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# Carcinogenic risk of emerging persistent organic pollutant perfluorooctane sulfonate (PFOS): A proposal of classification

Ricardo Arrieta-Cortes [a, *], Paulina Farias [b], Carlos Hoyo-Vadillo [a, c], Mina Kleiche-Dray [a, d]

[a] Science, Technology and Society, Cinvestav-IPN, Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, C.P. 07360, Mexico City, Mexico
[b] Centro de Investigación en Salud Poblacional, INSP, Universidad No. 655, Colonia Santa María Ahuacatitlán, Cerrada Los Pinos y Caminera, Cuernavaca, Morelos, 62100, Mexico
[c] Pharmacology Department, Cinvestav-IPN, Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, C.P. 07360, Mexico City, Mexico
[d] CEPED (IRD - Université Paris V Descartes), 19 rue Jacob, Paris, 75006, France

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ABSTRACT

Perfluoroalkyls are stable synthetic chemicals, able to repel oils, fats and water. These compounds have been used in the manufacturing of products such as Teflon®, lubricants, paints, fire-fighting foams, coatings for pans, carpets, clothes, and paperboard for packaging, among others. It is believed that populations are exposed constantly to them. Its regulation in the world is under development and several controversies are in the course of litigation. One occupational study shows bladder cancer risk. This paper intends to review scientific information on the most critical perfluoroalkyl compound and proposes a procedure to get a cancer-risk categorization which PFOS can cause to populations. Methods: As a guiding axis, we used the IARC process for developing monographs of carcinogenic risks. We used the SIGN guides for evaluating the quality of studies in human populations; and finally, we used the Squire method for evaluating studies in laboratory animals. Inadequate evidence of carcinogenicity was found in human studies mainly due to chance, threshold effect and confounders. In experimental animal studies, inadequate evidence of carcinogenicity was found in view of the number of affected species, different types of neoplasms, dose-response relationship and genotoxicity found in *in-vivo* and *in-vitro* studies. In this proposal, we concluded that cancer risk for PFOS, according to the IARC method, is not classifiable as carcinogenic to humans (group 3).

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## 1. Introduction

Perfluorinated compounds (PFCs) are synthetic chemicals that possess unique properties, such as high stability and extremely low surface tension. Many PFCs are insoluble in water and organic solvents, and can repel dust, water and oils (Jensen and Leffers, 2008). According to a study by the Organization for Economic Co-operation and Development (OECD), around 850 PFCs are known, including, perfluorooctane sulfonic acid or perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) (Fig. 1), which are considered the most important due to their high health-risk potential (Schulte, 2006), and especially due to their widespread use as Teflon®.

The PFCs molecules consist of a hydrophobic/lipophilic carbonated chain and a hydrophilic functional group. The hydrogen atoms of the carbonated chain are completely replaced by fluorine atoms. The 2-p orbital of fluorine is larger than the 1s orbital of hydrogen (Fig. 1), resulting in a decrease of surface tension properties, so these PFCs can repel dust, water, oils and fats, and also have a high chemical, thermal, biological and UV rays stability (Hansen et al., 2001; Arsenault et al., 2004). Because of these unique properties, the PFCs are used in different industrial processes and products such as refrigerating agents, fire-fighting foams, hydraulic fluids for the aviation industry, leather products, metal plating, for food packaging, floor polishes, coatings and additives for carpets and fabrics, and in the photographic and photolithography industry (Paul et al., 2009).

In general, it is considered that average global levels are around 20–30 ng PFOS/mL in blood samples (Fig. 2), and the levels of PFOA and other perfluoroalkyl carboxylic acids are below this range (Jensen and Leffers, 2008; Kannan et al., 2002 and Martin et al.,

* Corresponding author.
*E-mail addresses:* rarrieta@cinvestav.mx (R. Arrieta-Cortes), paulina.farias@insp.mx (P. Farias), citocromo@cinvestav.mx (C. Hoyo-Vadillo), Mina.Kleiche@ird.fr (M. Kleiche-Dray).

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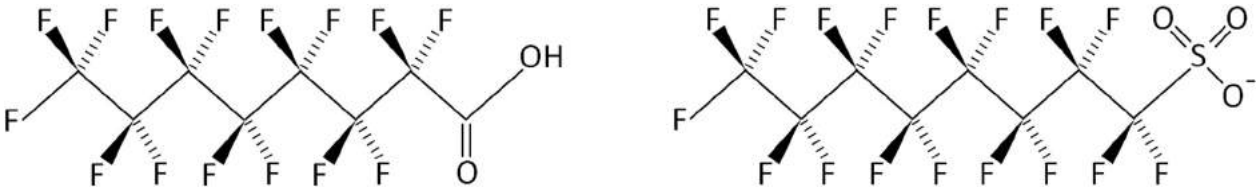

**Fig. 1.** Formulae of PFOA (left) and PFOS (right).

2010). Also, the environmental stability, persistence, bioaccumulative tendency and lack of biodegradation of PFOS and its related products are considered to have led to increased concerns over their body burden in humans and wildlife (Renner, 2001; Deon and Mabury, 2007; Lau et al., 2007).

Some studies suggest multiple toxicities correlated with PFOS, such as immunotoxicity, hepatotoxicity, carcinogenicity, and developmental and reproductive effects (Lau et al., 2007; Wang et al., 2010, 2012). Epidemiologic studies have shown an association between exposure to PFOS and the incidence of bladder cancer (Alexander and Olsen, 2007). In May 2009, PFOS and related compounds were listed in Annex B of the Stockholm Convention as Persistent Organic Compounds (POPs) candidates (Martin et al., 2010; Stockholm Convention, 2011; 2012).

The risk of exposure to PFOS was estimated by examining the probability of exceeding points of departure, or toxicity reference values. This is a function of PFOS concentration in human blood samples (Yeung et al., 2006). Protective values, the benchmark internal concentrations (BMICs), for risk characterization are as follows: immunotoxicity of 1.3 ng/mL (Grandjean and Budtz-Jørgensen, 2013), 33 μg/mL weight gain during lactation (3M Company, 2003), 44 μg/mL for liver toxicity (3M Company, 2003) and 62 μg/mL for liver tumor formation (Seacat et al., 2003). Concentrations of PFOS in 95% of the U.S. population were less than 100 ng/mL in blood serum (Olsen et al., 2003). In 85% of the Chinese population, concentrations were less than 100 ng/mL but in the 95th percentile, PFOS concentration could increase to 146 ng/mL (Yeung et al., 2006). These margins of exposure suggest that PFOS posed little or no intermediate risk to the population except for immunotoxicity risk (1.3 ng/mL) that even for the average global levels (around 20–30 ng PFOS/mL) this burden seems to be easily achieved.

Regarding studies performed in animals, there only exists one chronic test, which was carried out with Sprague-Dawley rats (Butenhoff et al., 2012), but according to Chang et al. (2014), the association seen between thyroid follicular cell adenoma and PFOS exposition should be considered a spurious finding in light of the absence of any response in the corresponding highly exposed group. So, in this case it is proposed to review some other animal studies exposed to PFOS, which even though subchronic, use an appropriate method of evaluation.

Respecting occupational and environmental studies performed in humans, Chang et al. (2014) again performed a critical review of four studies of PFOS in occupationally exposed workers and six studies in environmentally exposed communities. In these, the authors observed weak, inconsistent offset by negative associations, not in keeping with a positive exposure-response gradient and not coherent with the toxicological findings (Chang et al., 2014). But in this document, there is no mention of neither the flaws or defects present in those studies nor if there was a possibility to find a different conclusion if the study could have overcome those problems. This document proposes using a proper method to perform critical appraisal of cohort and case-control studies.

The present research paper aims to use different methods to assess and properly classify the cancer risk of PFOS, using as a


**Fig. 2.** PFOS in human blood reported by several countries (ng/mL). Source: Jensen and Leffers, 2008; Kannan et al., 2002 and Martin et al., 2010.

guiding axis the guidelines of "The IARC Monographs on the Evaluation of Carcinogenic Risk to Humans" from the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO), which still has not considered carrying out the corresponding monograph for this agent (IARC, 2008; IARC, 2014), as a way of settling existing controversies on this topic.

## 2. Methods

Herein, we propose following the directives of the program for preparation of the IARC monographs which claim to be the first step in developing the evaluation of carcinogenic risks. The document mentions that a cancer "hazard" is an agent able to cause cancer under certain circumstances, while a cancer "risk" is the probability of cancer occurring, taking into account the level of exposure to the agent. Therefore, these directives are a means of evaluating the "cancer hazard" of a substance and identifying cancer hazards even when the risks are very low at current exposure levels, because new uses or unexpected exposures could promote risks that are significantly higher (IARC, 2006).

As can be seen, the monographs conclude with a categorization of the agent through the appraisal of existing scientific information and reflecting the robustness of evidence derived from studies in humans and experiments with animals among other relevant data (IARC, 2006).

Thus, this assessment is intended to be conducted using a method for studies in humans and another one for animal experiments. In the case of systematic and critical evaluation of scientific information concerning the studies which have been performed with respect to PFOS in humans, this review is intended to use the critical appraisal guides of the Scottish Intercollegiate Guidelines Network (SIGN). The SIGN was created in 1993 and develops and disseminates evidence based on clinical practice guidelines. These guidelines contain recommendations for effective practice based on current evidence. In this case, we are proposing to use the SIGNs for cohort and case-control studies (SIGN, 2012), and additionally, for the classification of studies of carcinogens in animals also performed for PFOS, the proposed method is the one published by Robert A. Squire (1981). The proposal is to work with all existing studies for PFOS that are found in the Web of Sciences so far.

Articles eligible for inclusion were the original epidemiologic studies reporting association between exposure to PFOS and health outcome of cancer through Web of Science (http://apps.webofknowledge.com.access.biblioteca.cinvestav.mx) data base using the following terms: "PFOS" or "perfluorooctan*", successively combined with "cancer", "case-control", "cohort", "community", "mortality", "occupational", "risk assessment", "tumor", "malignan*" or "neoplas*". We considered only studies on human subjects published in English until October 2015. This strategy yielded 741 references from which titles and abstracts were assessed for identifying potentially relevant articles containing a description of its design, study subject, exposure assessment, outcome assessment, population, participants and statistical results for a complete review, and at the end, ten studies were selected for the current analysis.

For the selection of scientific information on PFOS in human studies, sources were classified as occupational and general population (non-occupational) studies (Figs. 3 and 4). The latter were further classified in cohort and case-control studies (Figs. 5 and 6). Subsequently, two matrices were developed (one for cohort studies and another one for case-control studies) to grade each study based on its compliance with the SIGN critical appraisal guidelines criteria and, thus, determine an overall assessment of High quality [+ +], Acceptable [+] or Low quality [0] for each one (Figs. 5 and 6).

In order to get a proper IARC conclusion on human studies, the analysis for both occupational and non-occupational studies was done objectively, and using scientifically accepted, uniform and pre-established criteria by means of the SIGN for cohort and case-study as a guide. Also, each study's risk rates, confidence intervals, significance, and statistical power, among others, was discussed.

Articles on PFOS in animals were considered eligible for inclusion if they were original toxicological studies reporting association between exposure to PFOS and health outcome of cancer through the Web of Science (http://apps.webofknowledge.com.access.biblioteca.cinvestav.mx) database using the following terms: "PFOS" successively combined with "animal test", *in vivo*" and "*in vitro*". We considered only studies on animal subjects published in English from January 2002 through October 2015. This strategy yielded 190 references from which, titles and abstracts were assessed for identifying potentially relevant articles for a complete review. At the end ten studies were selected for the current analysis.

For all of those animals and other *in-vivo* and *in-vitro* studies, the points obtained for each of the six categories proposed by Robert A. Squire method were quantified (Squire, 1981). This method proposes using a system for ranking animal carcinogens consisting of six factors (from A to F) that have to be scored according to scientific evidence found from 0, 1, 10, and 15 per the degree of severity on each one. They are based on evidence from long-term carcinogenicity studies in animals and from genotoxicity tests and there is biological justification for including each of the factors (Squire, 1981). Finally, the application of the scoring system to the six factors will result in a total score varying from 13 to 100. The numerical value that results from this analysis can be grouped to rank animal carcinogens into a class from I to V. For class I and II, chemicals would also have the highest priority for regulation. Classes from III to V may permit many options including approvals for limited uses, labeling, or public education programs. Findings for each factor related to PFOS are shown in Fig. 7 and finally, the total score and selection of the proper class are shown in Table 1.

At the end, a consolidation of both results was performed to proceed to the overall assessment of both aspects in order to propose an IARC category for the PFOS agent so this data could be useful to act as a preamble for performing guidelines or regulations in countries or institutions interested in the subject.

## 3. Results

### 3.1. Studies performed in humans

Out of a total of 741 studies found, ten were selected after review, as mentioned in the Methods section (Fig. 3). Of these, four epidemiological studies associated with occupational exposure were found (Alexander et al., 2003; Olsen et al., 2004; Alexander and Olsen, 2007; Grice et al., 2007), all of which were carried out at a 3M (Minnesota Mining and Manufacturing Company) facility in Decatur, Alabama. The summary of these studies contents can be seen in Fig. 3.

A cohort study published in 2003 (Alexander et al., 2003) points to an increase in the number of deaths from bladder cancer in workers ever employed in high exposure jobs (standardized mortality ratio (SMR) = 12.7 [2.63–37.35]). But according to SIGN critical appraisal guide (see Fig. 5, item number 1.13), due to the few cases observed (N = 3), and lack of identification of potential confounders in the study's design these results can't be clearly attributed to fluorochemical exposure or to any other occupational or non-occupational exposure to known or unknown bladder carcinogens (e.g. 4,4-methylene-dianiline, orthotoluidine, benzidine salts, butylbenzyl phthalate or even smoking habits). Thus, this is considered a low-quality study. In this case, conducting more

| Reference | Study nature | Studied subjects | Cancer forms being evaluated | Measures of association | Results |
|---|---|---|---|---|---|
| **Alexander et al., 2003** | Retrospective cohort mortality | 2,083 workers | Digestive organs, esophagus, colon, rectum, liver, pancreas, bronchi, trachea, lungs, breast, prostate, urinary system | High exposed mean serum PFOS level of 0.9ppm.<br>Standardized mortality ratio (SMR)<br><br>Bladder cancer ever high:<br>SMR = 12.77 (95% CI, 2.63-37.35)<br>Bladder cancer ≥1 year high:<br>SMR = 16.12 (95% CI, 3.32-47.14) | With only three observed cases the possibility of a chance finding cannot be ruled out. No exposure-response trend was detected. |
| **Olsen et al., 2004** | Retrospective cohort | 1,311 workers | Colon, rectum, thyroid, liver, prostate, kidney, respiratory system | Risk ratio episodes of care (RREpC), high exposed mean serum PFOS level 0.5-2ppm.<br>Benign colonic polyps:<br>RREpC = 2.4 (95% CI, 1.3-4.5)<br>Malignant colon neoplasm:<br>RREpC = 12 (95% CI, 0.8->100)<br>Malignant rectum neoplasm:<br>RREpC = 11 (95% CI, 0.8->100)<br>Malignant skin melanoma:<br>RREpC = 10 (95% CI, 0.7->100) | *Non-a-priori* associations among the fluorochemical plant workers related with bladder cancer. This study should only be considered a screening study. |
| **Alexander and Olsen 2007** | Retrospective cohort mortality | 2,083 workers | Kidney | High exposed mean serum PFOS level of 1.3-1.97ppm.<br>Standardized incidence ratio (SIR)<br><br>Bladder cancer ever high exp.:<br>SIR = 1.74 (95% CI, 0.64-3.79)<br>Bladder cancer ever low exp.:<br>SIR = 2.26 (95% CI, 0.91-4.67) | Little support for an association between bladder cancer and PFOS and no exposure-response trend detected. |
| **Grice et al., 2007** | Case-control | 1,400 workers of 1,895 eligible | Colon, melanoma, prostate | High exposed mean serum PFOS level of 1.3-1.97ppm.<br>Odds ratio (OR)<br><br>Colon cancer high exp. (>1 yr):<br>OR = 1.69 (95% CI, 0.68-4.17)<br>Melanoma high exp. (>1 yr):<br>OR = 1.01 (95% CI, 0.25-4.11)<br>Prostate cancer high exp. (>1 yr):<br>OR = 1.08 (95% CI, 0.44-2.69) | Did not observe association between working in a PFOS-exposed job and various cancers. |

**Fig. 3.** Summary of epidemiologic studies associated with occupational exposure to PFOS.

studies is suggested in order to confirm the findings because chance cannot be entirely ruled out.

In 2004, a study comparing episodes of care between fluorochemical plant workers and film plant workers (low exposure) was performed in order to find *a priori* associations with several cancer types (Olsen et al., 2004). In this case, no chemical workers presented an episode of care for bladder cancer. On the other hand, only significant differences were found in episodes of care for malignant melanoma of the skin (5 episodes observed vs. 2.2. expected), prostate cancer (5 episodes observed vs. 3.1 expected) and benign colonic polyps just restricted to long-term workers (26 episodes observed vs. 11 expected). Even though confounders are considered in the design of the study, episodes of care are not properly used for epidemiologic research because the metric does not provide a definitive measure of risk since it could include incident cases, prevalent cases, tentatively diagnosed cases, and misclassified cases that are the routine consequence of differential diagnoses that individuals could undergo in the course of disease diagnosis, treatment, and management (Olsen et al., 2004). In addition, it is evident that the confidence intervals for the risk ratio episodes of care (RREpC) for all cancer types are very wide and include the null value. Only benign colonic polyps seem to show a significant risk (RREpC = 2.4 [1.3–4.5]), but these are not relevant for this analysis. So, in this case, as observed in Fig. 5 (items 1.4, 1.11 and 1.12), this study is considered as low quality for determining an association between exposure and outcome.

| Reference | Study nature | Studied subjects | Measures of association | Results |
|---|---|---|---|---|
| **Eriksen et al., 2009** | Prospective case-cohort | 713 cases of prostate cancer, 332 cases of kidney cancer, 128 cases of liver cancer. 772 control subjects. | Max. plasma conc. PFOS = 130.5 ng/mL.<br>Incidence rate ratio (IRR)<br>Prostate cancer:<br>Q4 IRR = 1.38 (95% CI, 0.99-1.93)<br>TrendIRR = 1.05 (95% CI, 0.97-1.14)<br>Bladder cancer:<br>Q4 IRR = 0.70 (95% CI, 0.46-1.07)<br>TrendIRR = 0.93 (95% CI, 0.83-1.03)<br>Pancreatic cancer:<br>Q4 IRR = 0.91 (95% CI, 0.51-1.65)<br>TrendIRR = 0.99 (95% CI, 0.86-1.14)<br>Liver cancer:<br>Q4 IRR = 0.59 (95% CI, 0.27-1.27)<br>TrendIRR = 0.97 (95% CI, 0.79-1.19) | Plasma concentrations of PFOS in the Danish general population appear not to be associated with risk of prostate, bladder, pancreatic, or liver cancer. No relevant IRR and no exposure-response trend detected. |
| **Vassiliadou et al., 2010** | Cross-sectional | 40 cancer cases in hospital of Saint Savas. 142 control subjects. | ANOVA, $p > 0.05$<br>Median (range) plasma conc. PFOS Athens group:<br>Males = 13.69ng/mL (6.97 - 30.36 ng/mL)<br>Females = 7.03ng/mL (2.27 - 16.63 ng/mL)<br>Median (range) plasma conc. PFOS Argolida group:<br>Males = 10.47 ng/mL (3.46 - 40.36 ng/mL)<br>Females = 8.47ng/mL (2.63 - 26.36 ng/mL)<br>Median (range) plasma conc. PFOS cancer patients:<br>Males = 11.33 ng/mL (4.98 - 26.38 ng/mL)<br>Females = 8.00ng/mL (2.12 - 25.70 ng/mL) | No correlation was found between age and PFOS levels among cancer patients. |
| **Bonefeld-Jorgensen et al., 2011** | Case – control | 31 Inuit women with breast cancer. 115 control subjects. | Median serum PFOS level of breast cancer patients of 45.6 (range = 11.6-124) ng/mL.<br>Only for PFOS:<br>OR unadjusted = 1.01 (95%CI, 1.003-1.02)<br>OR adjusted = 1.03 (95% CI, 1.001-1.07)<br>For the sum of perfluorosulfonated acids:<br>OR unadjusted = 1.013 (95%CI, 1.002-1.023)<br>OR adjusted = 1.03 (95% CI, 1.00-1.05) | PFCs might be risk factors in the development of breast cancer in Greenlandic Inuit. Weak association between PFOS exposure and breast cancer risk. |
| **Yeung et al., 2013** | Cross-sectional | 66 cadaveric liver tissues. 9 normal liver control tissues, 25 serum samples. | Kruskal-Wallis rank test, $p > 0.05$<br>PFOS concentration in serum (ng/mL)<br>Control:<br>Median = 7.29, range = 1.43-34.9<br>Hepatocellular carcinoma cases:<br>Median = 11.5, range = 4.36-48.4<br>Cirrhosis cases:<br>Median = 13.7, range = 1.12-126<br>Hepatocellular carcinoma + cirrhosis cases:<br>Median = 11.4, range = 4.04-26.4<br>PFOS concentration in liver (ng/g)<br>Control:<br>Median = 5.03, range = 1.30-10.08<br>Hepatocellular carcinoma cases:<br>Median = 4.96, range = 1.92-13.7<br>Cirrhosis cases:<br>Median = 2.35, range = 0.375-12.5<br>Hepatocellular carcinoma + cirrhosis cases:<br>Median = 4.12, range = 2.28-42.5<br>Correlated serum and liver tissue (S1 table S3):<br>Hepatocellular carcinoma, $\rho = -0.064$<br>Cirrhosis, $\rho = 0.699$<br>Hepatocellular carcin. + cirrhosis, $\rho = 0.503$ | PFOS exposure seems to be more correlated with cirrhosis than hepatocellular carcinoma. Weak evidence of association between PFOS exposure and liver cancer. |

**Fig. 4.** Summary of epidemiological studies associated with non-occupational exposure to PFOS.

| Reference | Study nature | Studied subjects | Measures of association | Results |
|---|---|---|---|---|
| **Hardell et al., 2014** | Case-control | 200 cases of prostate cancer.<br>185 control subjects. | PFOS ≤ 8.3 ng/mL whole blood (control median), Odds ratio (OR) = 1.0 Referent<br><br>PFOS > 8.3 ng/mL: OR = 1.0 (95% CI, 0.6-1.5)<br><br>PFOS > 8.3 ng/mL: OR = 0.7 (95% CI, 0.4-1.3) Gleason score 2 – 6<br><br>PFOS > 8.3 ng/mL: OR = 1.1 (95% CI, 0.7-1.9) Gleason score 2 – 7<br><br>PFOS > 8.3 ng/mL: OR = 1.2 (95% CI, 0.7-2.0) PSA ≤ 10 ng/mL<br><br>PFOS > 8.3 ng/mL: OR = 0.8 (95% CI, 0.4-1.3) PSA ≥ 11 ng/mL<br><br>PFOS ≤ 8.3 ng/mL whole blood (no family history), OR = 1.0 Referent<br><br>PFOS > 8.3 ng/mL: OR = 1.2 (95% CI, 0.6-2.5) no family history<br><br>PFOS ≤ 8.3 ng/mL: OR = 0.9 (95% CI, 0.5-1.4) family history<br><br>PFOS > 8.3 ng/mL: OR = 2.7 (95% CI, 1.04-6.8) family history | Results are not consistent with PFOS exposure and prostate cancer, but a higher risk for prostate cancer was found in cases with heredity as a risk factor. |
| **Innes et al., 2014** | Cross-sectional | 208 cases of colon and/or rectal cancer.<br>47,151 control subjects. | All cases adjusted for metabolic/physiologic profile :<br>Q1 (0.25-13.5 ng/mL serum PFOS)<br>OR = 1.00 Referent<br>Q2(13.6 – 20.1 ng/mL )<br>OR = 0.38 (95% CI, 0.25-0.59)<br>Q3 (20.2 – 29.1 ng/mL )<br>OR = 0.27 (95% CI, 0.17-0.42)<br>Q4 ( ≥ 29.2 ng/mL)<br>OR = 0.24 (95% CI, 0.16-0.37) *P*-trend<0.00001<br><br>Residents since 1995 or before<br>Q1 OR = 1.00 Referent<br>Q2 OR = 0.19 (95% CI, 0.09-0.38)<br>Q3 OR = 0.13 (95% CI, 0.06-0.27)<br>Q4 OR = 0.12 (95% CI, 0.06-0.23)<br>*P*-trend<0.00001 | Found a strong, inverse association between PFOS and likelihood of colorectal cancer. |

**Fig. 4.** (*continued*).

In 2007, another attempt was made to identify additional bladder cancer cases using the same cohort as Alexander et al. (2003), but adding a self-administered questionnaire provided to all living members of the cohort and using death certificates for those deceased, in order to identify the diagnosis of bladder cancer and smoking habits. A standardized incidence ratio (SIR) of 1.74 [0.64–3.79] for bladder cancer was found for those classified as ever being highly exposed, and a SIR of 2.26 [0.91–4.67] for bladder cancer for those classified as ever being in a low exposure category. In this case, confounders were taken into account in the design of the study (Fig. 5), as required by the SIGN guide, but SIRs confidence intervals in all cases included the null value, so there is not statistical power to support the association and no exposure trend was observed (Alexander and Olsen, 2007).

And finally, Grice et al. (2007), putting aside the bladder cancer issue, conducted a case-control study in order to determine, using retired and former workers, other types of cancer outcomes. Very weak associations, expressed as odds ratios (OR), were found in highly exposed groups for the following cancer types: colon cancer = 1.69 [0.68–4.17], melanoma = 1.01 [0.25–4.11], and prostate cancer = 1.08 [0.44–2.69]. Even though the SIGN guide for case-control studies considers this design as an acceptable quality study (Fig. 6), in this case, all ORs included the null value in the confidence interval, so again a lack of statistical power is observed and an association between exposure and outcome cannot be established.

| Item number | Descriptor for cohort studies | Alexander et al., 2003 | Olsen et al., 2004 | Alexander and Olsen 2007 | Eriksen et al., 2009 |
|---|---|---|---|---|---|
| 1.1 | The study addresses an appropriate and clearly focused question.(Yes, No, Can't say) | Yes | Yes | Yes | Yes |
| 1.2 | The two groups being studied are selected from source populations that are comparable in all respects other than the factor under investigation. (Yes, No, Does not apply) | Yes | Yes | Yes | Yes |
| 1.3 | The study indicates how many of the people who asked to take part, did so in each of the groups being studied (Yes, No, Does not apply) | Does not apply | Does not apply | Yes | Yes |
| 1.4 | The likelihood that some eligible subjects might have the outcome at the time of enrolment is assessed and taken into account for the analysis. (Yes, No, Can't say, Does not apply) | Can't say | No | Yes | Yes |
| 1.5 | What percentage of individuals or clusters recruited into each arm of the study dropped out before the study was completed. | 0% | 0% | 26.1% | 0% |
| 1.6 | Comparison is performed between full participants and those lost to follow up, by exposure status. (Yes, No, Can't say, Does not apply) | Does not apply | Does not apply | Yes | Does not apply |
| 1.7 | The outcomes are clearly defined. (Yes, No, Can't say) | Yes | Yes | Yes | Yes |
| 1.8 | The assessment of outcome is made blind to exposure status. If the study is retrospective, this may not be applicable. (Yes, No, Can't say, Does not apply) | Does not apply | Does not apply | Does not apply | Can't say |
| 1.9 | Where blinding was not possible, there is some recognition that knowledge of exposure status could have influenced the assessment of outcome. (Yes, No, Can't say) | Can't say | Can't say | Can't say | Can't say |
| 1.10 | The method for assessment of exposure is reliable. (Yes, No, Can't say) | Yes | Yes | Yes | Yes |
| 1.11 | Evidence from other sources is used to demonstrate that the method of outcome assessment is valid and reliable. (Yes, No, Can't say, Does not apply) | Does not apply | No | Yes | Can't say |
| 1.12 | Exposure level or prognostic factor is assessed more than once. (Yes, No, Can't say, Does not apply) | No | No | Yes | No |
| 1.13 | The main potential confounders are identified and taken into account in the design and analysis. (Yes, No, Can't say) | No | Yes | Yes | Yes |
| 1.14 | Have confidence intervals been provided? (Yes, No) | Yes | Yes | Yes | Yes |
| 2.1 | How well was the study done to minimize the risk or bias or confounding? (High quality [+ +], Acceptable [+], Low quality [0]) | 0 | 0 | + + | + |
| 2.2 | Taking into account clinical considerations, your evaluation of the methodology used, and the statistical power of the study, do you think there is clear evidence of an association between exposure and outcome? (Yes, No, Can't say) | Can't say | No | No | No |
| 2.3 | Are the results of this study directly applicable to the patient group targeted in this guideline? (Yes, No) | No | No | No | No |
| 2.4 | Notes | See Fig. 3 | See Fig. 3 | See Fig. 3 | See Fig. 3 |

**Fig. 5.** Rates for methodological quality of cohort studies, according to SIGN checklist.

Concerning cancer risk associated with non-occupational exposure, six studies of this type were found (Eriksen et al., 2009; Vassiliadou et al., 2010; Bonefeld-Jorgensen et al., 2011; Yeung et al., 2013; Hardell et al., 2014; Innes et al., 2014). The summary of their contents can be seen in Fig. 4.

Based on occupational studies regarding PFOS exposure, a suggestive but inconsistent association between bladder and prostate cancers was found. Melanoma of the skin is supposedly associated with an incorrect use of safety equipment and around 1997, a heightened awareness for colon cancer screening among chemical plant employees supposedly arose, according to Olsen et al., 2004; so, it should not be considered at a later date. In 2009, a case-cohort study performed in the Danish Population (Eriksen et al., 2009) was published in which no association was found between PFOS plasma concentration and an increased risk of prostate cancer (incidence rate ratio (IRR) = 1.38 [0.99–1.93]), bladder cancer (IRR = 0.7 [0.46–1.07]), pancreatic cancer (IRR = 0.91 [0.51–1.65]), or liver cancer (IRR = 0.59 [0.27–1.27]). Although the SIGN guide shows an

| Item number | Descriptor for case-control studies | Grice et al., 2007 | Vassiliadou et al., 2010 | Bonefeld-Jorgensen et al., 2011 | Yeung et al., 2013 | Hardell et al., 2014 | Innes et al., 2014 |
|---|---|---|---|---|---|---|---|
| 1.1 | The study addresses an appropriate and clearly focused question.(Yes, No, Can't say) | Yes | Yes | Yes | Yes | Yes | Yes |
| 1.2 | The cases and controls are taken from comparable populations. (Yes, No, Can't say) | Yes | Yes | Yes | Yes | Yes | Yes |
| 1.3 | The same exclusion criteria are used for both cases and controls. (Yes, No, Can't say) | Yes | Yes | Yes | Yes | Yes | Yes |
| 1.4 | What percentage of each group (cases and controls) participated in the study? | Overall 73.9% | Cases: 100% Controls: 100% | Cases: 80% Controls: 100% | Cases: 100% Controls: 100% | Cases: 79% Controls: 54% | Cases: 71.2% Controls: 95.6% |
| 1.5 | Comparison is performed between participants and non-participants to establish their similarities or differences. (Yes, No, Can't say) | Yes | Can't say | No | Can't say | No | No |
| 1.6 | Cases are clearly defined and differentiated from controls. (Yes, No, Can't say) | Yes | Yes | Yes | Yes | Yes | Yes |
| 1.7 | It is clearly established that controls are non-cases. (Yes, No, Can't say) | Yes | Yes | Yes | Yes | Yes | Yes |
| 1.8 | Measures will have been taken to prevent knowledge of primary exposure influencing case ascertainment. (Yes, No, Can't say, Does not apply) | No | Can't say | Does not apply | Does not apply | Can't say | No |
| 1.9 | Exposure status is measured in a standard, valid, and reliable way. (Yes, No, Can't say) | Yes | Yes | Yes | Yes | Yes | Yes |
| 1.10 | The main potential confounders are identified and taken into account in the design and analysis. (Yes, No, Can't say) | Yes | No | Yes | No | Yes | Yes |
| 1.11 | Confidence intervals are provided. (Yes, No) | Yes | No | Yes | No | Yes | Yes |
| 2.1 | How well was the study done to minimize the risk of bias or confounding? (High quality [+ +], Acceptable [+], Low quality [0]) | + | 0 | + | 0 | + | + |
| 2.2 | Taking into account clinical considerations, your evaluation of the methodology used, and the statistical power of the study, do you think there is clear evidence of an association between exposure and outcome? (Yes, No, Can't say) | No | No | No | No | No | Yes (inverse) |
| 2.3 | Are the results of this study directly applicable to the patient group targeted by this guideline? (Yes, No) | No | No | No | No | No | No |
| 2.4 | Notes | See Fig. 4 | See Fig. 4 | See Fig. 4 | See Fig. 4 | See Fig. 4 | See Fig. 4 |

**Fig. 6.** Rates for methodological quality of case-control studies, according to SIGN checklist.

acceptable quality study (Fig. 5), all IRR confidence intervals include the null value, so it is difficult to establish any difference due to agent exposure.

Vassiliadou et al. (2010), in a cross-sectional study of different groups of adults living in Greece does not find a difference in median serum PFOS concentrations of men or women from Athens (13.69 ng/mL [6.97–30.36 ng/mL] and 7.03 ng/mL [2.27–16.63 ng/mL], respectively), a rural area in Argolida (10.47 ng/mL [3.46–40.36 ng/mL] and 8.47 ng/mL [2.63–26.36 ng/mL], respectively) and cancer patients (11.33 ng/mL [range = 4.98–26.38 ng/mL] and 8.00 ng/mL [range = 2.12–25.70 ng/mL], respectively). In addition, the SIGN guide designates it as a low-quality study due to a lack of confounders identified in the design of the study showing few details in the selection of the participants (Fig. 6, items 1.5 and 1.10).

A case-control study of breast cancer risk in Greenlandic Inuit population was published by Bonefeld-Jorgensen et al. (2011), and positive but weak associations were found for PFOS only, (OR

| A. Number of different species affected. Result: Two or more. Score:15 | | |
|---|---|---|
| **Reference** | Species | Test duration |
| **Benninghoff et al., 2012** | Rainbow trout | 12.5 months |
| **Butenhoff et al., 2012** | Sprague Dawley rats | 2 years |
| **Elcombre et al., 2012** | Sprague Dawley rats | 1, 7 and 28 days |
| **Wang et al., 2015a** | Albino Wistar rat dams | 1 day gestational age to 7 days postnatal age |
| **B. Number of histogenetically different types of neoplasms in one or more species. Result: Three or more. Score: 15** | | |
| **Reference** | Neoplasms found | |
| **Benninghoff et al., 2012** | Liver tumor | |
| **Butenhoff et al., 2012** | Hepatocellular adenoma, thyroid follicular cell adenoma, mammary carcinoma | |
| **Elcombre et al., 2012** | Not reported | |
| **Wang et al., 2015a** | Not reported | |
| **C. Spontaneous incidence in appropriate control groups of neoplasms induced in treated groups. Results: From 1 to 10%. Score: 1** | | |
| **Reference** | Neoplasms found in control groups | |
| **Benninghoff et al., 2012** | Liver tumor 0% | |
| **Butenhoff et al., 2012** | Hepatocellular adenoma and/or carcinoma = 0% males and females<br>Thyroid follicular cell adenoma = 0% males and females<br>Thyroid follicular cell carcinoma = 5% males and 0% females<br>C-cell adenoma = 20% females<br>Mammary fibroadenoma = 33.3% females<br>Mammary adenoma = 11.7% females<br>Mammary carcinoma = 18.3% | |
| **Elcombre et al., 2012** | Not applicable | |
| **Wang et al., 2015a** | Not applicable | |
| **D. Dose-response relationships (cumulative oral dose equivalents per kilogram of body weight per day for 2 years). Result: From 1 microgram to 1 milligram. Score: 10** | | |
| **Reference** | Dose-response | |
| **Benninghoff et al., 2012** | Not applicable | |
| **Butenhoff et al., 2012** | Hepatocellular adenoma and thyroid follicular cell adenoma = 0.984 mg/kg/day mean for males and 1.251 mg/kg/day mean for females | |
| **Elcombre et al., 2012** | Not applicable | |
| **Wang et al., 2015a** | Not applicable | |
| **E. Malignancy of induced neoplasms. Result: No malignancy. Score: 1** | | |
| **Reference** | Percentage of cancer cases | |
| **Benninghoff et al., 2012** | Hepatocellular carcinoma 0%, cholangiocellular carcinoma 0%. | |
| **Butenhoff et al., 2012** | At highest exposure:<br>Hepatocellular carcinoma = 0% males, 1.7% females<br>Thyroid follicular cell carcinoma = 1.7% males, 0% females | |
| **Elcombre et al., 2012** | Not applicable | |
| **Wang et al., 2015a** | Not applicable | |

**Fig. 7.** Ranking animal carcinogens scores for PFOS.

unadjusted = 1.01 [1.033–1.02] and OR adjusted = 1.03 [1.001–1.07]). According to SIGN guide, this study is considered as one with acceptable quality (Fig. 6) but the ORs and confidence intervals are very close to the null value, making difficult to

| F. Genotoxicity. Result: Incompletely positive. Score: 10 | | | | | |
|---|---|---|---|---|---|
| **Reference** | Test design | Cell tissue | PFOS conc. / exposition time | Genotoxicity | Findings |
| **Florentin et al., 2011** | *In vitro*, Comet assay Micronucleus assay | HepG2 human cells. | 5, 10, 50, 100, 200, 400, 600, 800 μM / 1hr y 24hrs | No | Cytotoxic effect was found in HepG2 cells produced by PFOS, but only at high concentrations and prolonged periods. |
| **Jacquet et al., 2012** | *In vitro*, Cell transformation assay Comet assay | Syrian hamster embryo cells | 0, 2 x $10^{-5}$, 2 x $10^{-4}$, 2 x $10^{-3}$, 2 x $10^{-2}$, 2 x $10^{-1}$, 2, 20 y 50 μg/mL | No | It is found expression of PPAR gene and it is proposed that carcinogenic potential is due to a non-genotoxic mechanism. |
| **Celik et al., 2013** | *In vivo*, Comet assay in bone marrow | Bone marrow albino rat | 0.6, 1.25 y 2.5 mg PFOS/kg body wt. | Yes | PFOS has genotoxic potential because it causes damage to the DNA in the bone marrow of rats. |
| **Wang et al., 2013** | *In vitro*, Cytotoxicity assay Mutant assay Caspase assay Assay for mitochondrial membrane potential | human–hamster hybrid cell | 1–200 μM PFOS / 1 to 16 days | No | The mitochondria are involved in the apoptosis and the oxidative stress induced by PFOS. |
| **Liu et al., 2014** | *In vivo*, Comet assay DNA diffusion assay Micronucleus test | Green mussels (*Perna viridis*) | 0.1, 1, 10, 100 and 1000 μg/L / 7 days | Yes | PFCs induce more genotoxicity when increasing dose and time. PFOS presents a major genotoxic potential. |
| **Wang et al., 2015b** | *In vitro* and *in vivo*, $Spi^-$ Mutation Analysis. | *gpt* delta transgenic mouse embryonic fibroblast | 0, 1, 5, 10 y 20μM / 24hrs | Yes | DNA strand breaks and gene mutation mediated by $H_2O_2$ by means of abnormal peroxisomal oxidation of fatty acids. |

**Fig. 7.** (*continued*).

establish an association between non-occupational exposure to PFOS and breast cancer in this community.

Yeung et al. (2013), presents a cross-sectional study about the content of PFOS in liver tissue and serum of patients with liver cancer and cirrhosis. In this case, evidence of association between PFOS exposure and liver cancer is weak and no correlation was observed between liver tissue (median PFOS concentration in liver of controls = 5.03 ng/mL [range = 1.30–10.08] and hepatocellular

**Table 1**
Score summary for animal studies related to PFOS.

| Factor | Score |
|---|---|
| A | 15 |
| B | 15 |
| C | 1 |
| D | 10 |
| E | 1 |
| F | 10 |
| Total | 52 |

General result: Corresponding to a class IV carcinogen (vigilance).

carcinoma cases = 4.96 ng/mL [range = 1.92–13.7]) and serum content (median PFOS conc. serum of controls = 7.29 ng/mL and hepatocellular carcinoma cases = 11.5 ng/mL). Considering the SIGN guide, this study is determined a low-quality one due to problems considering confounders in the design and not providing comparisons between participants (Fig. 6, items 1.5 and 1.10).

After that, a case-control study was performed in Sweden (Hardell et al., 2014) in order to study the risk of prostate cancer and exposure to perfluorinated alkyl acids. Results of this study suggest that exposure does not increase the risk of developing prostate cancer (PFOS > 8.3 ng/mL: OR = 1.0 [0.6–1.5]), except when a heredity risk factor was also present (PFOS family history > 8.3 ng/mL: OR = 2.7 [1.04–6.8]). This study is of acceptable quality (Fig. 6), but the suggested increase in risk is dependent upon a gene-PFOS interaction.

And lastly, an inverse association is shown by Innes et al. (2014), for colorectal cancer and serum levels of PFOS. In this cross-sectional study, a group of residents from several PFOA-contaminated water districts in mid-Ohio Valley completed a health survey where levels of PFOS were measured. A strong inverse association was found between PFOS and colorectal cancer (All cases adjusted: First quartile OR = 1 [referent], second quartile OR = 0.38 [0.25–0.59], third quartile OR = 0.27 [0.17–0.42] and fourth quartile OR = 0.24 [0.16–0.37]. In this particular case, the SIGN guide shows an acceptable study (Fig. 6) but these observations collectively suggest that altered perfluoroalkyl acids absorption due to colorectal cancer and colorectal cancer treatment is unlikely to explain the robust inverse associations observed in this study. Reverse causality remains a possibility and additional limitations include lack of information on the colorectal cancer stage at diagnosis and on certain risk factors for colorectal cancer, including inherited genetic alterations, history of inflammatory bowel disease, and specific dietary factors (Innes et al., 2014).

As it can be seen, in these epidemiological studies on worker populations, the main routes of exposure to PFOS are by inhalation and dermal contact, and the levels of exposure are higher than those found in the general population. On the other hand, in the latter population, the routes of exposure to PFOS are mainly the intake of contaminated water and food, and dermal contact with products and powders containing them and which are used in everyday life.

### 3.2. Studies performed in experimental animals

As for carcinogenesis studies performed in experimental animals for determining PFOS, including *in vivo* and *in vitro* studies as mentioned in the Methods section, ten related studies (Florentin et al., 2011; Benninghoff et al., 2012; Butenhoff et al., 2012; Elcombe et al., 2012; Jacquet et al., 2012; Celik et al., 2013; Wang et al., 2013, 2015a, 2015b; Liu et al., 2014) were found.

These papers mention that through their experiments it is possible to observe generation of liver tumors in rats by the activation of the following xenosensor nuclear receptors: peroxisome proliferator-activated receptor α (PPARα), constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) (Butenhoff et al., 2012). In other cases mentioned in the above papers, evidence shows that PFOS is able to induce DNA strand breaks of some cells exposed to the agent (Wang et al., 2015b), as well as change the microRNAs (miRNAs) expression in developing rat liver, supporting the hypothesis that PFOS induces alterations in some miRNAs and the expression of these altered miRNAs contribute to carcinogenesis (Wang et al., 2015a). The result of the analysis, and the scores of the studies mentioned above according to Squire (1981) are shown in the following section.

#### 3.2.1. Classification of PFOS according to animal carcinogenesis

PFOS was first mentioned to have properties of a peroxisome proliferator in rodents by Sohlenius et al. (1993). Since then, several studies have confirmed PFOS as an activator of PPARα, CAR and PXR but have not observed such properties in humans (Elcombe et al., 2012). In order to avoid being repetitive, studies from 2011 to the present were considered, so that for the current work, as mentioned before, ten studies were selected, of which four of them are animal experiments and six of them are *in vivo* and *in vitro* studies; the summary of its contents can be seen in Fig. 7.

Instead of rodents, Benninghoff et al. (2012) used the rainbow trout to mimic human insensitivity to peroxisome proliferation to investigate alternative mechanisms of action. Results showed that PFOS caused a minor increase in liver tumor incidence and that the mechanism of action for the promotion of hepatocarcinogenesis likely involves interaction with the hepatic estrogen receptor.

Butenhoff et al. (2012), for their part, performed a chronic toxicity and carcinogenicity study with potassium PFOS ($K^+$PFOS) in Sprague-Dawley rats and found several non-neoplastic effects in the liver including hepatocellular hypertrophy, with a proliferation of endoplasmic reticulum, vacuolation, and increased eosinophilic granulation of the cytoplasm. Additionally, statistically significant increases in hepatocellular adenoma were observed in males ($p = 0.0456$) and females ($p = 0.0386$) in the 20 ppm treatment group (highest exposed group).

Furthermore, Elcombe et al. (2012) developed an additional Sprague-Dawley rat test just to confirm the involvement of PPARα and CAR/PXR in the hepatic hypertrophic and hyperplastic response of rats to dietary treatment with $K^+$PFOS.

Recently, Wang et al. (2015a and 2015b) published a study performed in developing rat livers explaining that exposure to PFOS and other peroxisome proliferators is related to fatty acid catabolism, hepatocyte hypertrophy/proliferation, and tumor induction, but the mode of action leading to liver tumor formation is not fully understood. Findings in this study report that PFOS induced change in aberrant oncomiRs (miRNAs associated with cancer) and tumor-suppressor miRNAs (as a proposed mode of action), showing that PFOS might be a likely carcinogen.

As an attempt to apply the results of animal studies to possible effects on humans, Florentin et al. (2011) used human hepatoma (HepG2) cells (*in vitro* assay) to evaluate the cytotoxic and genotoxic effects of PFOA and PFOS and the intracellular generation of reactive oxygen (ROS) species in the same cell line. PFOA and PFOS have cytotoxic effect on human cells line HepG2 only at high concentrations and a long time of exposure. There was no finding of a relevant ROS generation, an increase of DNA damage, or a micronucleus on HepG2 at the range of concentrations tested. However, endocrine disruption potency is shown.

Another *in vitro* assay performed by Jacquet et al. (2012) in Syrian hamster embryo cells explored DNA damage in single cells in relation to PFOS exposure. The results of this study confirmed the non-genotoxic character of PFOS and pointed out a significant and

high impact on peroxisome proliferator-activated receptors gene expression, additionally corroborating rodent studies which revealed adenoma and carcinoma in liver, thyroid and mammary glands in rats (OECD, 2002). Lastly Jacquet et al. (2012) summarize that this study suggests a carcinogenic potential of PFOS through a non-genotoxic mechanism.

On the other hand, Celik et al. (2013) developed an *in vivo* study with bone marrow tissue of male Swiss albino rats in order to evaluate genotoxic and cytotoxic effects of PFOS in single cells and evaluate protective effects of curcumin against damages incurred by PFOS. According to results, PFOS has a potential genotoxic character caused by significant DNA damage in bone marrow tissue of male rats.

Wang et al. (2013) detected the mutagenic and apoptotic effects of PFOS using a human-hamster hybrid cell ($A_L$) line in an *in vitro* study and showed that exposure to PFOS does not induce the occurrence of CD59 gene (protects cells from self-destruction) mutation, indicating that damaged cells may avoid mutagenesis by undergoing apoptosis, so that mitochondria are involved in PFOS-induced apoptosis and oxidative stress. Thus, no mutagenic effects are found even with long-term treatment of $A_L$ cells.

Liu et al. (2014) performed an *in vivo* study in green mussels with the intent of studying the genotoxicity of several commonly detected perfluorinated chemicals. This was due to disagreements about PFOS as a non-genotoxic agent and considerations of other authors like Hagenaars et al. (2008) reporting that gene expression of important biological functions such as energy consumption and reproduction can be influenced by PFOS. In the case of green mussels, it was found that PFOS displays a higher genotoxic potential compared with other perfluorinated chemicals and that functional group is a major factor that affects the interaction of those compounds with genetic material.

Recently, Wang et al. (2015b) published an *in vivo* and *in vitro* study with *gpt* delta transgenic mouse mutation system to investigate the mutagenic response to PFOS and illustrate the contribution of hydrogen peroxide ($H_2O_2$) to PFOS genotoxicity. A $Spi^-$ Mutation Analysis was performed for both, *in vitro* testing with *gpt* delta transgenic mouse embryonic fibroblast and *in vivo* with livers and bone marrows from living *gpt* delta transgenic mice. Findings in this study indicate that PFOS-induction of DNA double strand breaks and gene mutations was mediated by $H_2O_2$ through abnormal peroxisomal fatty acid β-oxidation.

As mentioned in the Methods section, the five factors established by the Squire (1981) method were scored for ranking animal carcinogens (Fig. 7) with the information explained just above and in order to facilitate the discussion of related findings, an overall score was prepared as shown in Table 1.

## 4. Discussion

Exploring the quality of occupational and community studies performed in humans, it that flaws and defects probably led to problems of chance, bias and confounding; insufficient statistical power due to very wide confidence intervals and the inclusion of the null value in most cases makes it difficult to determine the association between PFOS exposure and the expected outcome. On the other hand, animal tests only showed mechanisms of peroxisome proliferators not present in humans, in such circumstances, the human evidence is critically important to establish if the exposure to the compound poses an increased risk of cancer to humans as proposed by Adami et al. (2011). So, the findings have to be seen in light of the IARC directives in order to establish a classification with the information available.

Although the procedure for elaborating IARC monographs proposes in its section A Chapter 5 the establishment of a working group (IARC, 2006), this article proposes a methodology to determine a classification for PFOS, since anyone, including a private citizen, can participate in the nomination process of a candidate agent (Pearce et al., 2015) and given that this compound has not yet been studied under the vision of the IARC monographs and has not even been included in any list of the upcoming compounds to be considered (IARC, 2008; IARC, 2014).

PFOS meets the criteria requested in the cited procedure in order to be considered as an agent for review. According to the procedure for elaborating monographs for the IARC in its section A, Chapter 3, the accomplishment of two main criteria is required, (a) that there is evidence of human exposure and (b) that there is some evidence or suspicion of carcinogenicity (IARC, 2006). According to the information selected, PFOS shows both types of evidence. Therefore, it is proposed that PFOS be reviewed and a classification is established to determine its cancer risk in order to facilitate decision-making and regulation at a local or national level.

As can be seen in the structure for evaluation of studies proposed by IARC 2006, two main categories of studies can be distinguished in humans and those in laboratory animals. For each case, an easy-to-follow methodology has been proposed since the information can be very diverse and its handling troublesome; a consideration of the methodology and other relevant data is required in order to upgrade, downgrade or confirm the final classification (Fig. 8).

| Category | Classification | Usage |
|---|---|---|
| Group 1 | The agent is carcinogenic to humans. | There is sufficient evidence of carcinogenicity in humans. |
| Group 2A | The agent is probably carcinogenic to humans. | There is limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals. |
| Group 2B | The agent is possibly carcinogenic to humans. | There is limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals. |
| Group 3 | The agent is not classifiable as to its carcinogenicity to humans. | For agents for which the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals. |
| Group 4 | The agent is probably not carcinogenic to humans. | There is evidence suggesting lack of carcinogenicity in humans and in experimental animals. |

**Fig. 8.** Classification Categories for the overall evaluation for the IARC monographs (IARC, 2006).

Evaluating the quality of studies on cancer in humans is an important part of a risk assessment process. By evaluating the scientific quality of a study, the weight that this study is given in the overall process is determined. The process proposed for this evaluation, as mentioned in the Methods section, is the use of the SIGN critical appraisal guidelines for cohort and case-control studies (Figs. 5 and 6).

According to SIGN critical appraisal guidelines, out of the ten selected studies only one is considered to be a high-quality [++] study (Alexander and Olsen, 2007), five are considered to be acceptable quality [+] studies (Grice et al., 2007; Eriksen et al., 2009; Bonefeld-Jorgensen et al., 2011; Hardell et al., 2014; Innes et al., 2014) and four are considered to be low-quality [0] studies (Alexander et al., 2003; Olsen et al., 2004; Vassiliadou et al., 2010; Yeung et al., 2013).

As it can be seen from Figs. 5 and 6, several criteria are not fulfilled for most of those studies according to SIGN critical appraisal guides, and in this case, it is seen that there are findings on occupational exposition through a number of approaches, from an analysis of a mortality database (Alexander et al., 2003), through an analysis of episodes of care (Olsen et al., 2004) and finally, on getting a proper cohort study (Alexander and Olsen, 2007). From non-occupational exposition studies, it is observed that some SIGN guides criteria are not properly met, probably due to the complexity of the communities where studies were performed, and there could have found difficulties in trying to design more extensive studies.

Regarding the occupational studies on PFOS, it is possible to track findings in a timeline in an effort to establish an association between exposure to PFOS and cancer. In May 2000, after 3M announced the voluntary phase-out of the production of perfluorooctanyl-related materials and after some animal studies reported findings on PFOS having peroxisome proliferator properties in rodents, the first cohort study was performed in a Decatur facility (Alexander et al., 2003). The study expected to observe an increase in liver cancer risk, but results pointed out an increase in bladder cancer (SMR = 12.7 [2.63–37.35]). With only three cases, the possibilities of confounding or chance could not be ruled out, so more studies were to be developed in order to confirm such a result. For example, smoking habits are considered to be associated with bladder cancer and this factor was not analyzed in this study (Fig. 5). Previous exposure to other chemicals is not considered either so it is difficult to establish an association.

Afterward, Olsen et al. (2004) tried to observe prior conditions for bladder cancer in the same cohort. However, only significant differences between episodes of care (during the 6-year study time period) were found for malignant melanoma of skin, prostate cancer and benign colonic polyps, and according to SIGN guide rate (Fig. 5), the findings did not correspond to animal studies or bladder cancer, so they do not support causation.

Another attempt was carried out by Alexander and Olsen (2007) to study cancer in the same cohort as the last two studies but, in this case, in addition to the supplied mortality records, a questionnaire was applied in order to diminish confounders (like smoking habits) and chance. In this case, the SIGN guide rate is high (Fig. 5) but a significantly elevated risk is not observed and an exposure-response trend was not found (ever high exposure SIR = 1.74 [0.64–3.79], ever low exposure SIR = 2.26 [0.91–4.67]). Additionally, the confidence intervals include, in all cases, the null value, so in the end, the difficulty in establishing an association persists.

A later study with the same cohort was performed, but this time trying to include several cancer types by means of more questionnaires and assessment of exposures (Grice et al., 2007). No association was found between exposure to PFOS and colon (OR = 1.69 [0.68–4.17]), melanoma (OR = 1.01 [0.25–4.11]) and prostate (OR = 1.08 [0.44–2.69]) cancers. Even though it is considered as an acceptable study according to SIGN guide (Fig. 6), in all cases the null value is included in the confidence intervals, so it is difficult to establish an association. Besides, as seen in Fig. 3, item number 1.8, and according to the authors, the extent of participation was associated with the exposures and outcomes. In addition, there is a possible bias regarding the systematically collected medical records which were not available for this population; thus, the self-administered questionnaires were used to ascertain health outcomes, and according to the hypothesis of the authors, it is conceivable that effects of PFOS and other fluorochemical exposures can manifest as recurrent problems rather than a single occurrence of relatively common events (Grice et al., 2007), so they limited the report to the first occurrence of the condition. Therefore, future studies exploring this hypothesis could find different results.

In spite of the occupational exposure being one or two orders of magnitude higher than community exposure, several studies have been performed on this type of population. Eriksen et al. (2009) case-cohort study in the Danish general population find exposure to PFOS is not associated with prostate (Q4 IRR = 1.38 [0.99–1.93]), bladder (Q4 IRR = 0.70 [0.46–1.07]), pancreatic (Q4 IRR = 0.91 [0.51–1.65]) and liver (Q4 IRR = 0.59 [0.27–1.27]) cancers, as seen in inconsistent IRRs and confidence intervals including the null value in all cases. Even though considered acceptable study (Fig. 5), a non-differential misclassification may have occurred when using a single measure of plasma concentration for PFOA and PFOS for each individual since concentration in one time-point might not reliably reflect the relevant plasma concentration decades ago or at other times (Eriksen et al., 2009). More studies measuring PFOA and PFOS exposure at relevant times for the potential development of cancer are needed in order to rule out (Fig. 5 item number 1.12) different conclusions.

There are three cross-sectional studies (Vassiliadou et al., 2010; Yeung et al., 2013; Innes et al., 2014) in which general cancer cases, liver cancer and colorectal cancers are evaluated. Only a weak association between PFOS exposure and liver cancer is found as well as a strong inverse association related to colorectal cancer. In this case, Vassiliadou et al. (2010) and Yeung et al. (2013) are considered low-quality studies (Fig. 6); no potential confounders are taken into account, and in the former, it was not possible to investigate the temporal trend in serum samples. Again, different results could possibly be found in further studies with more control of these last issues. Finally, the study of Innes et al. (2014) is the only one of these studies with an acceptable quality (Fig. 6), but as mentioned before, an inverse association is observed and a protective effect is excluded due to timing of serum collection when cancer is diagnosed, the colorectal cancer stage at diagnosis and other risk factors not considered.

Two case-control studies (Bonefeld-Jorgensen et al., 2011; Hardell et al., 2014) evaluate breast and prostate cancers, respectively. In both cases, a weak association is found and risk factors are established, as in the sum of perfluorosulfonates in breast cancer (OR adjusted = 1.03 [1.00–1.05]) and heredity in prostate cancer (OR = 2.7 [1.04–6.8]). Both are considered acceptable studies (Fig. 6), but in the first one, a poor statistical power is observed according to authors due to the few number of subjects involved and in the second one, the timing of sample collection could be a flaw.

In summary, none of the available occupational or non-occupational cancer studies in humans find associations, but these can't be ruled out due to problems in population sample size, confounders not being considered, timing of exposure measurement, timing and stage of diagnosis, and/or exposure to additional agents not accounted for, among others, promoting problems of

chance, bias and confounding. As such, available cancer studies in humans are considered to provide "inadequate evidence of carcinogenicity".

However, further studies are necessary in order to verify that a different conclusion could or could not be found on the topics previously discussed. For example, studies complying with more SIGN guidelines criteria could be performed in emerging occupational settings like China, Russia or India (Wang et al., 2014), and continuing the follow-up of the already explored cohorts to ascertain cancer incidence in order to increase our knowledge on the risk that PFOS presents may provide different conclusions on risk of cancer.

In relation to studies about animal carcinogenesis caused by PFOS, the one by Butenhoff et al. (2012) in rodents is the only chronic one identified. The study found several non-neoplastic effects associated with $K^+$PFOS exposure and a statistically significant increase in hepatocellular adenoma (males $p = 0.0456$ and females $p = 0.0386$). The effects observed are consistent with the expected activation of the xenosensor nuclear receptors PPARα, CAR and PXR, but this mechanism is not expected to operate in humans.

The rest of the animal studies identified are sub-chronic (Benninghoff et al., 2012; Elcombe et al., 2012; Wang et al., 2015a). The first one, performed in rainbow trout in order to mimic human insensitivity to peroxisome proliferation, found that promotion of hepatocarcinogenesis likely involves interaction with the hepatic estrogen receptor, but this mechanism is not confirmed in the studies evaluated here. The second study confirms the peroxisome proliferation mechanism operating in rodents, and the third one expands the knowledge about the mechanism of action of PFOS induced change of aberrant oncomiRs and tumor-suppressor miRNAs in the carcinogenesis process.

Only two *in vivo* and *in vitro* studies were performed in cells with some human component (Florentin et al., 2011; Wang et al., 2013). In the first one, a cytotoxic effect in relation to exposure to PFOS was found, and in the second one, an involvement of the mitochondria in apoptosis and oxidative stress induced by PFOS was seen, confirming the impossibility of having the peroxisome proliferation mechanism in humans.

The rest of the studies show a genotoxic potential of PFOS (Celik et al., 2013; Liu et al., 2014; Wang et al., 2015b). In the study by Wang et al., 2015b, the fact that the DNA strand breaks and that gene mutations are mediated by $H_2O_2$ by means of abnormal peroxisomal oxidation is better explained. Only the study by Jacquet et al. (2012) performed in Syrian hamster embryo cells does not consider PFOS as genotoxic, but proposes that the carcinogenic potential is due to a non-genotoxic mechanism.

Using the information previously discussed and the score obtained in Table 1 by the Squire method (1981), it is evident that the overall score of 52, corresponding to class IV, could be equivalent to an IARC classification of "Inadequate Evidence of Carcinogenicity" in available animal experiments. In this category, the data suggest a carcinogenic effect but is inadequate for making a definitive evaluation, in this case, because the agent increases the incidence of only benign neoplasms or lesions of uncertain neoplastic potential, or the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

## 5. Conclusions

From what is mentioned in the procedure for elaborating IARC monographs, and distinguishing between the assessments of the available information on PFOS and carcinogenesis from epidemiological and from toxicological studies, the following is concluded:

- In terms of occupational and non-occupational studies, all information provides *Inadequate evidence of carcinogenicity* in humans and,
- Information from animal carcinogenicity studies provides *Inadequate evidence of carcinogenicity* in experimental animals.
- The evidence regarding mechanisms of PFOS-associated carcinogenesis is considered not to be relevant to carcinogenicity potential, because hepatocarcinogenesis by PPARα, CAR and PXR activators clearly demonstrated in rodents does not seem to occur in humans. In contrast, the half-life of PFOS in humans (4.8 years) than in rodents (1–2 months) and its ubiquity could be the only proven facts for a moderate consideration of this topic, which did not lead to a change in the overall classification of PFOS.

Therefore, it is suggested to classify PFOS as a Group 3 agent (*not classifiable as to its carcinogenicity to humans*) according to the IARC's scale for purposes of local or national regulation as required.

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## Transparency document

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