Prof. Giovanni Costa
Medico Chirurgo
Specialista in Medicina del Lavoro
Cattedra di Medicina del Lavoro
Università degli Studi di Verona

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Egr. Signor
Dr. Carlo Gloria
Direzione
MITENI S.p.A.
Trissino (VI)

Report on the APME APFO *ad hoc* Toxicology Working Group – Meeting held on Tuesday 13th November 2001 at the Henderson's Wharf Hotel, Baltimore, USA

1. Participants

D. Farrar - Ineos Chlor (Chairman – for AGFP)
J. Butenhoff and D. Bacon - 3M
G. Costa - Miteni
S. Murphy - AtoFina
C. Elcombe - University of Dundee
G. Kennedy, G. Jepson and R. Hope - DuPont Haskell Labs
G. Malinverno - Ausimont
H. Iwai- Daikin

2. Feedback from the meeting with US EPA

G. Kennedy (DuPont) reported about the recent meeting between FMG and EPA held in Washington DC on October 2, 2001.

In this meeting EPA was up-to-dated as concerns toxicology and descriptive biology of APFO, in particular: acute toxicity, repeated dose toxicity, developmental toxicity, reproductive toxicity, genetic toxicity, monkey study, human health experience, pharmacokinetic studies in progress, ecotoxicity, adsorption/desorption, environmental fate.

He read a message sent by EPA after the meeting in which they appreciate the update given concerning the ongoing studies on APFO. They expressed their concern about some data concerning its persistence in the environment and their willing to have as much as possible information in order to explore the problem of fluorinated compounds in general. They consider also the study on monkey very important and they are waiting for the final report.

G. Kennedy (DuPont) and D. Farrar (IneosChlor) believe it important to reply by giving more information concerning the differences between PFOS and PFOA, as there is the feeling

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that they are still not considering properly the different toxicology and environmental distribution of the two compounds.

J. Butenhoff (3M) believes it also important to add some data concerning the environmental biomonitoring that 3M has collected in the last year (see point 9).

These questions will be addressed next Friday in the SPI-APME meeting that will be held in Baltimore.

G. Malinverno said that Ausimont has just started a large biomonitoring study in air (around plants in the place of production) and water (*blank* and water discharged and, possibly, in the nearby river). The data will be ready by next December.

3. "Biomonitoring"/environmental distribution of APFO (review of existing data, adequacy of existing data, next steps for APME/SPI)

A. Sharif (DuPont) has circulated a document concerning the environmental distribution of PFOA (and PFOS) according to the studies of Giese and co-workers. The report in attached. The conclusion are: "PFOA is not as widely distributed in aquatic (freshwater and marine) life and in mammals and birds that feed on fish as PFOS. Furthermore, in general, where detected the levels of PFOA in animal tissues are considerably lower than PFOS".

All agreed on considering this document as the basis for further integration and discussion, particularly as concerns the clarification of the differences between PFOA and PFOS. G. Kennedy pointed out that the LOQ (Limit of Quantification) is not the same in all the studies, so it is necessary to clarify this point, as the results can be interpreted differently according to the lower or higher LOQ used.

There are many other recent studies carried out by 3M that analysed the distribution of PFOS and PFOA in water and blood. In the ongoing SETAC meeting in Baltimore, a Canadian group of researchers (Mabury and co-workers) presented other data, that should be included in the report.

D. Farrar stressed the necessity to build up a table of comparison, having all the documents and studies comparable in terms of methods.

J. Butenhoff (3M) thinks that a document on human monitoring should also be prepared, either because it is a form of bio-monitoring and as it is relevant for the credibility of the report both at scientific and regulatory level.

R. Hope (Dupont) says that now the most important end point in the environmental issues is to find out the to separate PFOA from other materials.

D. Farrar added that the main charge of the group is to demonstrate that we can use PFOA in a responsible way, and that the preliminary question to be answered is: "Do we have sufficient information to convince the authorities that APFO is not widely distributed and, if not, what do we have to do to complete these information?"

Therefore, it is necessary to have a solid database in order to explain why the distribution is different and what the concentrations found mean in terms of risk assessment.

D. Farrar and G. Kennedy proposed that A. Sharif and J. Butenhoff work together in updating and preparing such document, with different chapters concern wildlife, humans, etc.,

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and facing two main aspects: 1) to respond to EPA, 2) to try to explain the PFOA distribution in the environment.

4. Current testing programme - environmental end points (toxicity study in fish and daphnia; adsorption/desorption study (definition of protocol)

The group agreed on the protocols that P. Thomas (Atofina) has sent before the meeting concerning the toxicity studies on Daphnia magna and trout prepared by CIT laboratory (Paris). The lab asked some information about the "stability" in the test media (that is if the condition remain stable during the period, or if the PFOA can be absorbed by some material).

The reply by the group is that we do not need any preliminary study on "stability" as the previous studies show that it is stable and not absorbed by any material.

3M will send the test material (both linear and branched). Daphnia studies will start in January and end in February 2002; studies on trout will start in April and end in May 2002.

As concerns the adsorption/desorption study, it has been proposed to choose the DuPont Haskell Lab for its experience and reliability. P. Thomas (Atofina) and R. Hope (DuPont) will supervise the study.

5. Clarification of physico-chemical data (water solubility, vapour pressure).

There is an old data concerning vapour pressure defined by DuPont many years ago. It has been decided to ask Dupont to repeat the measure and also to establish the sublimation temperature.

As concerns water solubility there is a recent 3M data reporting a solubility for APFO >100 g/l.

6. Update on progress of current toxicology testing programme.

6.1. ADME/mass balance, PBK model for rats and humans Protein binding studies, and .

G. Jepson (DuPont) presented some data of *an interim* report on the ongoing toxicokinetic studies in rats.

The preliminary results show some clear sex differences: females clear very rapidly (40 hours), males very slowly (several days), consequently the Mass balance is higher in males than females (blood: males 10, female <LOQ). In rat liver PFOA is found more in nuclei, mitochondria and microsomi in male rats, while more in cytosol in female rats. No difference was seen between male and female kidneys. For transport in plasma, probably alfa2globulines are important. In female urines all PFOA is free, in males is linked with proteins.

G. Jepson (DuPont) has circulated a review of the present knowledge about the protein binding of PFOA and possible models of toxicokinetics, trying to define a PBK (Physiologically Based Kinetic) model including multiple exposure and storage

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compartments, with the aim of explaining the inter-species and sex differences in distribution, metabolisation and toxic effects.

6.2. Mechanisms of pancreatic tumour formation

C. Elcombe University of Dundee) is going to continue next January his project aimed at elucidating the mechanism of induction of the pancreatic acinar cell tumours.

It has been suggested that APFO is a promoter of acinar cell neoplasia. That is, APFO induces endogenously initiated acinar cells to proliferate and expand clonally leading to tumour formation. According to his experiment on rats, which were fed by APFO (300 ppm) in the diet for up to one year, there was an increased incidence of hypertrophic, basophilic acinar cell foci, but a generalised increased pancreatic cell replication was not evident. The lack of a generalized hyperplasia does not support the hypothesis that a chronic increase in cell replication was the cause of the pancreatic tumours seen in the previous study in rats. It is possible that the hypertrophic foci observed following APFO treatment of rats are a metaplastic change that represents the plasticity of the acinar cells and not a pre-neoplastic change.

6.3. Feedback on pathology review of pancreatic lesion CRE

J. Butenhoff (3M) confirmed that the planned meeting of the three expert pathologists, who have to evaluate the typology of the pancreatic lesions, is scheduled for November 28th.

Such joint evaluation seem very important, as the single pathologists have different ideas and concepts about cellular lesions and hyperplasia, which of course can be more or less relevant for human health.

7. Update on progress of 2-generation reprotox study

J. Butenhoff (3M) confirmed the positive data presented in July in Verona showing no effect of APFO both on 1st and 2nd generation of rats. These data have been also showed at the recent EPA-SPI meeting (see photocopies enclosed).

Now they are concluding the study by examining all the tissues and organs of the animals. The report will be completed by the end of the year.

8. COVANCE primate study

J. Butenhoff (3M) confirmed that P. Thomford (Covance) will send the two final reports by the end of the year. The report on Range-finding study is due by December 17th and the Six-month study by December 29th. There was a delay due to the late arrival of some dosages from other laboratories.

J. Butenhoff (3M) and G. Kennedy (DuPont) have finished the preparation of the paper concerning this study, that will be submitted for publication to an important journal of toxicology (probably Toxicological Sciences) by the first of December. I was given a draft copy for final comments (see copy enclosed).

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Elcombe (University of Dundee) is going on with the follow-up study on the monkey liver. His preliminary data show a mythocondrial proliferation, whereas the DNA content appears decreased, thus suggesting that there is no hyperproliferation. The expert liver pathologists will end the review of the livers next week. J. Butenhoff said that one worker of 3M plant, with high PFOA blood level, showed the same picture at liver biopsy.

As concerns the 3M elimination kinetics studies, J Butenhoff said that the final report will be ready in two months. The males appear to be a little bit more efficient in elimination (22 vs 30 days) than females.

9. Update of 3M biomonitoring programme

J. Butenhoff informed that they are preparing the final reports concerning the blood level of APFO found in US children and red cross donors. The summary of these data have been already presented at the Verona meeting in July. The reports will be ready in 1-2 months and sent to the regulatory agencies.

10. Update on Miteni biomonitoring programme

I have summarised the data of the biomonitoring on MITENI workers carried out last Spring and already presented at the Verona meeting. Our data appear higher than 3M and DuPont workers; also Ausimont has started a biomonitoring program and the first data appear quite low except for two cases. I told the group that MITENI has planned a new control next winter, that is extended also to previously exposed workers, in order to have some more information on APFO clearance.

G. Malinverno (Ausimont) confirmed his agreement in having a meeting to discuss the data by the end of November in Milano; also G. Kennedy (DuPont) and J. Butenhoff (3M) expressed their agreement for a joint meeting with their occupational health physicians in order to compare all the data and evaluate human exposure and common biological parameters. This meeting will be probably scheduled in connection with the next meeting of the APFO *ad hoc* group (see point 15.)

D. Bacon (technical manager of 3M) agreed in sending me their method for the APFO environmental monitoring, in order to have the possibility to carry out both environmental and biological monitoring in the next control session.

11. Discussion of 3M BLV for APFO

According to their experimental and human data, several months ago 3M proposed a corporate Biological Limit Value (BLV) of 5 ppm. This document has been circulated in the group. The value was set considering a "safety" factor of 10 in relation to the experimental LOEL. The report says that " the BLV for perfluorooctanoate is established at a level which, based on current available data, would not be expected to be associated with a significant risk of adverse health effects in occupationally-exposed workers. By establishing this BLV at 5 ppm, the Committee does not intend to imply that serum levels of perfluorooctanoate greater

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than the BLV immediately pose a significant risk of adverse health effects. However, if an employee's serum perfluorooctanoate level either meets or exceeds the BLV, it should be understood that corrective actions (which may include temporary removal from the immediate work area) may need to be applied, on a case-by-case basis, at the direction of 3M Occupational Medicine".

There was some doscussion about the methodology of extrapolation of this level, but there was a general agreement on this level, that at present can be acceptable as an "action level", that is corrective and preventive measure have to be undertaken.

DuPont does not have any standard level; they found no apparent effect in workers up to 6 ppm. Some caution has to be taken considering the possible cutaneous skin transfer.

12. Feeddback from SETAC Meeting in Baltimore

From Saturday 11th to Thursday 15th the 22nd Congress of the SETAC (Society of Environmental Toxicology and Chemistry) has been held at the Convention center in Baltimore. I attended the session on Perfluoninated compounds, held on Monday, where there were 10 oral presentations and 7 posters.

Most of the presentations concerned PFOS, with some reports confusing or equalising PFOS and PFOA as concerns toxicology. There was a rather curious presentation saying that PFOA is 160 times more toxic than PFOS, made by Richard Walker, former toxicologist at 3M, based on an old study on female rats. The 3M members of the group believe that the scientific evidence shows exactly the contrary, but that it was not convenient to argue during the presentation; G. Kennedy (3M) suggested to send him the available data on PFOS and PFOA and to ask him if he is able to confirm his previous statement.

Ast some people can be confused by such kind of communications, that can also influence the regulatory authorities, everybody believe that this shows once more the importance of preparing a comparative document with clear and plain information for business people and authorities.

13. Proposal to prepare an EU-style Risk Assessment.

It was agreed that the Dupont document, prepared for US EPA, can be used as basis for starting and EU-style risk assessment.

There are sufficient information, coming from Japanese, 3M and Canadian studies, that allow the preparation of a draft document.

G. Kennedy (DuPont) will circulate the dossier, thereafter it will be decided which European companies with specific expertise in preparing such kind of dossier have to be contacted.

14. Other business

G. Malinverno (Ausimont) informed that EURO CHLOR has recently sent an e-mail to their members announcing that OSPAR (Oslo Paris Commission), an European organisation

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responsible for the North Sea (it is not a governmental agency, but it is connected with them), issued a list of 17 compounds that they intend to check for environmental pollution in the North Sea; among these there are also some perfluorinated compounds, and in particular 335-67-1 (ottanoic-pentadecafluor acid). They will contact users and producers.

G. Malinverno will circulate the letter and the list (see in attachment). D. Farrar will ask APME to respond as a group, but G. Malinverno believes that MITENI has to reply directly in relation to the specific compounds produced.

15. Date and location of next meeting

The chairman proposed to hold the next meeting on Thursday 14th, 2002, in a city of the east cost (probably Philadelphia), before the Congress of SOT (Society of Toxicology), that will be held in Nashville from 18th to 22nd, 2002.

With kind regards

Prof. Giovanni Costa