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Report on the meeting of the Plastics*Europe* APFO AD-HOC TOXICOLOGY WORKING GROUP, held on 16-17 November 2006 in Brussels (CEFIC)

Participants:

D Farrar (Chairman), Ineos Chlor (for Asahi Glass Fluoropolymers)

J Butenhoff, 3M

I Colombo, Solvay Solexis

G Costa, University of Milan (for Miteni)

W De Wolfe, DuPont

C Elcombe, CXR University of Dundee

P Hoff, 3M

H Iwai, Daikin

R Jung, Clariant (for Dyneon)

G Kennedy, DuPont

S Murphy, Arkema (teleconference)

M Neal, PlasticsEurope

B Schmit, Solvay

G. Malinverno, Solvay (only 17th)

1. Interactive Science Forum (ISF) – update on progress

G. Malinverno (Solvay), who had to finalise the organisation of the Forum, said that the original objectives of the Forum are now changed in the light of the recent proposal, made by the Commission on the Environment, Public Health and Food Safety of the European Parliament, for a new EU Directive (Provisional 2005/0244(COD)) related to restrictions on the marketing and use of PFOS and PFOA. He pointed out that now we have to be prepared to respond properly to the statements of the Directive as soon as it will be put on the table for discussion, rather than holding a scientific forum, which in many cases Legislators and Regulators do not take in proper account.

He said that a pure scientific event is now not useful, but a more regulatory and political debate plus science could be more effective. Therefore, Solvay is prepared to support logistically this event provided that it has regulatory and institutional connections.

D. Farrar (Ineos Chlor) said that the aim of this APFO ad hoc Committee has mainly scientific aims, and that regulatory business deal with other Committees and, moreover, Classification & Labellling and Reprotoxicity are two key-points that can influence also political decisions.

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OECD group is already concluded, and PFOA was not considered in the latest discussion list. However, as this "advice" will be sent to the EU Chemical Agency, we have to be prepared to give our view as soon as they start discussing under REACH umbrella (maybe in 1-2 years). On the contrary, the key issue is now the recent proposal of the EU Directive (in particular as concerns the risk assessment and the statement saying that PFOA is "suspected to be similar to PFOS"); in his opinion, the APFO *ad hoc* group has the aim to support scientifically companies, politicians and regulators for improving this Directive. He also said that the PE main Committee will have soon to define priorities as concerns monetary resources to be given to risk assessment and not simply to toxicological evaluation.

G. Malinverno and and W. de Wolf (DuPont) agreed that Reprotoxicity is now the main issue, and the group should try to avoid the classification in cat. 2, because this is influencing any further action.

G. Kennedy (DuPont) explained what was the development of the situation in USA: at the beginning PFOA was evaluated at the same as PFOS, then companies and scientist promoted meetings for information of the Regulators (e.g. EPA) about the differences between the two substances; this was followed by a lot of discussion and confrontation between industry and institutions with subsequent interaction and some agreements.

D. Farrar said that the forthcoming workshop on PFOA to be held in Arlington (Virginia) in February 2007 (see point 5.) will have the same aim as the ISF could have in Europe. He proposed to organise a workshop on occasion of the forthcoming Eurotox Conference, to be held in Amsterdam in September 2007. G. Maliverno offered to support this proposal (similar to Arlington) at the next meeting of the Eurotox officers in order to try to add this issue in the next Conference.

C. Elcombe (CXR Biosciences) said the British Toxicology Society could also organise a workshop on this topic. After a long discussion about pros and cons of such event and connections, the group agreed on this proposal: D. Farrar and C.Elcombe will meet the Chair of the British Toxicology Society next week in order to organise a 2-day meeting on toxicity of PFOA and addressing also the PBT issue, possibly by June 2007.

2. EPA Risk Assessment on PFOA and Science Advisory Panel

2.1. Liver growth (peroxisome proliferation) study

This study, carried out by CXR Bioscience (C. Elcombe) on behalf of PE and aimed at responding to EPA question on possible causes of liver damage in rats, confirmed previous results. The administration of dietary APFO (compared to Wyeth14,643, which is a well known carcinogen) lead to decreased body weights after 7 and 28 days of exposure, but without any adverse clinical observations. These data clearly demonstrate that APFO exhibits the prototypical properties of a "peroxisome proliferator." The administration of APFO to rats leads to hepatomegaly characterised by hypertrophy and hyperplasia. These changes are preceded by interaction of APFO with members of the nuclear hormone super family, particularly PPAR, CAR and PXR.

As next steps C. Elcombe gave recommendations for a study on guinea pig, who is not a respondent species for "peroxisome proliferators": this is to explain the possible alternative mechanism according to EPA questions. It was suggested to use Phenobarbital (enzyme inductor) as control substance.

A 2nd proposal on interspecies comparison of APFO effects, in particular in primary hepatocyte cultures (3 guinea pigs and 3 human donors), was also presented. He also gave the financial costs of such two studies (77000 and 34000 English Pounds).

The group agreed that these two studies, already foreseen as phase 3 of the general protocol proposed, would add further contribution to a better understanding of carcinogenicity of PFOA, but also argued that he financial costs of such studies have to be evaluated in the light of the priorities that the main Committee will establish (see also point on pancreas issues).

2.2. Update on immunotoxicity study on APFO

G. Kennedy (DuPont) showed the main results of the report presented in July 2006 to EPA by Dr. Scott E. Loveless (Director of the Mammalian Toxicology Unit at Haskell Lab., DuPont). He reviewed all the studies on this subject and the conclusions say that APFO does not cause primary effects on the immune system in rats or mice. In particular, in rats, no immune-related changes occurred, even at doses causing significant systemic toxicity; in mice, no immune-related changes occurred at doses that did not produce a stress response (0.3-1 mg/kg), while immune-related changes occurred at doses causing significant systemic toxicity and stress (10-30 mg/kg).

2.3. Volume of distribution publication

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(Dayneon) argued that all studies compared internal doses and not exposure data (high or low levels), so it is not possible to have a proper evaluation of the volume of distribution, and this is a crucial point for the evaluation of the body burden, in particular as concerns what are the practical consequences of different doses (ppm or ppb).

J. Butenhoff (3M) said that the revision of the study on elimination in steady state conditions in monkeys poses the question if there is another compartment where PFOA can accumulate. In the forthcoming SOT meeting (see point 5.) some experts will be invited to discuss this point

By mid March 2007 J. Butenhoff (3M), G. Kennedy (DuPont) and R. Jung (Dyneon) will prepare a position paper on toxico-kinetics. By Spring 2007 J. Butenhoff will provide a proposal and budget for a low dose kinetic study in primates

3. Review of CXR pancreatic transcriptional profiling studies

The conclusions of the three studies carried out by CXR Biosciences were:

Procarcinogenic gene expression changes observed in the whole pancreas, reported in the first study (CXR0213), in response to dietary treatment with Wyeth 14643 (50ppm) and APFO (300ppm) did not occur in pancreas acinar cells isolated from rats treated with these agents. By contrast, gene expression changes in the gluconeogenesis/pancreatitis genes (PEPCK, DUSP6) and pancreatitis associated protein 1 (PAP1), showed similar time dependent changes in both whole pancreas and isolated acinar cells in response to these treatments. As duct cells represent the only other major cell type in the pancreas apart from acinar cells, and contribute ~10% of the total RNA isolated from whole pancreas, it is likely that this cell type is the site of the procarcinogenic gene expression changes that were observed in whole pancreas.

Pathways analysis of gene expression changes associated with the procarcinogenic treatment (Wyeth 14643 50 ppm), identified the altered expression of a number of genes, (MEN1, NFKB2, BID, CASP2, CASP6 and SMAD5, that were indicative of the potential for suppression of apoptosis and cellular differentiation. This could have a promoting effect on cells that may have sustained initiating mutations in cancer genes at an earlier time point. Similar changes to MEN1, BID, CASP2, CASP6 and SMAD5 were observed following exposure to APFO, however the effects of DEHP treatment on these genes was less marked (not significant above the 99% confidence limit , P>0.01).

The anti-inflammatory gene PAP1 was markedly down-regulated by the carcinogenic treatment (Wyeth 50 ppm) and APFO (300ppm) (but not the non-carcinogenic treatment, DEHP). This effect could predispose the pancreas to inflammation and tissue damage associated with autodigestion by activation of digestive proenzymes.

Conclusion for the future:

According to C. Elcombe some relevant results and clarifications have been reached but many things have still to be analysed and clarified. The rationale for going further is clear, but the priorities can be different: liver for some people, pancreas cancer for others.

Data achieved can be considered sufficient/improved for some aspects, compared with the hypothesis given some years ago (e.g. liver mechanism of proliferation) , but still insufficient for other aspects (e.g. pancreas).

C. Elcombe proposed to further studies able to clarify these points, in particular:

- A laser capture microdissection of acinar cells and ducto-acinar cells and repeat gene expression changes;

- A bioinformatics analysis to fllter out possible dye-swap artefacts

- A quantification and confirmation of protein expression in pancreas tissue

- A correlation of transcription/protein changes in whole pancreas with those in specific cells

- To reproduce in vivo effects in isolated/cultured human cells.

Obviously, these studies have to be decided according to the priorities the main committee will decide to take (see point 2.1).

4. OECD activities.

4.1. OECD Stockholm workshop

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Farrar, J. Butenhoff (3M) and W. de Wolf (DuPont) will be there W. de Wolf summarised the paper he prepared concerning bioconcentration and bioaccumulation (see working document in paper given). The document has an appendix with the OECD risk assessment on PFOA. The conclusions are that PFOA has a low bioaccumulation and biomagnification potential, so it has not to classified as "B". Its findings in remote area (i.e. poles) means only exposure through several transfer/transport media (i.e. water, air, animals) and not bioaccumulation through the food chain.

On the contrary, C11 e C12 show a significant bioaccumulation.

4.2. Others activities

W. de Wolf said that Germany has updated SIAR and discuss it at Science24 (April 27 in Paris) and got a consensus about human risk.

G. Malinverno said that it is important to monitor (and circulate) what is going on everywhere in the world about APFO that could be relevant in the perspective of the forthcoming EU Directive on restrictions.

5. Proposed SoT Current Concepts in Toxicology Seminar

J. Butenhoff (3M) said that the programme of the SOT seminar (Current Concepts in Toxicology of APFO) to be held in Arlington (Virginia) on February 14-16th, 2007 (see www.toxicology.org) and sponsored by 3M, DuPont, PE, SOT and EPA is completed.

D. Farrar proposed to have a meeting of the APFO-WG on Friday afternoon and Saturday morning, after the conference.

6. Update on cholesterol issue

No news.

7. Levels of APFO in blood.

R. Jung has informed that German MAK has set the Biological Limit Value for PFOA in worker's blood at 5 mg/l. He will sent me a connected documentation as soon as possible. (I remind you that 3M had recently set its corporate BLV to 2 ppm).

8. PFOA/PFOS comparison

No news; the document is almost ready and will be put soon in the PE extranet folder.

9. Need for study on Avian reproductive affects

P. Hoff (3M) is preparing a short report on this issue and he said that there were some presentations at Dioxin 2006, but none about PFOA. He said also that avian reprotoxicity data are not required by REACH, so there are no reasons for a specific reprotox study on PFOA unless it is specifically required by regulators. J. Butenhoff (3M) added that previous studies on PFOS and PFOA do not support the relevance of bird for human exposure; they are mainly indicators of pollution (the paper will be put on extranet).

10. APFO – EU classification & labelling

D. Farrar reported about the meeting of the EU Technical Committee Classification and Labelling of Dangerous Substances, concerning Health Effect of Existing Substances, held in Arona, 4-5 October 2006.

The discussion among member States about PFOA dealt with:

Carcinogen Category 2: Norway reviewed data that was basis for their R45 proposal. UK preferred Category 3 as there was not enough confidence to support Category 2, as did Netherlands and Italy. Sweden and Denmark supported Norway. Germany said there no supportive evidence, and therefore Category 3 was appropriate. Belgium and France supported this. Norway said there were 2 different studies in one species at differing levels, and this fulfilled the Category 2 criteria. Netherlands asked if the mechanism had been taken into account, i.e. was it a Genotoxic or non-genotoxic mechanism. Norway said it was borderline between Category 2 and Category 3. Cefic said there has been an independent review of the studies and there were no excess mammary gland tumours. Other work was ongoing.

classified, the studies were on a complex mixture. ECB concluded that the substances can be treated as a group. Netherlands questioned whether they could be treated as a group. Cefic explained the substance being marketed.

Reproductive toxicity – development: Category 2. Norway reviewed a mouse study on development and were proposing Category 2 - R61. UK said the findings were confounded by maternal toxicity, and therefore Category 3. Norway responded that in their view this was not the case. Sweden supported Norway. Germany said there were different effects at lower doses and no indication that pup mortality was due to maternal toxicity: therefore Category 2 applied. Cefic said the effects in mice were compromised by maternal toxicity. Netherlands supported Category 2 due to the effects at lower doses. UK said there was maternal toxicity at all doses, and therefore it was Category 3. ECB concluded Category 2.

Reproductive toxicity – Fertility. Norway advised that this was concluded at the last meeting with no classification.

Risk phrase: R48/23. Cefic said this is an inhalation study and considered R48/20/22 more appropriate on a weight of evidence approach. Norway said the data supported R48/23 for inhalation and considered the oral route should be R48/25. Germany could not follow the Cefic rationale. The criteria have different cut-offs and considered R48/23 appropriate. Cefic explained the data and reviewed it. ECB concluded that R48/23 was agreed.

Risk phrase: R48/22. Netherlands proposed R48/22 and this was supported by the UK. ECB concluded that R48/22 was agreed.

Risk phrase: R22. ECB said that this had previously been agreed for the ammonium salt and was agreed for all the compounds. Netherlands expressed concern over inhalation route. Finland asked if this was academic and which salts are marketed? They were not keen on read across if the substance was not marketed. Germany preferred to cover all substances where possible. Cefic advised what was manufactured in the EU and their uses. ECB concluded to read across R22 to all compounds. Comments were requested in the follow up. Apply R20/22.

Risk phrase: R48/24. Sweden additionally proposed this for the dermal route. Norway said they had not proposed it as the data was limited. ECB said a proposal was needed and it cannot be concluded. Germany said that if there is data it only leads to R48/21. Cefic said there is no absorption in human skin, and a study can be made available. ECB said to be concluded in the follow up, provisionally no classification.

Risk phrase: R36. ECB advised it had previously been agreed for the ammonium salt and read across to other compounds.

ECB concluded as classified R61, R40, R48/23, R48/22, R20/22, R36.

This recommendation for classification and labeling will be passed to the European Chemical Agency for REACH, and this could take several years to become effective.

According to G. Malinverno and R. Jung (Dayneon) APFO is going to be under authorization irrespective than it is PBT or not. High priority will be given to PBT substances, then the other substances. Solvay has already started considering this, despite there are still many aspects to be clarified.

The critical point is Category 2 for developmental toxicity. Many states did not have any position, so they did not disagree with the Norwegian proposal. The PE position was not very strong. D. Farrar suggested to contact directly representatives of the member States and discuss with each one their position. J. Butenhoff said that it is necessary to understand better the mechanism of reprotoxicity and further studies are necessary on this issue. However, it is clear that PFOS causes malformation, PFOA does not.

11. Update on activities in Germany

R. Jung reported that German authorities have a lot of ongoing activities, also towards PFs, following some data on pollution of the Rhine river by APFO and measurements made in drinking waters of North Rhein Wesfalia.

Various federal states picked these information and made several measurement programmes in water, food, breast milk and other biota. Some publications appeared in newspapers and federal sheets. German Commission on drinking water has issued a tolerable life-time acceptable value (0.1 mcg/liter). Next week there will be an authority meeting among UBA, University, Ministers, MAK Commission; J. Butenhoff and R. Jung have been invited as experts.

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formal ban of PFOA and asked R. Jung to check if this is true; it has also to be taken into account that Germany is going to act as President of the EU Commission for the next 6 months.

12. UK activities

ACHS/UK Chemical Stakeholder Forum

W. de Wolf participated in a debate on PBT issues. "P" was agreed, "non-B" was also agreed, there are still some doubts about "T". Further discussion will be done in December, also concerning the long half-life and reprotoxicity. According to D. Farrar, for UK environment agency this seems not to be a concern. The UK-COT (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment) set a TDI (tolerable daily intake) limit of 3 microgram/kg-bw/day .

13. Update on C9 studies.

No news

14. Other b Business

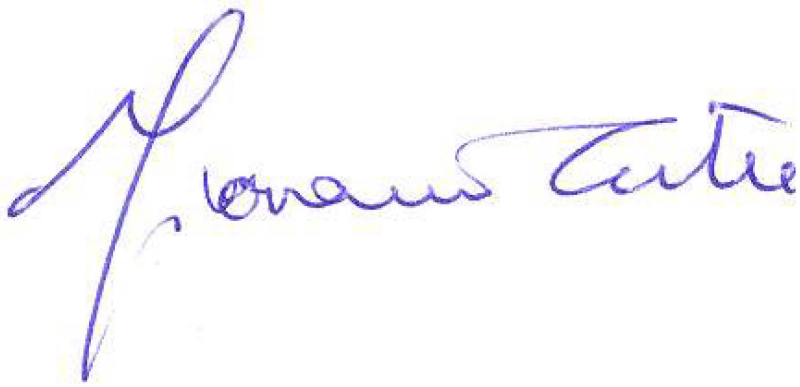
Cohort mortality study on DuPont Washington works

G. Kennedy reported the preliminary results of the DuPont Washington Works Plan Epidemiology Study on cohort mortality analysis in the period 1984-2002. They examined any cause of death, and in particular ischemic heart disease deaths and their possible association with APFO exposure (job and cumulative years of exposure). The external references were the general mortality data of the DuPont Region 1 (West Virginia) and USA population. 773 death among men and 33 deaths among women were recorded. The calculated mortality rates well within expected values. A decrease of SMRs (Standard Mortality Ratios) for prostate cancer anf cerebrovascular disease was seen. No significant increase of SMRs for kidney cancer was recorded. Only for diabetes the SMRs were significantly higher in men. The conclusion was "no effect related to APFO exposure".

15. Date and location of the next meeting

Next meeting will be held in Arlington (Virginia) , at the end of the SOT meeting, on Friday 16th afternoon and Saturday 17th, 2007.

Kind regards



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