b]		Precision[a]			
			QCL		QCH	
Analyte	NHANES 2003–2004	NHANES 1999–2000	NHANES 2003–2004	NHANES 1999–2000	NHANES 2003–2004	NHANES 1999–2000
PFOSA	0.2	0.05	2.7 (14.9)	2.4 (14.1)	13.0 (16.3)	12.4 (12.5)
Me-PFOSA-AcOH	0.6	0.2	3.4 (15.5)	3.1 (14.2)	9.1 (16.7)	9.0 (13.5)
Et-PFOSA-AcOH	0.4	0.2	3.8 (17.2)	3.5 (14.3)	8.3 (19.2)	8.1 (15.6)
PFBuS	0.4	ND	4.4 (18.2)	ND	14.6 (15.1)	ND
PFHxS	0.3	0.1	2.5 (16.4)	2.1 (16.6)	11.9 (12.9)	11.2 (12.3)
PFOS	0.4	0.2	8.9 (10.4)	8.8 (8.4)	31.4 (10.1)	31.6 (7.1)
PFHpA	0.3	0.4	7.6 (17.0)	6.8 (13.5)	15.8 (14.3)	15.5 (12.0)
PFOA	0.1	0.1	3.2 (10.0)	3.1 (8.5)	14.7 (10.9)	15.1 (7.3)
PFNA	0.1	0.1	2.5 (15.0)	2.6 (15.4)	12.7 (13.2)	13.0 (10.9)
PFDeA	0.3	0.2	2.4 (17.5)	2.2 (13.9)	8.5 (18.2)	8.4 (13.1)
PFUA	0.3	0.2	1.9 (22.0)	2.0 (19.1)	9.9 (19.8)	10.6 (16.2)
PFDoA	1.0	0.2	2.2 (25.6)	2.4 (22.4)	8.5 (25.7)	9.1 (19.3)

ND, not determined.
[a]Mean concentration (% coefficient of variation) of repeated measurements (minimum of 20) over time of quality-control calf serum materials of low (QCL) and high (QCH) concentrations. [b]The NHANES 1999–2000 samples were analyzed by using the approach described in Kuklenyik et al. (2005), whereas the NHANES 2003–2004 samples were analyzed by using the Kuklenyik et al. (2004) approach.

PFHpA (6.2%), and Et-PFOSA-AcOH, (3.4%); their geometric mean and selected percentile concentrations are given as Supplemental Material in Tables S1–S6 (online at http://www.ehponline.org/docs/2007/10598/suppl.pdf). For the two analytes detected in < 1% of the samples (PFDoA, < 0.1%; PFBuS, 0.4%), we could not calculate the 95th percentile of concentrations.

Statistically significant correlations ($p < 0.001$) existed between the log-transformed concentrations of PFOS and PFOA (Pearson correlation coefficient $r = 0.66$), PFHxS ($r = 0.56$), and PFNA ($r = 0.50$); between PFOA and PFHxS ($r = 0.46$) and PFNA ($r = 0.55$); and between PFHxS and PFNA ($r = 0.17$).

The final models included sex ($p < 0.01$), age, race/ethnicity, and age-by-race/ethnicity interaction ($p = 0.01$) for PFOS; sex, race/ethnicity, age, education, sex-by-age ($p < 0.01$), sex-by-race/ethnicity ($p = 0.03$), and education-by-age ($p = 0.04$) interactions for PFOA; sex, race/ethnicity ($p = 0.01$), age, and sex-by-age interaction ($p = 0.02$) for PFHxS; and sex ($p < 0.01$), race/ethnicity, age, education ($p = 0.02$), smoking status ($p = 0.02$), and race/ethnicity-by-age ($p < 0.01$) and

Table 2. Geometric mean and selected percentiles (95% confidence intervals) of perfluorooctanesulfonate (PFOS) concentrations in serum (μg/L) for the U.S. population 12 years of age and older: data from NHANES 2003–2004.

Variable	Geometric mean	10th percentile	25th percentile	50th percentile	75th percentile	90th percentile	95th percentile	No.
All	20.7 (19.2–22.3)	9.8 (9.0–10.8)	14.6 (13.8–15.2)	21.1 (19.8–22.4)	29.9 (27.5–32.8)	41.2 (35.5–48.9)	54.6 (44.0–65.9)	2,094
12–19 years	19.3 (17.5–21.4)	9.9 (9.5–10.9)	14.4 (12.5–15.7)	19.9 (17.6–21.9)	27.1 (23.6–30.2)	36.5 (28.6–45.6)	42.2 (35.1–52.1)	640
20–39 years	18.7 (17.3–20.1)	8.9 (8.2–10.2)	12.6 (11.2–14.2)	18.7 (17.7–20.4)	27.4 (24.9–29.7)	36.9 (33.6–41.3)	44.3 (38.6–60.8)	490
40–59 years	22.0 (19.7–24.5)	10.6 (9.2–12.3)	15.3 (14.1–18.0)	22.2 (20.2–24.2)	32.2 (27.4–35.4)	43.8 (33.5–62.7)	61.5 (43.8–81.8)	387
≥ 60 years	23.2 (20.8–25.9)	9.9 (7.7–13.0)	16.6 (15.0–17.9)	23.9 (20.9–27.2)	34.7 (30.0–39.3)	50.3 (40.8–68.9)	69.4 (49.6–90.0)	577
Mexican American	14.7 (13.0–16.6)	7.4 (5.6–7.9)	10.3 (8.3–11.8)	15.9 (13.4–17.9)	21.1 (18.7–23.5)	28.1 (24.1–35.0)	35.5 (28.9–38.5)	485
Non-Hispanic black	21.6 (19.1–24.4)	9.9 (7.5–11.9)	14.8 (12.5–16.8)	22.0 (19.5–24.9)	32.2 (28.1–36.2)	43.8 (37.2–57.3)	57.5 (43.8–78.4)	538
Non-Hispanic white	21.4 (19.9–23.1)	10.5 (9.5–11.5)	15.0 (14.4–16.0)	21.9 (20.5–23.0)	30.2 (27.7–33.0)	41.3 (35.7–49.6)	55.9 (44.0–69.4)	962
Female	18.4 (17.0–20.0)	9.0 (7.8–9.9)	12.4 (11.5–13.8)	18.2 (16.8–19.7)	27.3 (23.6–30.0)	39.7 (34.4–42.6)	45.7 (42.3–61.5)	1,041
Male	23.3 (21.1–25.6)	12.3 (10.4–13.5)	17.7 (15.9–18.9)	23.9 (22.3–25.3)	32.1 (28.7–35.7)	45.3 (35.5–62.7)	62.7 (43.8–81.8)	1,053

Table 3. Geometric mean and selected percentiles (95% confidence intervals) of perfluorooctanoate (PFOA) concentrations in serum (μg/L) for the U.S. population 12 years of age and older: data from NHANES 2003–2004.

Variable	Geometric mean	10th percentile	25th percentile	50th percentile	75th percentile	90th percentile	95th percentile	No.
All	3.9 (3.6–4.3)	1.9 (1.8–2.1)	2.7 (2.6–3.0)	4.0 (3.8–4.4)	5.8 (5.2–6.3)	7.8 (6.7–9.6)	9.8 (7.4–14.1)	2,094
12–19 years	3.9 (3.5–4.4)	2.2 (1.9–2.3)	2.9 (2.6–3.2)	3.9 (3.3–4.4)	5.4 (4.6–6.1)	6.9 (5.6–9.2)	8.6 (5.9–12.6)	640
20–39 years	3.9 (3.6–4.2)	1.8 (1.5–2.1)	2.7 (2.5–3.0)	4.1 (3.7–4.5)	5.8 (5.4–6.1)	7.6 (7.3–8.4)	9.6 (8.4–11.1)	490
40–59 years	4.2 (3.8–4.8)	2.0 (1.8–2.4)	2.9 (2.6–3.2)	4.2 (3.9–4.8)	6.3 (5.3–7.2)	8.2 (6.8–10.7)	10.6 (7.4–16.9)	387
≥ 60 years	3.7 (3.3–4.1)	1.8 (1.5–2.1)	2.7 (2.4–2.9)	3.9 (3.5–4.3)	5.4 (4.9–5.9)	7.2 (6.0–9.5)	9.5 (6.9–14.1)	577
Mexican American	3.1 (2.8–3.4)	1.4 (1.1–1.8)	2.2 (1.9–2.5)	3.3 (3.0–3.6)	4.4 (4.1–5.1)	6.7 (5.7–7.3)	7.6 (6.7–10.5)	485
Non-Hispanic black	3.4 (3.0–3.8)	1.2 (1.1–1.6)	2.2 (1.9–2.5)	3.7 (3.1–4.2)	5.1 (4.4–6.1)	7.7 (5.3–10.9)	9.3 (6.5–13.9)	538
Non-Hispanic white	4.2 (3.9–4.5)	2.1 (2.0–2.3)	3.0 (2.6–3.2)	4.2 (3.9–4.6)	5.9 (5.4–6.6)	7.8 (7.2–9.1)	9.8 (7.6–13.3)	962
Female	3.5 (3.2–3.8)	1.6 (1.5–1.9)	2.5 (2.2–2.7)	3.6 (3.2–3.9)	5.2 (4.6–5.7)	7.1 (6.3–8.2)	8.4 (7.4–10.6)	1,041
Male	4.5 (4.1–4.9)	2.3 (2.0–2.4)	3.2 (3.1–3.5)	4.6 (4.2–5.0)	6.3 (5.6–7.1)	8.3 (6.8–11.8)	10.4 (7.4–17.5)	1,053

Table 4. Geometric mean and selected percentiles (95% confidence intervals) of perfluorohaxanesulfonate (PFHxS) concentrations in serum (μg/L) for the U.S. population 12 years of age and older: data from NHANES 2003–2004.

Variable	Geometric mean	10th percentile	25th percentile	50th percentile	75th percentile	90th percentile	95th percentile	No.
All	1.9 (1.7–2.2)	0.7 (0.6–0.7)	1.0 (0.9–1.2)	1.9 (1.6–2.1)	3.3 (2.8–3.9)	5.9 (4.8–7.2)	8.3 (7.1–9.7)	2,094
12–19 years	2.4 (2.1–2.9)	0.6 (0.5–0.8)	1.2 (1.0–1.4)	2.3 (1.7–3.0)	4.8 (3.9–6.0)	9.5 (6.8–12.5)	13.1 (9.9–19.6)	640
20–39 years	1.8 (1.6–2.0)	0.5 (0.5–0.6)	1.0 (0.9–1.2)	1.7 (1.5–2.0)	2.8 (2.5–3.3)	4.8 (3.9–6.1)	6.7 (4.9–9.4)	490
40–59 years	1.9 (1.6–2.2)	0.7 (0.5–0.8)	1.0 (0.9–1.2)	1.6 (1.4–2.0)	3.1 (2.3–4.5)	5.5 (4.3–6.9)	6.7 (5.5–8.2)	387
≥ 60 years	2.0 (1.7–2.4)	0.8 (0.5–0.9)	1.1 (1.0–1.3)	1.9 (1.6–2.1)	3.2 (2.6–3.7)	7.2 (4.3–9.7)	10.2 (7.0–12.6)	577
Mexican American	1.4 (1.2–1.7)	0.5 (0.3–0.7)	0.7 (0.5–0.9)	1.4 (1.2–1.7)	2.3 (1.9–2.7)	4.2 (3.1–5.1)	5.4 (4.0–8.9)	485
Non-Hispanic black	1.9 (1.6–2.3)	0.5 (0.3–0.7)	1.1 (0.9–1.3)	1.9 (1.5–2.2)	3.4 (2.7–4.3)	6.0 (5.0–7.1)	8.2 (6.3–12.0)	538
Non-Hispanic white	2.0 (1.8–2.3)	0.7 (0.6–0.8)	1.1 (1.0–1.3)	1.9 (1.6–2.1)	3.3 (2.8–4.0)	6.0 (4.6–7.8)	8.1 (6.9–10.1)	962
Female	1.7 (1.6–1.9)	0.6 (0.5–0.6)	0.9 (0.8–1.0)	1.5 (1.4–1.8)	2.9 (2.5–3.5)	5.8 (4.6–6.9)	8.2 (6.7–10.0)	1,041
Male	2.2 (1.9–2.5)	0.8 (0.7–1.0)	1.3 (1.1–1.4)	2.0 (1.8–2.4)	3.3 (2.8–4.4)	6.1 (4.6–8.1)	8.5 (6.4–10.5)	1,053

Table 5. Geometric mean and selected percentiles (95% confidence intervals) of perfluorononanoate (PFNA) concentrations in serum (μg/L) for the U.S. population 12 years of age and older: data from NHANES 2003–2004.

Variable	Geometric mean	10th percentile	25th percentile	50th percentile	75th percentile	90th percentile	95th percentile	No.
All	1.0 (0.8–1.1)	0.4 (0.3–0.4)	0.6 (0.5–0.6)	1.0 (0.9–1.1)	1.5 (1.2–1.7)	2.2 (1.6–3.8)	3.2 (1.8–7.7)	2,094
12–19 years	0.9 (0.7–1.0)	0.3 (0.3–0.4)	0.5 (0.5–0.6)	0.7 (0.6–0.9)	1.2 (0.9–1.5)	1.9 (1.2–3.3)	2.7 (1.3–6.3)	640
20–39 years	1.0 (0.8–1.1)	0.3 (0.2–0.5)	0.6 (0.6–0.7)	0.9 (0.8–1.1)	1.4 (1.2–1.7)	2.1 (1.7–2.7)	2.8 (1.9–6.1)	490
40–59 years	1.1 (0.9–1.4)	0.5 (0.4–0.5)	0.7 (0.6–0.7)	1.0 (0.9–1.2)	1.7 (1.2–2.4)	2.7 (1.6–5.9)	4.3 (1.7–9.3)	387
≥ 60 years	0.8 (0.7–1.0)	0.3 (0.2–0.3)	0.5 (0.5–0.6)	0.9 (0.8–1.0)	1.3 (1.1–1.5)	1.9 (1.5–3.0)	3.0 (1.6–6.5)	577
Mexican American	0.7 (0.6–0.8)	0.2 (0.1–0.2)	0.5 (0.4–0.5)	0.7 (0.5–0.8)	1.0 (0.9–1.3)	1.6 (1.2–1.8)	2.0 (1.6–2.8)	485
Non-Hispanic black	1.1 (0.8–1.5)	0.4 (0.3–0.6)	0.6 (0.5–0.8)	1.0 (0.8–1.4)	1.6 (1.2–2.7)	3.1 (1.5–6.5)	4.7 (2.1–9.3)	538
Non-Hispanic white	1.0 (0.8–1.1)	0.4 (0.3–0.4)	0.5 (0.5–0.6)	0.8 (0.8–0.9)	1.5 (1.2–1.7)	2.2 (1.6–3.4)	2.9 (1.8–6.2)	962
Female	0.9 (0.7–1.0)	0.4 (0.3–0.4)	0.6 (0.5–0.6)	0.9 (0.7–0.9)	1.2 (1.0–1.6)	2.2 (1.4–3.3)	3.0 (1.7–6.1)	1,041
Male	1.1 (0.9–1.3)	0.5 (0.4–0.5)	0.6 (0.6–0.7)	1.0 (0.9–1.2)	1.6 (1.3–1.8)	2.4 (1.7–4.8)	4.0 (1.8–8.7)	1,053

age-by-smoking status ($p = 0.04$) interactions for PFNA. Because of these interactions with age, concentrations were compared at the 25th (age = 26 years), 50th (age = 41 years), 75th (age = 55 years), and 90th (age = 70 years) percentiles of age.

LSGM concentrations provide geometric mean estimates for a demographic variable after adjustment for the model covariates (Table 6). The statistical significance values when comparing these LSGM concentrations are shown in the Supplemental Material, Table S7 (online at http://www.ehponline.org/docs/2007/10598/suppl.pdf). PFOS LSGM concentrations were significantly higher ($p < 0.01$) in males than in females. Similarly, for PFOA and PFHxS, males had significantly higher LSGM concentrations than females except at the 90th percentile of age (Table 6). LSGM concentrations of PFHxS were significantly lower for Mexican Americans than for non-Hispanic blacks ($p = 0.01$) and non-Hispanic whites ($p < 0.01$); LSGM concentrations did not differ significantly between non-Hispanic whites and non-Hispanic blacks ($p = 0.49$). PFOS and PFNA LSGM concentrations were significantly lower in Mexican Americans than in non-Hispanic blacks (PFOS, $p < 0.01$; PFNA, $p < 0.01$–0.03) and non-Hispanic whites (PFOS, $p < 0.01$; PFNA, $p < 0.01$–0.02), regardless of age; LSGM concentrations between non-Hispanic whites and non-Hispanic blacks differed significantly only at the 75th and 90th percentiles of age (Table 6). Non-Hispanic whites had significantly higher PFOA LSGM concentrations ($p < 0.01$), regardless of sex, than Mexican Americans. The differences between Mexican-American males and non-Hispanic black males and between non-Hispanic white males and non-Hispanic black males were not statistically significant.

We used a two-sample t-test to compare the difference of the two geometric mean concentrations (on the log scale) of PFOS, PFOA, PFHxS, and PFNA during NHANES 1999–2000 and NHANES 2003–2004 (Table 7), taking into account their associated standard errors and degrees of freedom, by age, sex, and race/ethnicity, using SAS. The differences were all statistically significant ($p < 0.05$), except for PFHxS in Mexican Americans ($p = 0.21$) (Table 7). We analyzed the NHANES 2003–2004 samples first and then the NHANES 1999–2000 samples (Calafat et al. 2007) using two methods that differed in the manner in which PFCs were extracted and preconcentrated from the serum (Kuklenyik et al. 2004, 2005). In both methods, we used tandem mass spectrometry with $^{18}O_2$-PFOS, $^{13}C_2$-PFOA, and $^{18}O_2$-PFOSA (only for NHANES 1999–2000) for quantification, the same multiple reaction monitoring transitions for quantification for PFOA (413/369) and PFOS (499/99), the same QC materials and analytical standards. $^{18}O_2$-PFOSA was not commercially available when the 2003–2004 NHANES samples were analyzed. Except for PFNA and PFOA, for which the LODs were the same regardless of the method, the method used for NHANES 1999–2000 (Kuklenyik et al. 2005) had slightly lower LODs than the method used for NHANES 2003–2004 (Kuklenyik et al. 2004) (Table 1). To estimate whether method differences could account for the differences in concentrations, we analyzed QC samples from low and high concentration pools and 124 split samples using both methods. The two methods showed good agreement from the results of the split sample analysis [presented for PFOA in Figures S1 and S2 in Supplemental

Table 6. Least-square geometric mean concentrations (μg/L) (95% confidence intervals) of PFOA, PFOS, PFHxS, and PFNA in various demographic groups.

Group	PFOA	PFOS	PFHxS	PFNA
Female		18.5 (17.1–20)		0.9 (0.7–1)
Male		23.6 (21.8–25.7)		1.1 (0.9–1.3)
Female: age P25	3.4 (3.1–3.7)		1.7 (1.5–1.9)	
Female: age P50	3.5 (3.3–3.8)		1.7 (1.5–1.9)	
Female: age P75	3.7 (3.4–4)		1.7 (1.5–2)	
Female :age P90	3.8 (3.4–4.2)		1.7 (1.5–2)	
Male: age P25	5.1 (4.7–5.5)		2.4 (2–2.8)	
Male: age P50	4.5 (4.2–4.9)		2.2 (1.9–2.6)	
Male: age P75	4.1 (3.7–4.5)		2.1 (1.8–2.4)	
Male: age P90	3.7 (3.2–4.2)		1.9 (1.6–2.3)	
MA			1.4 (1.1–1.7)	
NHB			1.9 (1.6–2.3)	
NHW			2.0 (1.8–2.3)	
Female, MA	2.6 (2.3–3)			
Female, NHB	2.8 (2.5–3.2)			
Female, NHW	3.8 (3.5–4.1)			
Male, MA	3.6 (3.3–3.9)			
Male, NHB	4.1 (3.5–4.8)			
Male, NHW	4.6 (4.2–5.1)			
MA: age P25		13.9 (12.5–15.5)		0.7 (0.6–0.8)
MA: age P50		15.1 (13.6–16.8)		0.7 (0.6–0.8)
MA: age P75		16.3 (14.4–18.4)		0.7 (0.5–0.8)
MA: age P90		17.7 (15.3–20.6)		0.6 (0.5–0.8)
NHW: age P25		20.1 (18.6–21.8)		1 (0.8–1.2)
NHW: age P50		21.2 (19.6–22.9)		1 (0.8–1.1)
NHW: age P75		22.3 (20.5–24.3)		0.9 (0.8–1.1)
NHW: age P90		23.5 (21.3–26)		0.9 (0.8–1)
NHB: age P25		19.9 (17.9–22.1)		1.1 (0.8–1.4)
NHB: age P50		22.6 (20.1–25.5)		1.2 (0.9–1.6)
NHB: age P75		25.5 (22.1–29.5)		1.3 (1–1.9)
NHB: age P90		29.0 (24.3–34.7)		1.5 (1–2.1)
NonSMK: age P25				1 (0.8–1.1)
NonSMK: age P50				1 (0.8–1.1)
NonSMK: age P75				1 (0.8–1.1)
NonSMK: age P90				1 (0.8–1.2)
SMK: age P25				1.1 (0.9–1.3)
SMK: age P50				1 (0.8–1.1)
SMK: age P75				0.9 (0.8–1)
SMK: age P90				0.8 (0.7–1)
< HS				0.7 (0.6–0.8)
= HS				1 (0.8–1.1)
> HS				1.2 (0.9–1.7)
< HS: age P25	3.7 (3.4–4.1)			
< HS: age P50	3.7 (3.4–4.1)			
< HS: age P75	3.7 (3.3–4.1)			
< HS: age P90	3.7 (3.3–4.2)			
= HS: age P25	4.4 (4.1–4.7)			
= HS: age P50	4 (3.7–4.3)			
= HS: age P75	3.7 (3.3–4.1)			
= HS: age P90	3.3 (2.8–4)			
> HS: age P25	4.2 (3.8–4.6)			
> HS: age P50	4.1 (3.7–4.5)			
> HS: age P75	4.1 (3.6–4.6)			
> HS: age P90	4 (3.4–4.7)			

Abbreviations: HS, high school; MA, Mexican American; NHB, non-Hispanic black; NHW, non-Hispanic white; NonSMK, nonsmoker; P25, 25th percentile of age = 26 years; P50, 50th percentile of age = 41 years; P75, 75th percentile of age = 55 years; P90, 90th percentile of age = 70 years; SMK, smoker.

Material (online at http://www.ehponline.org/docs/2007/10598/suppl.pdf)]. Results were similar for all other analytes (data not shown). In general, analysis of the QC pools showed mean concentrations and coefficients of variation which were similar between the two methods (Table 1).

Discussion

We detected PFOS, PFOA, PFHxS, and PFNA in > 98% of persons in this representative sample of the civilian, noninstitutionalized U.S. population, ≥ 12 years of age. These findings confirm that measurable serum concentrations of these compounds were prevalent in the United States in 2003–2004, even after 3M in 2002 discontinued its industrial production of PFOS and related compounds, including the ammonium salt of PFOA. Direct and indirect sources of PFOA still exist in the United States, although since 1999, global emissions of PFOA reportedly have decreased by more than half as of 2004 (Prevedouros et al. 2006), and current producers have committed to reducing manufacturing emissions of PFOA and its salts and precursors (U.S. EPA 2006).

Other PFCs, however, were detected infrequently. For example, PFBuS was detected in < 0.5% of the samples. PFBuS is a final degradation product of perfluorobutanesulfonyl fluoride, now used in the manufacture of materials as a replacement for POSF-related chemicals [C-6 (e.g., PFHxS) and C-8 (e.g., PFOS)] that were phased out beginning in 2000. Similarly, in a study involving 18 volunteer employees from 3M Company, PFBuS was detected only in workers with production-related duties, whereas PFOA, PFOS, and PFHxS were detected in most workers (Ehresman et al. 2007). The lower frequency of detection of PFBuS than PFOS, PFOA, and PFHxS suggests that human exposures to PFBuS are indeed lower, and/or that pharmacokinetic factors, which might include increased urinary elimination, are different.

PFOS showed the highest geometric mean and 95th percentile concentrations, followed by PFOA, PFHxS, and PFNA. For PFOS, PFOA, and PFNA, however—unlike lipophilic POPs whose serum concentrations increase with age (Needham et al. 2006)—concentrations were quite similar among the four age groups (Tables 2–5), a finding that agrees with previous data (Calafat et al. 2007; Olsen et al. 2003, 2004b, 2004c). By contrast, for PFHxS, the geometric mean and 95th percentile concentrations were higher for adolescents than for adults, as previously reported (Calafat et al. 2007; Olsen et al. 2004b). The higher concentrations of PFHxS in children and adolescents could be related to their increased contact with carpeted floors containing PFHxS, which is used for specific postmarket carpet-treatment applications (Olsen et al. 2004b).

In agreement with previous reports (Calafat et al. 2006a, 2007; Fromme et al. 2007; Harada et al. 2004; Midasch et al. 2006; Yeung et al. 2006), we observed sex and race/ethnicity differences. Females had significantly lower LSGM concentrations of PFOS than did males (Table 6). For PFOA and PFHxS, sex differences also existed but were not as pronounced for the elderly (Table 6). Mexican Americans had the lowest LSGM concentrations of PFHxS and non-Hispanic whites and non-Hispanic blacks had similar concentrations (Table 6). Racial differences for PFOS and PFNA were age dependent, whereas those for PFOA were sex dependent (Table 6). These sex and racial differences may reflect variability in exposure patterns as a result of differences in factors such as lifestyle, diet, and use of products containing PFCs that may contribute to the observed serum concentrations of PFCs.

To evaluate whether the discontinued production of PFOS and related compounds by 3M Company in 2002 and technologic changes implemented by other companies have led to a subsequent decrease in serum PFC concentrations in the general U.S. population (Olsen et al. 2007b), we compared NHANES data of 1999–2000 with NHANES data of 2003–2004. The distribution of serum concentrations of PFOS, PFOA, PFHxS, and PFNA by sex, race/ethnicity, and age in 2003–2004 (Tables 2–5) was similar to that for the general U.S. population in 1999–2000 (Calafat et al. 2007). However, the geometric mean concentrations for PFOS, PFOA, and PFHxS in 2003–2004 were lower than for 1999–2000. For PFNA, 2003–2004 levels were higher than those found in 1999–2000. These concentrations differed significantly for all demographic groups except for PFHxS in Mexican Americans (Table 7). Various concentration percentiles similarly decreased for PFOS, PFOA, and PFHxS. We analyzed the NHANES 1999–2000 and 2003–2004 samples by using two different methods; however, these approaches provided equivalent results [Table 1; Figures S1 and S2 in the Supplemental Material (online at http://www.ehponline.org/docs/2007/10598/suppl.pdf)], indicating that the differences cannot be attributed to changes in the analytical methodology. The decrease in serum concentrations of PFOS and PFOA during this time interval agreed with the reported reductions in PFOS and PFOA concentrations for a group of Red Cross blood donors in the United States (Olsen et al. 2007b) and in PFOS (temporal trends for PFOA were not examined) in Arctic ringed seals in the same time (Butt et al. 2007). These decreases in serum concentrations of PFOS and PFOA in humans and wildlife had been related to the phaseout of POSF-based materials in 2000–2002 (Butt et al. 2007; Olsen et al. 2007b).

For PFHxS, although the geometric mean concentrations were lower in 2003–2004 than in 1999–2000, the differences were less evident, and in some cases they reversed at the higher concentration percentiles for some demographic categories. These findings may be related to the lower concentrations of PFHxS than of PFOS or PFOA and to differences in the estimated geometric mean serum

Table 7. Geometric mean concentrations (95% confidence intervals) of PFOA, PFOS, PFHxS, and PFNA in NHANES 1999–2000 and NHANES 2003–2004 for the whole population and different demographic groups.[a]

	PFOS		PFOA		PFHxS		PFNA	
Variable	1999–2000	2003–2004	1999–2000	2003–2004	1999–2000	2003–2004	1999–2000	2003–2004
All	30.4 (27.1–33.9)	20.7 (19.2–22.3)	5.2 (4.7–5.7)	3.9 (3.6–4.3)	2.1 (1.9–2.4)	1.9 (1.7–2.2)	0.5 (0.5–0.7)	1.0 (0.8–1.1)
12–19 years	29.1 (26.2–32.4)	19.3 (17.5–21.4)	5.5 (5.0–6.0)	3.9 (3.5–4.4)	2.7 (2.1–3.4)	2.4 (2.1–2.9)	0.5 (0.4–0.5)	0.9 (0.7–1.0)
20–39 years	27.5 (24.9–30.2)	18.7 (17.3–20.1)	5.2 (4.7–5.7)	3.9 (3.6–4.2)	2.0 (1.7–2.3)	1.8 (1.6–2.0)	0.5 (0.4–0.6)	1.0 (0.8–1.1)
40–59 years	33.0 (28.0–38.8)	22.0 (19.7–24.5)	5.4 (4.7–6.2)	4.2 (3.8–4.8)	2.1 (1.8–2.3)	1.9 (1.6–2.2)	0.6 (0.4–0.7)	1.1 (0.9–1.4)
≥ 60 years	33.3 (28.5–38.8)	23.2 (20.8–25.9)	4.8 (4.3–5.5)	3.7 (3.3–4.1)	2.2 (1.9–2.5)	2.0 (1.7–2.4)	0.6 (0.5–0.8)	0.8 (0.7–1.0)
Female	28.0 (24.6–31.8)	18.4 (17.0–20.0)	4.8 (4.3–5.3)	3.5 (3.2–3.8)	1.8 (1.6–2.1)	1.7 (1.6–1.9)	0.5 (0.4–0.6)	0.9 (0.7–1.0)
Male	33.4 (29.6–37.6)	23.3 (21.1–25.6)	5.7 (5.2–6.3)	4.5 (4.1–4.9)	2.6 (2.3–3.0)	2.2 (1.9–2.5)	0.6 (0.5–0.7)	1.1 (0.9–1.3)
Mexican American	22.7 (19.8–25.9)	14.7 (13.0–16.6)	3.9 (3.6–4.2)	3.1 (2.8–3.4)	1.5 (1.1–1.9)	1.4 (1.2–1.7)	0.3 (0.3–0.4)	0.7 (0.6–0.8)
Non-Hispanic black	33.0 (26.2–41.6)	21.6 (19.1–24.4)	4.8 (4.1–5.6)	3.4 (3.0–3.8)	2.2 (1.6–2.9)	1.9 (1.6–2.3)	0.8 (0.6–1.0)	1.1 (0.8–1.5)
Non-Hispanic white	32.0 (29.1–35.2)	21.4 (19.9–23.1)	5.6 (5.0–6.2)	4.2 (3.9–4.5)	2.3 (2.0–2.5)	2.0 (1.8–2.3)	0.6 (0.5–0.7)	1.0 (0.8–1.1)

[a]For PFOS, PFOA, and PFNA, all differences between NHANES 1999–2000 (Calafat et al. 2007) and NHANES 2003–2004 geometric mean concentrations are statistically significant ($p < 0.001$). For PFHxS, except for Mexican Americans ($p = 0.209$), all other differences are also statistically significant with $p < 0.001$, except for females ($p = 0.037$), persons ≥ 60 years of age ($p = 0.016$), persons 12–19 years of age ($p = 0.004$), and non-Hispanic blacks ($p = 0.004$).

elimination half-life (PFHxS, 7.3 years; PFOA, 3.5 years; and PFOS, 4.8 years) (Olsen et al. 2007a). Furthermore, the correlation between the serum concentrations of PFOS and PFOA was higher than correlations of PFHxS and either PFOA or PFOS, suggesting potential common exposure pathway(s) for PFOA and PFOS, but probably not for PFHxS (mostly used in carpet-treatment applications (Olsen et al. 2004b). Pharmacokinetic factors may also contribute to these differences. The transformation of certain POFS-related sulfonamides to PFOS and potentially to PFOA in the atmosphere was suggested as a common mechanism for formation of both PFOS and PFOA, which would account at least partly for the high correlation in serum concentrations (Olsen et al. 2007b). On the other hand, PFOA and other perfluorocarboxylates (e.g., PFNA), but not PFOS, might be formed from the biodegradation of the volatile fluorotelomer alcohols (Ellis et al. 2004).

Current manufacturing practices exclusively use fluorotelomers for the synthesis of perfluorocarboxylates (Prevedouros et al. 2006). Perfluorocarboxylates, including PFNA, were present as reaction by-products in POSF-based materials (Prevedouros et al. 2006). Interestingly, our data suggest that PFNA geometric mean concentrations in 2003–2004 approximately doubled over those of 1999–2000. However, because human exposure data for PFNA are more limited than they are for PFOS, PFOA, and even PFHxS, these results must be interpreted with caution. In 2004, the estimated annual production of the ammonium salt of PFNA, primarily used as a processing aid in the manufacture of such fluoropolymers as polyvinylidene fluoride, was 15–75 tonnes (Prevedouros et al. 2006). Information about efforts to reduce manufacturing emissions for PFNA, estimated at about 10% of the amount produced, was not found (Prevedouros et al. 2006). As a comparison, global manufacturing emissions of PFOA were about 15 tonnes in 2004, down from about 45 tonnes in 1999 (Prevedouros et al. 2006).

For most PFCs, these NHANES 2003–2004 results are consistent with reduced population exposure because of recent efforts of industry and government. U.S. and worldwide efforts continue in attempts to reduce exposures to PFCs, including PFOS and PFOA, and many halogenated POPs, including polybrominated diphenyl ethers. We will continue to assess exposure to these and other chemicals in the U.S. population through NHANES, an effort that will provide unique information on trends of exposure to these chemicals over time. In addition, we are analyzing pooled serum samples from 3- to 11-year-old children to fill data gaps for mean PFC concentrations in this age range.

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Use of Newborn Screening Program Blood Spots for Exposure Assessment: Declining Levels of Perfluorinated Compounds in New York State Infants

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Temporal biomonitoring studies can assess changes in population exposures to contaminants, but collection of biological specimens with adequate representation and sufficient temporal resolution can be resource-intensive. Newborn Screening Programs (NSPs) collect blood as dried spots on filter paper from nearly all infants born in the United States (U.S.). In this study, we investigated the use of NSP blood spots for temporal biomonitoring by analyzing perfluorooctane sulfonate (PFOS), perfluorooctane sulfonamide (PFOSA), perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) in 110 New York State (NYS) NSP blood spot composite specimens collected between 1997 and 2007, representing a total of 2640 infants. All analytes were detected in ≥90% of the specimens. Concentrations of PFOS, PFOSA, PFHxS, and PFOA exhibited significant exponential declines after the year 2000, coinciding with the phase-out in PFOS production in the U.S. Calculated disappearance half-lives for PFOS, PFHxS, and PFOA (4.4, 8.2, and 4.1 years, respectively) were similar to biological half-lives reported for retired fluorochemical workers. Our results suggest sharp decreases in perinatal exposure of NYS infants to PFOS, PFOSA, PFHxS, and PFOA and demonstrate, for the first time, the utility of NSP blood spots for assessment of temporal trends in exposure.

Introduction

Temporal human biomonitoring studies can provide a means to track trends in exposure to environmental chemicals for the broader population. This capability can help set priorities for exposure research and toxicity evaluation, inform government and industry decision makers about emerging exposures and the efficacy of mitigation efforts, and provide data that can be integrated with health surveillance data. Among the challenges faced by biomonitoring studies of the broader population are the difficulty and expense associated with collection of human specimens. For characterization of inherent variability in contaminant levels across diverse populations, large numbers of specimens must be collected to constitute an adequately representative sample. For detection of temporal trends in population exposures, the challenges of biomonitoring studies are compounded by the need for ongoing or repeating surveys with adequate temporal resolution.

Statewide population-based specimen collection programs have been in place in the U.S. for >40 years; newborn screening programs (NSPs) collect blood specimens from more than 98% of all newborn infants in the U.S. for the detection of biomarkers of congenital disorders (*1*). Whole blood collected by heel stick from an infant 1−2 days after birth is spotted on a standardized filter paper card. After collection, an infant's card is dried and sent to a state NSP laboratory, where a portion of one or more blood spots is removed for analysis. The New York State (NYS) NSP receives dried blood spots from approximately 250 000 newborns annually and began archiving unused residual blood spots from all newborns in 1997, for health-related follow-up testing and public health research.

Whole-blood concentrations of chemical blood constituents can be estimated from analysis of a dried blood spot, because a disk (or "punch") removed from the center of a dried blood spot on standardized filter paper represents a volumetric measurement of blood similar to a liquid measure (*2*). The evaluation of dried blood spots for exposure measurement has historical precedent. Burse et al. (*3*) demonstrated that dichlorodiphenyl dichloroethylene (DDE) was present in 10 individual NSP blood spots from the state of Texas. Dua et al. (*4*) measured organochlorine pesticides in blood spots collected from adult pesticide applicators in India. The usefulness of dried blood spots for assessment of children's lead exposure has been debated (*5*). Recently, as biomonitoring has become increasingly established as a public health tool, the use of blood spots for exposure assessment has received renewed attention (*6*).

Perfluorinated compounds (PFCs) are chemicals for which there is considerable interest in potential temporal exposure changes for the general population. PFCs have been used in a variety of industrial and consumer products, including surfactants, insecticides, and protective coatings for paper, textiles, carpets, and apparel. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and related PFCs have been found in human blood (*7, 8*) and higher-trophic-level wildlife (*9*).

The toxicity of PFCs is not well characterized, but studies of laboratory animals exposed to PFOS and PFOA suggest liver effects, developmental toxicity, and cancer (*10*). Recently, inverse associations were reported between cord blood concentrations of PFOA and birth weight (*11*), and PFOS and PFOA and birth weight and size (*12*).

In 2000, the primary manufacturer of PFOS-related compounds, 3M, announced its intention to voluntarily phase out production by the end of 2002 (*13*). 3 M eliminated its electrochemical fluorination manufacture of the ammonium salt of PFOA at the same time, and production commenced by another company using a process with lower environmental releases (*14*). The efficacy of efforts to mitigate environmental releases and human exposures (e.g., through

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reduced chemical production, and changes in manufacturing practices) can be evaluated through temporal human biomonitoring. Human biological half-lives for PFOS, PFOA, and perfluorohexane sulfonate (PFHxS) have been estimated at 4.8, 3.5, and 7.3 years, respectively (*15*), and recent data suggest that levels of some PFCs in the general population may have begun to decrease (*16, 17*).

PFOS, perfluorooctane sulfonamide (PFOSA), PFOA, and perfluorononanoic acid (PFNA) can cross the human placenta (*18, 11, 19–21*). Resistance to degradation and low vapor pressures of the target PFCs suggested that they would be stable in archived NSP dried blood spots. The current study quantified PFOS, PFOSA, perfluorohexane sulfonate (PFHxS), PFOA, and PFNA in NYS NSP blood spots and evaluated the utility of archived spots for determination of temporal trends in population exposures.

Methods and Materials

Chemicals and Reagents. Potassium salts of PFOS (>95%), PFOSA (95%), PFHxS (99.9%), perfluorobutane sulfonate (PFBS) (99%), and PFOA (98%) were provided by the 3M Company (St. Paul, MN). PFNA (>95%) was obtained from Fluorochem Ltd. (Derbyshire, UK), and ^{13}C4-PFOS and ^{13}C4-PFOA (both >98%) from Wellington Laboratories (Guelph, ON, Canada). Organic solvents and reagents were HPLC grade and ACS grade, respectively (J. T. Baker, Phillipsburg, NJ).

Blood Spot Specimens. After analysis for congenital disease biomarkers by the NYS NSP, blood spot cards with unused residual blood spots were stored at 4 °C under desiccating conditions, indexed by date of receipt (generally, a few days after birth), in polyethylene bags. We selected cards from 11 dates spanning the full temporal range of the NYS NSP archive (1997 to 2007). For evaluation of seasonal variations in exposures, potentially resulting from usage patterns for PFC-containing waterproofing treatments and similar products, dates were chosen to represent winter (December 31, ±9 days; 7 dates) or summer (i.e., June 30, ±4 days; 4 dates). NSP cards from 240 individual infants were selected from approximately 1000 cards bundled together for each selected date; efforts were made to choose cards from throughout the bundle (i.e., every fourth card). Although cards had unique identifier numbers, the numbers were not recorded. Ten composite specimens of 24 1/4-in. (~6.0 mm) diameter NSP dried blood spot punches were collected in 15-mL polypropylene (PP) tubes for each of the 11 sample dates, representing a total of 2640 newborn infants.

To characterize the background levels of PFCs in the stock NSP filter paper, we created method blanks from 24 punches of the grade 903 specimen collection paper used by the NYS NSP (Schleicher & Schuell BioScience. Inc., Keene, NH). For each of the 11 dates of blood spots sampled, one NSP archive date-specific "field blank" was prepared as a composite of 24 1/4-in. punches from the unspotted portions of 24 of the 240 blood spot cards selected for that date. This study was approved by the NYS Department of Health Institutional Review Board for Protection of Human Subjects.

Extraction and Analysis. NSP blood spot composite specimens were desorbed in 3 mL of Milli-Q water, sonicated for 60 min, and allowed to sit overnight at 4 °C. Specimens were then sonicated for an additional 60 min, fortified with PFBS, ^{13}C4-PFOS, and ^{13}C4-PFOA as internal standards (^{13}C4-PFOS and ^{13}C4-PFOA were not available for the first 60 specimens), and extracted by an ion-pair extraction method previously described (*22*). In brief, specimens were extracted with 5 mL of methyl *tert*-butyl ether (MTBE), after the addition of 1 mL of 0.5 M tetrabutyl ammonium hydrogen sulfate solution (adjusted to pH 10) and 2 mL of 0.25 M sodium carbonate/sodium bicarbonate buffer. The MTBE layer was separated by centrifugation at 4000 rpm for 4 min, quantitatively transferred into another PP tube, evaporated to dryness under a gentle stream of nitrogen, and reconstituted with 0.5 mL of methanol. The extract was vortexed and transferred into an autosampler vial for analysis.

Analytes were detected and quantified with an Agilent 1100 series high-performance liquid chromatograph (HPLC) coupled with an Applied Biosystems API 2000 electrospray triple-quadrupole mass spectrometer (ESI-MS/MS). Extracts (10 μL) were injected onto a 100 × 2.1 mm (5 μm; Thermo Electron Corporation, Bellefonte, PA) Betasil C18 column, with a 50 × 2.1 mm Betasil C18 precolumn. The mobile phase was 2 mM ammonium acetate/methanol (flow rate of 300 μL/min) starting at 10% methanol, increasing to 100% methanol at 10 min, holding for 3 min, before decreasing to 10% methanol. Target compounds were measured by multiple reaction monitoring. The mass transitions were set at 499→99 for PFOS, 503→99 for ^{13}C-PFOS, 498→78 for PFOSA, 399→80 for PFHxS, 299→80 for PFBS, 413→369 for PFOA, 417→372 for ^{13}C-PFOA, and 463→219 for PFNA.

Methods for sample preparation and analysis were validated with freshly drawn blood from adult volunteers (as approved by the NYS DOH Institutional Review Board for the Protection of Human Subjects) and newborn calf serum (NBCS) spotted on standard NSP filter paper. Method detection limits (LODs) (expressed as ng/mL on a whole-blood basis), were calculated as 3 standard deviations of matrix blanks (24 punches from spots of NBCS). No substitutions were made for NSP blood spot specimen concentrations below the LOD. Native spike recoveries in blood spots were determined by measurement of PFCs in a composite of four 75-μL spots of spiked adult blood (300 μL, total blood volume), and 300 μL specimens of unspotted, unspiked blood. Method precision was determined through analysis of ten 24-punch specimens made from adult blood spots.

Statistical Analysis. All statistical analyses were carried out with SAS v. 9.1 (SAS Institute Inc., Cary, NC). Differences in mean concentrations by date, over the temporal range of the archive, were assessed by one-way analysis of variance (ANOVA). Differences in PFC concentrations between two time intervals were evaluated with a two-sample *t* test. Half-lives corresponding to decreasing PFC concentrations ("disappearance half-lives") were calculated from the regression slopes of natural log-transformed mean blood PFC concentrations for each date versus years since 2000. To evaluate the effect of season of birth on PFC levels, we introduced a dichotomous variable representing winter and summer, along with years since 2000 as a continuous variable, into a multivariable linear regression model to predict natural log concentration for each PFC.

Results and Discussion

Initial method development and validation work with adult blood indicated that target analyte recoveries in dried blood spots were similar to those for liquid whole blood. Analyte recoveries for adult blood spots averaged 60%, 82%, 73%, 112%, and 72% for PFOS, PFOSA, PFHxS, PFOA, and PFNA, respectively (see Supporting Information). These recoveries suggest that concentrations in NSP blood spot punch composite specimens may be biased low for some PFCs (e.g., PFOS). Each 1/4-in. NSP punch was estimated to be equivalent to 13.4 μL of whole blood, based on a typical infant hematocrit level and NYS NSP spot size, and previous blood spot volume assessments (*23*). Internal standard recoveries for NSP specimens averaged 103%, 86%, and 85% for ^{13}C-PFOS, PFBS, and ^{13}C-PFOA, respectively; measured PFC concentrations in NSP composite specimens were not adjusted for these recoveries. Method precision was good, with relative standard deviations (RSDs) for 10 extractions of 24-punch adult blood spot specimens <10% for PFOS, PFHxS, and PFNA, and 21% and 26% for PFOSA and PFOA,

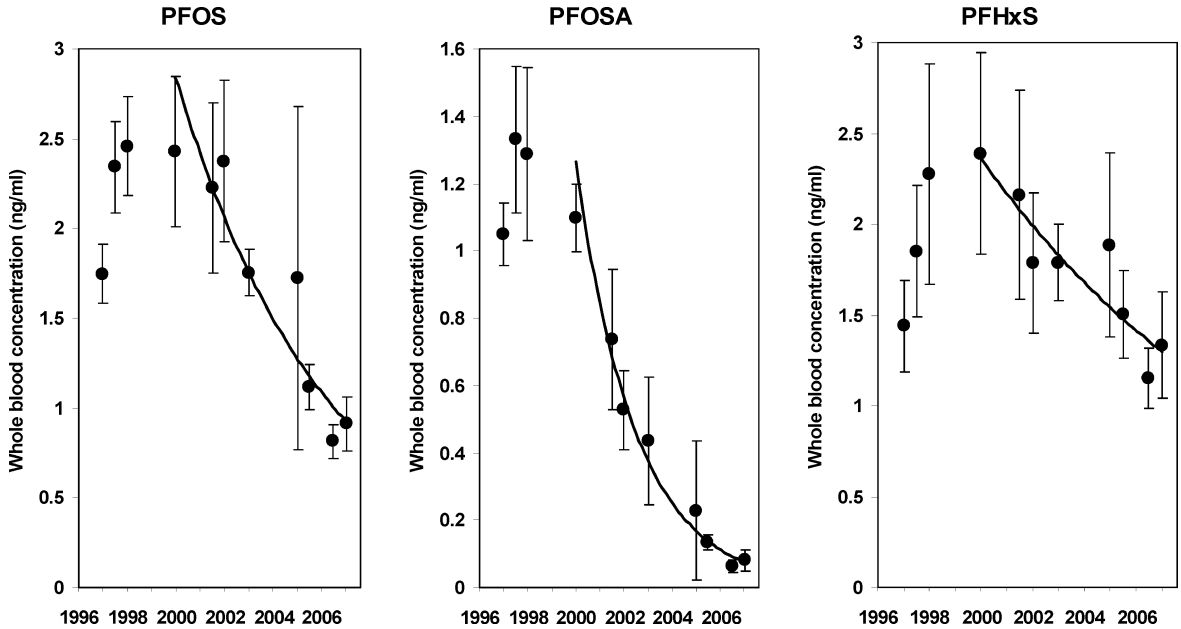

FIGURE 1. Temporal trends in mean PFOS, PFOSA, and PFHxS blood concentrations for ten NSP blood spot punch composite specimens, collected for each of 11 time points between years 1997 and 2007. Error bars represent 95% confidence intervals. Concentrations after year 2000 fit with curves of exponential decrease.

respectively. LODs, expressed in units of whole blood equivalents, were 0.2 ng/mL for PFOS, PFHxS, and PFNA, 0.04 ng/mL for PFOSA, and 0.4 ng/mL for PFOA.

PFC levels in reagent blanks indicated some analytical background for PFOA and less so for PFNA. PFC levels in method blanks extracted with each batch of NSP specimens were similar to reagent-blank levels, demonstrating that the standard filter paper contained negligible levels of PFCs. Field blanks exhibited PFC levels that were only marginally higher than method blank levels, suggesting limited contamination from transportation, processing, and storage of cards. Date-specific field blank levels were subtracted from all measured NSP specimen concentrations prior to data analysis and presentation. After blank subtraction, concentrations of PFOS, PFOSA, PFHxS, PFOA, and PFNA were above the LOD in 100%, 98%, 100%, 90%, and 90%, respectively, of the NSP blood spot punch composite specimens. For those analytes with relatively higher analytical background (PFOA and PFNA), precision for blank-subtracted measurements may be lower than reported overall method precision.

Mean whole-blood concentrations for NSP specimens for each date ranged from 0.81 to 2.41 ng/mL for PFOS, 0.06 to 1.41 ng/mL for PFOSA, 1.22 to 2.46 ng/mL for PFHxS, 0.34 to 1.41 ng/mL for PFOA, and 0.27 to 0.51 ng/mL for PFNA (see Supporting Information). No previous studies reporting the levels of PFCs in dried blood spots or capillary whole blood from newborn infants are available for direct comparison of our results. The majority of biomonitoring data reported for PFCs thus far has been for adult serum or plasma. With the exception of PFOSA, measured infant whole blood levels reported here are lower than contemporaneous serum levels reported in the literature for U.S. adults (*24*).

PFOS, PFOSA, PFOA, and PFNA have been measured in cord blood serum or plasma (*18, 11, 19–21*). A study of 293 cord blood serum specimens collected between 2004 and 2005 reported geometric mean levels for PFOS, PFOSA, and PFOA of 4.9, <0.05, and 1.6 ng/mL, respectively (*18*). Geometric mean concentrations of PFOS, PFOSA, and PFOA in females (≥12 years old) reported for the 2003−2004 National Health and Nutrition Examination Survey (NHANES) were 18.4, <0.2, and 3.5 ng/mL, respectively. Matched maternal and cord blood serum results suggest that levels of PFOS in newborn infants may be about one-third (*19, 11*) to one-half (*20*) of the levels in their mother's blood. Results for PFOA varied considerably, with levels in cord blood serum from about 40% lower (*11*) to 30% higher (*20*) than matched maternal blood serum. Comparisons for PFOSA are more difficult, as no matched maternal/cord blood concentrations values have been reported. Twenty six percent of cord blood specimens reported by Apelberg et al. (*18*) had levels of PFOSA at ≥0.05 ng/mL, and the 90th percentile for females in 2003−2004 NHANES was 0.2 ng/mL.

In addition to compound-specific placental transfer efficiencies, differential distribution to blood constituents needs to be considered, in order to compare infant whole-blood concentrations with adult serum concentrations. Adult plasma or serum concentrations for PFOS, PFHxS, PFOA, and PFNA are greater than or equal to matched adult whole-blood concentrations (*25, 26*). In contrast, PFOSA serum levels were found to be less than 20% of whole blood levels (*26*). At the average adult hematocrit level of 43.5% (*28*), these results suggest a 10:1 preferential accumulation of PFOSA in the cellular blood volume. Since the cellular blood volume in capillary blood of infants aged 1−2 days (mean hematocrit 62% (*29*)) is 40% higher than the cellular blood volume of adults, serum levels of PFOSA in infants may be only 15% of whole blood levels. Considering the ratios reported by Kärrman et al. (*27*) and the difference between hematocrit levels in adults and newborn infants, NYS infant serum levels of PFCs can be estimated. Serum concentrations estimated for NSP specimens from 12/29/2004, i.e., 2.2 ng/mL for PFOS, 0.04 ng/mL for PFOSA, and 1.2 ng/mL for PFOA, are similar to contemporaneous mean U.S. concentrations for cord serum collected between 2004 and 2005 (*18*) of 4.9 ng/mL for PFOS, <0.05 ng/mL for PFOSA, and 1.6 ng/mL for PFOA.

Mean whole-blood concentrations for NSP blood spots differed significantly ($p < 0.0001$) by date for all target analytes, except PFNA ($p = 0.144$), allowing inferences to be made about temporal change. Over the temporal range of the archived NSP specimens, all PFCs except PFNA appeared to exhibit concentration maxima between 1998 and 2001 (Figures 1 and 2). Concentrations in blood spots from three dates between 12/30/1997 and 6/29/2001 ($n = 30$), approximately corresponding to these maxima, were signifi-

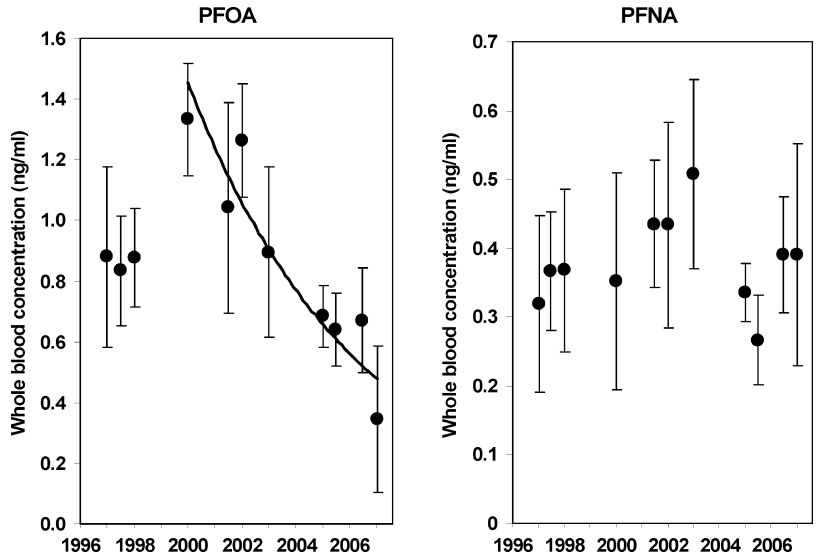

FIGURE 2. Temporal trends in mean PFOA and PFNA blood concentrations for ten NSP blood spot punch composite specimens collected for each of 11 time points between years 1997 and 2007. Error bars represent 95% confidence intervals. PFOA concentrations after year 2000 fit with curve of exponential decrease. Note that no significant decrease was observed for PFNA. One extreme outlier (>9 × SD above mean for all dates (n = 110)) for PFNA (6.14 ng/mL) was excluded. Including the outlier, the mean (95% CI) for 12/30/2002 was 1.07 (−0.21−2.35) ng/mL.

TABLE 1. Disappearance Half-Lives Based on Regression on Natural Log-Transformed Mean NSP Blood Spot Specimen Concentration and Years Since 2000 (eight specimen dates)

compound	r^2	p	disappearance half-life in years (95% CI)
PFOS	0.86	0.0008	4.1 (3.1−7.2)
PFOSA	0.96	<0.0001	1.7 (1.4−2.1)
PFHxS	0.81	0.0027	8.2 (5.4−16.2)
PFOA	0.82	0.0019	4.4 (3.0−7.9)

cantly higher (p < 0.0001) than concentrations in spots from the most recent three sampled dates, between 6/27/2005 and 1/8/2007 (n = 30), for all target PFCs except PFNA (p = 0.411) (see Supporting Information). The temporal patterns of concentrations of PFOS, PFOSA, PFHxS, and PFOA are suggestive of minor increases in concentration preceding the maximum concentrations, though the limited duration of the observed increase precludes inferences regarding longer term trends.

PFOSA levels exhibited a sharp and steady decline, starting between 12/30/1997 and 12/29/1999 and continuing into 2007, that approximated an exponential decrease (Figure 1). The temporal trends in concentrations of PFOS, PFHxS, and PFOA were also consistent with exponential decrease. Linear regression on natural log-transformed mean concentrations between 12/29/1999 and 1/8/2007 indicated that these exponential trends were significant (p < 0.0001) for PFOS, PFOSA, PFHxS, and PFOA. In contrast, PFNA appeared to exhibit no temporal trend in concentration, and a linear regression model of natural log-transformed concentrations and years since 2000 was not significant (p = 0.457). Season did not appear to influence PFC concentrations, as the dichotomous variable for winter or summer was not significant ($p \geq 0.280$) in any multivariable linear regression models.

Regression slopes for natural log concentration versus years since 2000 allowed the calculation of disappearance half-lives for PFOS, PFOSA, PFHxS, and PFOA (Table 1). The disappearance half-life was shortest for PFOSA at 1.7 years (95% confidence interval (CI), 1.4−2.1). Half-lives for PFOS and PFOA were similar to one another, at 4.1 years (95% CI, 3.1−7.2), and 4.4 years (95% CI, 3.0−7.9), respectively. PFHxS showed a slower rate of decline over this period, with a half-life of 8.2 years (95% CI, 5.4−16.2).

Exponential decreases in contaminant concentrations in human populations have been previously observed when exposures have been significantly curtailed (*30*). When a population exposure is nearly eliminated, the levels of the contaminant in the population may decrease according to the function(s) that characterize(s) the contaminant's pharmacokinetic elimination from the human body. A study of retired fluorochemical workers found that the decrease in serum levels of PFOS, PFHxS, and PFOA could be approximated by a first-order, single-compartment model (*15*). Calculated arithmetic mean and geometric mean half-lives for serum elimination in workers were, respectively, 5.4 years and 4.8 years for PFOS, 8.5 years and 7.3 years for PFHxS, and 3.8 years and 3.5 years for PFOA. The disappearance half-lives reported in this study for PFOS, PFOA, and PFHxS bear a strong resemblance to serum elimination half-lives reported by Olsen et al. (*15*), suggesting that maternal and subsequent fetal exposures to these three compounds were sharply curtailed in NYS around the year 2000.

Several other studies have evaluated temporal patterns in human levels of PFCs. Olsen et al. (*31*) found no significant changes in levels in serum, of any of the PFCs included in the current study, collected from selected U.S. populations between 1989 and 2001. Kärrman et al. (*27*) reported that concentrations of PFOS and PFHxS in breast milk from Sweden remained relatively unchanged between 1996 and 2004, although slightly lower levels of PFOS and PFHxS were evident for 2002 through 2004. Other studies reported increasing serum levels of PFOA and/or PFOS from the 1980s through 1999 in Japan (*32*) and through 2002 in China (*33*). Recent studies in the U.S. have reported findings that suggest that human exposure to some PFCs is declining. A study comparing PFC levels in two groups of Minnesota Red Cross blood donors, the first sampled in 2000 and the second in 2005, found that geometric mean serum concentrations of PFOS and PFOA were lower in the second group by 54% and 51%, respectively (*16*). Geometric mean PFOS, PFHxS, and PFOA serum concentrations for NHANES were 32%, 10%, and 25% lower,

TABLE 2. Mean and Median PFC Concentrations for NSP Blood Spot Punch Composite Specimens Collected for 1999−2000 (n = 10) and 2003−2004 (n = 20); Observed Relative Differences (%) Compared with Those Reported Between NHANES 1999−2000 and NHANES 2003−2004

compound	NSP 1999−2000 mean (median) (n = 10) concentration (ng/mL)	NSP 2003−2004 mean (median) (n = 20) concentration (ng/mL)	NSP relative difference in mean (median) concentrations from 1999−2000 to 2003−2004 (%)	NHANES relative difference in geomean concentrations from 1999−2000 to 2003−2004 (%) [a]
PFOS	2.43 (2.29)	1.74 (1.59)	−29 (−31)	−32
PFOSA	1.10 (1.08)	0.33 (0.28)	−70 (−74)	−80
PFHxS	2.40 (2.47)	1.84 (1.64)	−23 (−34)	−10
PFOA	1.33 (1.36)	0.80 (0.73)	−40 (−46)	−25
PFNA	0.35 (0.27)	0.38 (0.35)[b]	+8 (+31)	+100

[a] NHANES relative difference based on geometric mean serum concentrations for PFOS, PFHxS, PFOA, and PFNA, and reported 90th percentile concentrations for PFOSA (*17, 24*). Geometric mean NHANES PFOSA levels were below the limits of detection. [b] One extreme outlier (>9 × SD above mean for all dates (n = 110)) for PFNA (6.14 ng/mL) was excluded; mean and median NSP relative differences between 1999−2000 and 2003−2004 including the outlier are 100% and 41%, respectively.

respectively, in 2003−2004 than in 1999−2000, although PFNA was 100% higher (*17*).

The temporal changes in blood spot PFC concentrations observed in our study are consistent with changes reported by others. Blood spot levels from 12/29/1999 (n = 10), the midpoint of NHANES 1999−2000, can be compared with blood spot levels from 12/30/2002 and 12/29/2004 (n = 20), dates that bracket NHANES 2003−2004. Relative changes over this time interval in mean blood spot concentrations for PFOS, PFHxS, PFOA, and PFNA (−29%, −23%, −40%, and +8%, respectively) are similar to changes reported for the U.S. population, aged ≥ 12 years (Table 2). Calafat et al. (*24*) did not report changes in PFOSA levels, because geometric mean concentrations were below the detection limits, but a change in 90th percentile concentrations (−80%) can be calculated from reported data (*17, 24*) that is similar to the change in mean PFOSA levels observed over that time interval in our study (−70%).

The apparent temporal peak in human population exposure coincides with the reported peak in levels of PFCs in wildlife (*34, 35*). Disappearance half-lives, observed in seals after 2000, for PFOS and PFOSA were 3.9 years (average of reported values) and 2.0 years, respectively (*35*), similar to those for NYS infants reported here. Decreasing biological levels of PFOS, PFOSA, and PFHxS may be the result of significant reductions in global production of perfluorooctanesulfonyl fluoride (POSF) and ammonium perfluorooctanoate (APFO) by electrochemical fluorination. Our results suggest that exposures were very sharply curtailed around 2000, 2 years prior to the completion of the phase-out electrochemical fluorination by 3M. Although the phase-out was not concluded until 2002, elimination was completed by the end of 2000 for 88% of 3M global POSF production, and 100% of the 3M global production of products for (i) paper and packaging (including all food packaging) and (ii) textile, leather, and carpet treatment (*36*). Reduced levels of POSF production continued on into 2002 but only for industrial products and fire fighting foams. It is possible that the products eliminated in 2000, which accounted for 81% of global POSF chemistry production, were those that presented the greatest opportunities for direct human exposure and release to the environment. Prevedouros et al. (*14*) estimated global emissions of PFO/APFO from all sources dropped by only about 40% in 2000, and production of APFO by fluorotelomer iodide oxidation commenced in late 2002. PFNA was never manufactured by the discontinued electrofluorination process (*24*), and as a result, production and environmental releases of this compound may not have declined after 2000.

Little is known about the dominant exposure pathways for PFCs. PFOS bioaccumulates, and therefore dietary intake of animal-based food products is a potential exposure pathway (*37*). Exposure to PFOA may result from treated food packaging materials (*38, 39*), and fluoropolymer-based consumer product treatments (*14*). The rapid decline in exposure indicated by biomonitoring results of this study and others suggests that predominant exposure media are those that have effectively equilibrated rapidly to reflect reduced production and environmental releases. Such media could include short-life-cycle consumer products (food packaging, temporary surface treatments) or rapidly mixing environmental media like air but are less likely to include most animal-product foods which would more slowly respond to changes in emissions.

While our study demonstrates the feasibility of PFC measurement in blood spot specimens, there are a number of limitations for use of blood spots for measurement of PFCs or other contaminants. First, the small volume of blood represented by a single blood spot punch poses analytical challenges for detection. Although pooling of blood spot punches can provide a means of overcoming those analytical challenges, much of the information about the underlying distribution of individual punch concentrations is lost. The exact blood spot punch volumes and therefore corresponding blood concentrations are not known. Serum volume contained in a punch was found to vary by as much as 47%, depending upon the level of whole-blood hematocrit, spot size, and location of punch within the spot (*2*). The stability of analytes under blood spot storage conditions is an important consideration for studies of environmental exposure (*6*), and the stability of PFCs under multiyear storage at 4 °C has not been investigated. It should be noted that if losses of PFCs had occurred in archived specimens over the years of storage, the oldest specimens would have experienced the greatest losses, and the actual rate of concentration decrease in infants since the year 2000 would be faster than that observed. While method validation work with adult whole blood spots demonstrated good recovery for native analyte spikes, these recoveries may not accurately represent recoveries for the spotted blood of newborns. Further investigation of blood spot punch volumes and PFC concentrations in neonate whole blood, serum, and blood spots is needed.

In spite of these limitations, pooled blood spots are a viable biological medium for retroactive exposure studies of PFCs. The PFCs evaluated in the current study are nonvolatile and relatively stable compounds (*40*) and have been found to be stable in whole blood stored under a variety of conditions (*26*). Blood spots collected in state-based NSPs

are collected on filter paper that is tested for absorptive uniformity, with quality assurance overseen by the Newborn Screening Quality Assurance Program (NSQAP) at the Centers for Disease Control and Prevention (CDC). The effect of punch-to-punch variability in blood volume across individuals due to variability in hematocrit, spot size, punch location, and other factors is effectively averaged out by pooling punches into composite specimens in our study and is reduced further by analysis of multiple composite specimens. Pooling punches also preserves subject confidentiality, obviating special handling and precautions necessary in most studies of individual specimens.

In summary, our study presents the first characterization of temporal trends in PFC concentrations in a human population over several points in time since production of these chemicals peaked around the year 2000. Significantly higher concentrations of PFOS, PFOSA, PFHxS, and PFOA were measured in blood spots collected between December, 1997 and June, 2001, compared to concentrations in blood spots collected between June 2005 and January 2007. The changes in concentration of these four PFCs from 2000 onward approximated exponential decreases. Calculated disappearance half-lives for PFOS, PFHxS, and PFOA were similar to biological half-lives reported for individual retired fluorochemical workers, suggesting a sharp decrease in population exposure to these chemicals for NYS infants and, by extension, their mothers.

The similarity of temporal changes observed in this study and those based on extensive sampling programs suggests that blood spots are useful for inference of broad trends in population exposures. Archived blood spots represent a unique opportunity to characterize perinatal chemical exposure in a large population at a relatively low cost, by capitalizing on an existing biological specimen collection program.

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Supporting Information Available

Calculation of blood spot punch volume, method validation results, mean NSP specimen concentration by date, and comparison of temporal maximum PFC concentrations to the most recent concentrations. This information is available free of charge via the Internet at http://pubs.acs.org.

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PROCESS FOR PRIORITIZING CHEMICALS FOR CONSIDERATION UNDER PROPOSITION 65 BY THE "STATE'S QUALIFIED EXPERTS"

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

December 2004

BACKGROUND

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code section 25249.5 *et seq*.) requires the Governor to publish a list of chemicals known to cause cancer or reproductive toxicity. One of the mechanisms by which a chemical is placed on this list is a finding by the "state's qualified experts" that a chemical "has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity" (Health and Safety Code section 25249.8(b)). As the lead agency for the implementation of Proposition 65, the Office of Environmental Health Hazard Assessment (OEHHA) has formed the Science Advisory Board (SAB), which includes two committees of independent scientists and health professionals that serve as the state's qualified experts. These committees are the Carcinogen Identification Committee (CIC) and the Developmental and Reproductive Toxicant Identification Committee (DART IC).

This document describes the process used by OEHHA staff to identify chemicals for evaluation by the CIC and DART IC. The process is designed to ensure that the efforts of these committees are focused on chemicals that may pose significant hazards to Californians. As with the previous process (OEHHA, 1997), this process includes multiple opportunities for public input.

The CIC, at its December 2002 meeting, asked OEHHA to develop this process as an alternative to the random prioritization process that had been in use since 1997. The CIC specifically asked for an alternative process that could better take into account the level of exposure in California, the population potentially affected by various chemicals being reviewed by OEHHA, as well as the degree and extent of potential harm posed by the chemical. The CIC also asked OEHHA to address the deficiencies in the existing process and the costs of an alternative process. Deficiencies noted in the existing process included the significant length of time needed to conduct prioritizations, the considerable staff resources expended, and the public health importance of chemicals reaching the committees for consideration. The Chair of the CIC requested that two of its members informally assist OEHHA in developing an alternative procedure.

Since the prioritization process also affects the work of the DART IC, after consultation with the Director of OEHHA, the Chair of the DART IC asked that members of the DART IC also be involved in developing an alternative prioritization process. An informal workgroup comprised of OEHHA staff and a few members of the DART IC and CIC assisted OEHHA in this effort. The process laid out in this document is the result of a two-year proceeding. The workgroup developed a draft prioritization process following input from the DART IC, CIC and the public. Two public comment periods and a public workshop were held and finally the prioritization process outlined here was discussed and endorsed by the CIC at their November 1, 2004 public meeting and the DART IC at their November 4, 2004 public meeting, following additional public comment.

This prioritization process replaces the existing one described in OEHHA's 1997 document, "Procedure for Prioritizing Candidate Chemicals for Consideration under Proposition 65 by the 'State's Qualified Experts'" and is posted on the OEHHA Web site.

The goals of this process is to more quickly and efficiently prioritize chemicals for development of hazard identification materials for subsequent CIC and DART IC review.

The prioritization process is based on a preliminary appraisal of the evidence of hazard for the purpose of identifying chemicals for possible hazard identification materials preparation and committee review. The cost in staff resources and time required to conduct the proposed process are not expected to exceed those of the previous process.

The prioritization process described here is the primary method by which a chemical can reach the CIC or DART IC for consideration. As has always been the case, the Director of OEHHA at his or her discretion may decide to abbreviate or modify the process. For example, a member of the public or a committee member may petition OEHHA to abbreviate the process to respond to new information or an emerging public health issue. Following consultation by the Director with the appropriate committee chair, a chemical may be placed on the agenda for discussion at the next scheduled committee meeting. In all such cases, OEHHA will post public notices of any such action in the *California Regulatory Notice Register* and on its Web site, with appropriate notice periods.

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In addition, a chemical may be referred to the CIC or DART IC by OEHHA when it is found not to meet the criteria for listing by the authoritative bodies mechanism subsequent to the issuance by OEHHA of a Notice of Intent to List as provided in regulation (Title 22, Cal. Code of Regs. §12306). Finally, as appropriate, chemicals will be brought to the relevant committees that are listed in Title 22, Cal. Code of Regs. § 14000 (Health and Safety Code section 25249.8(c)) because they are required by State or Federal law to be tested for carcinogenicity or reproductive toxicity, once the required testing has been completed. If the resulting tests on a chemical provide data with strong evidence of cancer or reproductive toxicity, the chemical will typically be brought to the relevant committee for consideration.

PRIORITIZATION PROCESS

The following lays out the steps that OEHHA uses in selecting chemicals for consideration by the CIC and DART IC. This prioritization process is conducted on a periodic basis, with no set interval, and it is rerun as needed. Figure 1 is a flow chart of the prioritization process.

- ***Tracking database.*** OEHHA maintains tracking databases of chemicals that have come to OEHHA's attention for DART or carcinogenicity evaluation. Chemicals may come to OEHHA's attention through literature searches or suggestions from the CIC or DART IC, other state organizations, the scientific community or the general public. A chemical may be grouped with other, similar chemicals at various stages in the prioritization process. For example, groupings may result from similarity in chemical structure, mechanistic considerations, or the production of the same or similar proximate active dissociation products or metabolites. Examples of chemical groupings that have been reviewed by the CIC or DART IC are: aflatoxins and polychlorinated biphenyls (similarity of chemical structure and mechanism), inorganic oxides of arsenic (same active dissociation product), alcoholic beverages (same set of proximate carcinogens), and radionuclides (similarity of mechanism and active agent).

- ***Candidate Chemicals.*** Chemicals entered into the tracking database are investigated for the existence of relevant toxicity data and the potential for human exposure. Those with data suggesting they cause reproductive toxicity or cancer and have exposure potential in California become candidate chemicals.

  The toxicity evaluation at this stage involves the identification of one or more studies suggesting cancer or reproductive effects in animals or humans. The evaluation of exposure potential in California is qualitative and does not involve prediction of levels of exposure. Production, use, or monitoring data provide qualitative evidence of exposure potential. In the absence of information specific to California, data on production, use and environmental levels at the national level is generally assumed to reflect that in California. Examples of evaluations of exposure potential in California are given in OEHHA (2004).

- ***Proposed Chemicals for Committee Consideration.*** Candidate chemicals are screened using a focused literature review. All candidate chemicals initially undergo an epidemiology data screen. This involves the identification of those chemicals with epidemiological evidence suggesting they cause cancer or reproductive toxicity. The type of literature review screen will change over time. The literature review is typically based upon original research articles, literature compilations, or reviews. A chemical that does not pass this screen remains a candidate chemical, and will be reevaluated using future screens based upon other relevant criteria such as evidence from animal studies at a later time. This and future screens will be applied to all candidate chemicals. In

conducting the initial epidemiology screen, if OEHHA becomes aware of a chemical with very strong evidence from animal studies, that does not meet the epidemiology screen, but nevertheless poses a potentially significant hazard, that chemical will be proposed for committee consideration as well.

Chemicals selected by the screen undergo preliminary toxicological evaluation to determine whether they should be proposed for committee consideration for possible preparation of hazard identification materials. At this stage of the prioritization process, the overall evidence of carcinogenicity or reproductive toxicity of the chemical is considered, including epidemiologic, animal bioassay, and other relevant information (e.g., on pharmacokinetics, chemical structure, maternal toxicity, genotoxicity), as appropriate. This preliminary overall evaluation is typically based on original research articles, and literature compilations or reviews. Both positive and negative studies will be considered.

Factors considered in weighing the epidemiological evidence include the type of epidemiological study (e.g., case-control), study population, exposure situation, endpoint (e.g., tumor type, developmental effect), dose-response, possible roles of bias and confounding, and overall study quality. Greater weight will be given to analytical studies, and less weight to descriptive studies and case reports. Factors considered in weighing evidence from animal studies include the number of experiments, species tested, routes of administration, frequency and duration of exposure, numbers of test animals, and dose-response. Other relevant data such as from genotoxicity, pharmacokinetic and mechanistic studies, and maternal toxicity will also be considered in weighing the evidence. In accordance with guidelines of the U.S. Environmental Protection Agency (1991, 1996), adverse developmental effects that co-occur with maternal toxicity, and reproductive effects that co-occur with systemic toxicity are considered evidence of reproductive toxicity unless these toxicities are severe enough to preclude interpretation of the study. In animal data evaluations, effects are assumed to be relevant to humans, unless OEHHA determines there is sufficient evidence to the contrary.

It is unlikely that chemicals will be proposed for CIC or DART IC review that have been recently reviewed by an authoritative body and found to have insufficient evidence of carcinogenicity or reproductive toxicity, respectively. Exceptions to this generalization may occur, for example, if an authoritative body has evaluated a chemical but failed to review all relevant data, or compelling new data have become available since the evaluation. Also, a chemical may be taken to the CIC or DART IC if an authoritative body finds adequate evidence of carcinogenicity or reproductive toxicity but the evidence or formal identification does not meet the criteria for listing in regulation (Title 22, section 12306).

Public comment and submission of chemical list to the relevant committee. The list of chemicals proposed by OEHHA for CIC or DART IC consideration for potential preparation hazard identification materials is released to the public for comment, along

with the rationale for the selection. A notice identifying OEHHA's list of chemicals proposed for potential preparation of hazard identification materials is published in the *California Regulatory Notice Register* and posted on OEHHA's Web site. This begins a 60-day public comment period. The public may then comment on the scientific evidence pertaining to the selection of the chemical for prioritization. OEHHA then compiles public comments and sends them to the relevant committee for review, along with the list of chemicals proposed for potential preparation of hazard identification materials and related rationale.

Appraisals of the evidence to support a proposal for potential preparation of hazard identification materials for a given chemical for review by the CIC or DART IC is qualitative. This initial evaluation by its nature is abbreviated and is not as intensive or thorough as a hazard evaluation. It is simply a preliminary appraisal for the purpose of identifying chemicals for further evaluation, preparation of hazard identification materials and potential committee review. The in-depth review of toxicological data would occur at the later stage, when hazard identification materials are developed.

- ***Committee Consultation on Chemicals for Review.*** During the CIC and DART IC meetings, OEHHA will receive advice and consultation from the committees on the list of chemicals proposed for hazard identification materials preparation and eventual committee consideration. That is, the committee advises OEHHA on the chemicals that should undergo the development of hazard identification materials, committee review and eventual listing decision. The CIC advises OEHHA concerning chemicals for carcinogenicity hazard identification, and the DART IC advises OEHHA concerning chemicals for reproductive toxicity hazard identification. The committees may also suggest other chemicals that should undergo hazard identification materials preparation. At the committee meeting, the public is given the opportunity to comment on chemicals being proposed for hazard identification materials preparation and eventual committee consideration. The committees can vote on recommendations or provide less formal advice to OEHHA concerning which chemicals should be brought back for their consideration following preparation of hazard identification materials.

- ***OEHHA Selection of Chemicals for Preparation of Hazard Identification Materials.*** OEHHA selects the chemicals for the development of hazard identification materials. After receipt of committee advice and public comment, OEHHA will select those chemicals that appear to have evidence of reproductive toxicity or carcinogenicity sufficiently strong to warrant the development of hazard identification materials and subsequent CIC or DART IC review for possible listing.

The prioritization process ends with the selection of chemicals by OEHHA for the development of hazard identification materials. The next steps in the process, described below, are those of hazard identification for the purposes of Proposition 65.

- ***Data Call-In.*** OEHHA solicits information on the evidence for carcinogenicity or reproductive toxicity on chemicals selected for review. A "data call-in" notice published

in the *California Regulatory Notice Register* and posted on OEHHA's Web site requests information relevant to the preparation of hazard identification materials on the chemicals selected for review.

- ***Hazard Identification Materials on Chemicals for Committee Review.*** Hazard identification materials are prepared for CIC or DART IC consideration and released to the public for comment. OEHHA decides the order in which these materials are prepared based on committee advice, staff resources, and public health considerations. The public is invited to comment on the hazard identification materials during a 60-day public comment period. Approximately two weeks before the public meeting of the respective committee, the public comments are collated and sent to the committee for consideration along with the hazard identification materials developed by OEHHA.

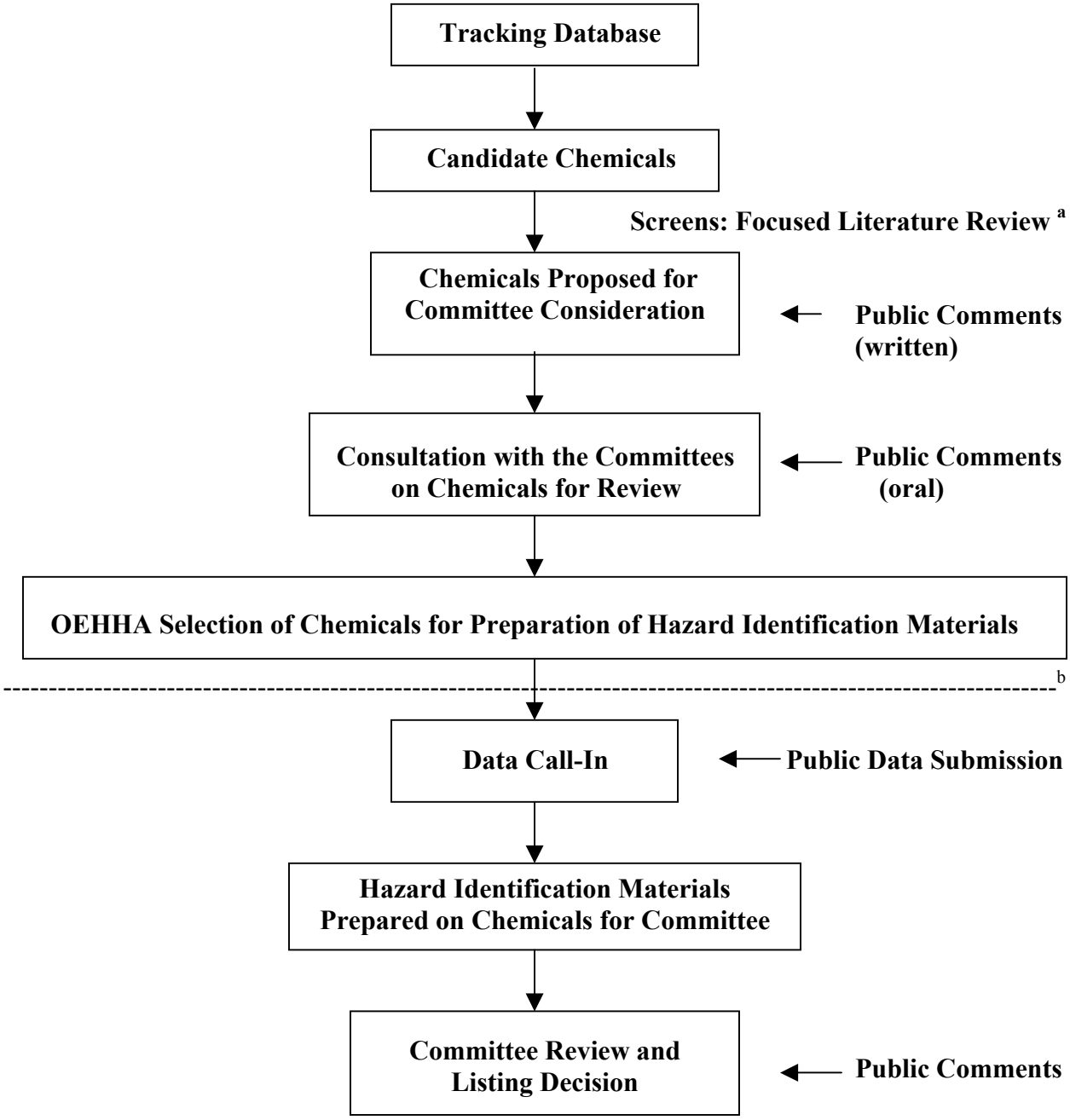
- ***Committee Review and Decision on Listing.*** The CIC or DART IC holds a public meeting to deliberate on whether the chemical has been clearly shown to cause cancer or reproductive toxicity. The hazard identification materials and the public comments received during the 60-day comment period are considered at the meeting. The public has a further opportunity to comment at the meeting. At the conclusion of the deliberations, the committee generally will render an opinion as to the developmental or reproductive toxicity or carcinogenicity of a chemical, as appropriate. In considering groups of chemicals, the committee may make findings for individual members of the group, or the group as a whole (e.g., arsenic [inorganic oxides]).

LEGAL CONSIDERATIONS

The prioritization process is, and has always been, intended to be used by OEHHA as a general process for prioritization of chemicals for committee consideration. The Director may abbreviate or otherwise modify the process. For example, the public or a committee member may petition the Director to abbreviate the prioritization process to respond to new information or an emerging public health issue, and the chemical may consequently be placed on the agenda of an upcoming committee meeting for discussion. In all such cases, OEHHA will post public notices of any such action in the *California Regulatory Notice Register* and on its Web site, and provide appropriate notice and comment periods. The prioritization process does not now have, nor has it ever had, the force of a regulation. Based upon Health and Safety Code section 25249.8(e), the development and implementation of the prioritization process is not subject to the provisions of the Administrative Procedure Act.

This prioritization process will not generally be applied to chemicals contained only in prescription or over-the-counter medications with mandatory cancer or reproductive toxicity warnings approved by the federal Food and Drug Administration, based on the California Supreme Court decision in *Paul Dowhal v Smith-Kline Beecham Consumer Healthcare et al.* (2004) 12 Cal. Rptr. 3d 262; 4 Cal. Daily Op. Serv. 3259, 2004 Daily Journal D.A.R. 4601.

Figure 1. Prioritization Process



[a] First screen based on epidemiological evidence; subsequent screens may be based on animal evidence.

[b] Dotted line indicates where the prioritization process ends and hazard identification process begins.

REFERENCES

Office of Environmental Health Hazard Assessment (OEHHA, 1997). Procedure for Prioritizing Candidate Chemicals for Consideration under Proposition 65 by the "State's Qualified Experts." OEHHA, Reproductive and Cancer Hazard Assessment Section, Sacramento.

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