B A N   A S B E S T O S   F R A N C E

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Patrick HERMAN **Tél/fax : 05 65 62 23 02**
Algues 12230 Nant **Email : patrick.herman@ free.fr**

Annie THEBAUD-MONY **Email:annie.thebaud-mony@wanadoo.fr**

About the report from "experts" on chrysotile and asbestos substitutes

The role of Biopersistence

Henri Pezerat*

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Presentation

In this report, Henri Pezerat, toxicologist, demonstrates that the methodology and the conclusions of Bernstein's research cannot support the hypothesis of the absence of danger when workers and population are exposed to chrysotile. The facts that we are gathering in every part of the world, with thousands of exposed workers to chrysotile who died of mesothelioma and other cancer, are demonstrating the contrary.

* Henri.pezerat@tele2.fr

About a new report from experts on chrysotile asbestos substitutes

The role of Biopersistence

Henri Pezerat*

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In November 2005, WHO convened a workshop on mechanisms of fibre carcinogenesis and assessment of chrysotile asbestos substitutes, which was held at the International Agency for Research on Cancer in Lyon (France).

The summary consensus report, published on 31 January 2006, contains the collective views of the international group of experts (1). This report appears in the context of a worldwide campaign, initiated by the producers of chrysotile and the International Chrysotile Association, suggesting that on the basis of the biopersistence of this mineral in the lung, chrysotile is innocuous. This leads some politics from Quebec to declare that one year after a three months exposure period to chrysotile, "no fibre remains in the human body and no adverse consequence remains". Such declarations are very drastic in terms of human health and contradictory to numerous epidemiological data, thus implying an in depth evaluation of the data on which they are based.

These data, published by Bernstein et al., report results of inhalation experiments on rats, studies which were first financed by Union Carbide and then by the asbestos producers.

The problem of biopersistence being tackled in the WHO consensus report, this question will be particularly developed in our comments.

More globally the WHO consensus report allows some renewal of the approach of certain points of fibre carcinogenesis which needs to be underlined.

I - CARCINOGENICITY TESTING

Sensitivity of the inhalation studies on animals with fibres

"From studies with asbestos, it is apparent that the sensitivity of the rat inhalation studies to fibre induced lung tumours is clearly lower than that of humans."

Comment from Henri Pezerat (H.P.) : It is necessary to point out that animal studies on chrysotile tended to underestimate the concentrations necessary to induce lung tumours in human.

* Henri.pezerat@tele2.fr

Place of the genotoxicity testing

"In vitro genotoxicity tests are mainly indicative of genotoxic effects involved in the first steps of tumour initiation. Effects related to biopersistence of fibres (such as continuous "frustrated phagocytosis") and secondary genotoxicity arising from reactive oxygen species and reactive nitrogen species and mitogen release by macrophages and inflammatory cells are not detected in routinely used genotoxicity tests. Therefore, negative results indicate a lack of primary genotoxicity, but do not exclude effects on later steps of carcinogenesis."

Comment from H.P.: In other words, particularly in the field of insoluble or sparingly soluble inorganic substances, it will not be wise to abandon animal experiments to the sole benefit of genotoxicity tests on cultured cells, politics which is promoted for the defence of animal species (rats, mice, hamsters).

II - FACTORS CONDITIONNING THE CARCINOGENIC POTENCY OF FIBRES

"The chemical composition of the substitutes is a key factor influencing structure and physico-chemical properties, such as surface area, surface reactivity, solubility, etc. Attention should be paid not only to the chemical composition of the fibres, their major and trace elements, but also to contaminants or accompanying elements, including their speciation. Fibre-derived free radical generation favours DNA damage and mutations. Surface properties are a determining factor in the inflammatory response. In relation to fibre dimension and deposition, one can assume that there exists a continuous variation on the carcinogenic potency of respirable fibre, which increases with length."

Comment from H.P.: At last, it is now recognised that the carcinogenic properties of certain fibres are not only associated to there dimensional characteristics and there persistence in biological medium . Several factors play a role in the existence and intensity of the carcinogenic properties of fibres, the main one being the surface reactivity which is closely linked to the chemical composition and the structure of fibres. This is also pertinent to all insoluble or sparingly soluble particles that can be inhaled (2, 3, 4). The dimensional characteristics and the biopersistence are only two additional parameters.

In this respect, it would have been useful to discuss the role of Fe^{2+} ion (and probably Fe^{3+} ion in certain coordination) at the interface between fibres (or particles) and biological medium. Fe^{2+} is an electron donor which generates some very reactive oxygen species which inevitably play a role in carcinogenesis. For example some Fe^{2+} ions are substituted for magnesium in Canadian chrysotile and in its major contaminant, nemalite.

Moreover, and this was pointed out by the WHO group of experts, the recognition that there is a continuous variation of carcinogenic properties of the fibres in relation to there length, raises the problem of the necessary prevention associated to fibres below 5 µm length. And, in agreement with our research works (5, 6), this report confirms that in carcinogenicity studies, nothing justifies that only the fibres above 20 µm long should be accounted for.

III – ROLE OF BIOPERSISTENCE (or clearance half-time of fibres in the lung)

"Biopersistence of a fibre increases tissue burden, and therefore, may increase any toxicity the fibre might possess. For synthetic vitreous fibres, there is evidence in animals that the

potential for carcinogenicity increases with biopersistence. This has not been demonstrated however for other fibres."

Comment from H.P.: The report of the WHO group of experts put the biopersistence factor back to its place, i.e. a parameter among others in the chain of events related to cancer development. The importance of this factors has only be demonstrated for fibrous synthetic fibres.

In a general way, this factor is associated to the chemical composition and the structure of the fibres and of theircontaminants.

Relation between biopersistence and chemical composition

The relation between biopersistence and chemical composition is evident for fibrous synthetic fibres. In mineral wools (glass wool, rock wool and slag wool) there are alkaline and alkaline-earth cations (sodium, calcium etc…) that have high affinity for water. The higher their concentration in the material, the quicker water will disaggregate it in biological medium; thus the biopersistence will be short. On the contrary, a very low to null concentration of these ions, such as in refractory ceramic fibres, make them highly persistent in biological media.

It is however not acceptable to consider that biopersistence of fibrous synthetic fibres is the sole parameter that determine their carcinogenicity. In a study (4) on six "historical" samples of mineral wool (material with low persistence) manufactured in firms where it has been possible to carry out an epidemiological study, we have demonstrated that:

- Three samples of glass wool containing less than 0.4% Fe^{2+}, devoid of a high oxidizing potency associated to oxygen radicals, were issued from firms in which no excess of lung cancer was detectable among the workers.
- Three samples of rock wool from 1949 to 1974, possessing a high potency to produce highly aggressive radical oxygen species in aqueous medium, having an Fe^{2+} ranging from 6.75 to 12 % as FeO, were issued from firms in which an excess of lung cancer was detected in the population concerned. The excess was directly linked to the Fe^{2+} content of these rock wools.

These studies demonstrate that some rock wools, despite a low biopersistence, possess a significant carcinogenic activity.

Relation between biopersistence, structure and fibre history

The relation between biopersistence and structure can be illustrated by the example of chrysotile.

The geological conditions prevalent during and after the formation of this mineral have not only an influence on the length and on the structure of the fibres, but also on the nature and the density of the crystal defects which govern the reactivity, so the solubility, of all the solids. The defects generate some discontinuity in the crystalline structure, thus creating zones of fragility which are more prone to hydration accompanied by breakage of long fibres into short fibres and dispersion of fibres into elementary fibril, isolated or in small numbers. The chrysotile from the mine of Calidria (USA) is one example of minerals where the structure has been strongly affected by the geological environment.. At this location, chrysotile is composed of only very short fibres with an external area three to four times greater than that of other commercial chrysotile with short fibres, meaning not only smaller diameters but also an opened porosity associated to numerous structural defects. It is evident that such a sample has undergone severe insults from lixiviation during its geological history and is therefore affected by numerous structural defects which renders it very fragile under biological conditions.

This explains the results of Bernstein (7-13) presenting for chrysotiles from different origins (Canada, USA, Brazil) different values of biopersistence. This type of results, in relation with the geological history of each mineral, are logical and known since a long time. . Even in the same mine, differences in density and nature of defects in a mineral occur, depending on the place where the samples are extracted.

The structural modifications which appeared during the geological phases can be amplified during the treatment of the fibres in industrial settings (i.e.: phenomena of torsion in the textile industry), and a fortiori in the laboratories, by grinding, aqueous treatment, heating; operations capable of inducing structural defects which will be zones of high fragility when aggressed by water in pulmonary medium. This explains the obvious relation between the nature and the intensity of preliminary treatments and the half life of the samples in pulmonary medium.

It must be noted that in his publications, Bernstein discusses poorly this problem and even in some cases, provides no information on the treatment of the fibres before their use as aerosols. In the 1994 article (7), always cited in the methodology of the other articles, he refers to a pre-selection of long fibres by a method of sedimentation in aqueous medium, treatment which will reduce the surface activity of these samples. In another manuscript from 1993 (9) about Canadian chrysotile, he describes a grinding method at high speed in which the sample is projected against a durable grinding surface; method which would be very aggressive for the structure of the mineral. In the subsequent literature (8, 10-13) the preliminary treatments are only referenced to these two articles. This raises numerous questions since lixiviation and severe grinding could seriously damage the structure of the mineral thus shortening its half life in pulmonary medium.

In addition, the reference made by Bernstein to the protocol published by the European Commission to evaluate the biopersistence of synthetic mineral fibres bring no information on the treatment sustained by the fibres before the formation of the aerosol used for exposure of the rats.

THE DIFFERENCES OF POINT OF VIEW AMONG AUTHORS ON THE BIOPERSISTENCE OF CHRYSOTILE FIBRES

There is strong disagreements between Bernstein et al. and other research teams on the clearance half time of chrysotiles in pulmonary medium, those data from the team of Bernstein being always shorter. For example, Kimizuka et al. (16), Roggli & Brody (17) and Rogli et al. (18) have reported an increase of the of retention time of the fibres in the lung related to the average length of the fibres. In the same way, Coin et al. (19-20) demonstrated an increase of the number of fibres above 16 µm in the lung with time after cessation of the exposure.

The most interesting comparison is that concerning the Canadian chrysotile investigated by both the teams of Bernstein (9, 12) and Coin (19-20). The two groups worked on different Canadian samples and have not indicated the nature of the treatment applied to the fibres before the production of the aerosols which were used for inhalation in rats. This is regrettable! It must be noted that Bernstein et al. provides some indication concerning the fibres used by Coin et al., but again without any reference. The results of these two studies, one month after cessation of exposure (6 hours by day during 5 days in Bernstein study, and a single treatment of 3 hours in the Coin study) were in complete opposition. In Bernstein study the half life for the long fibres (> than 20 µm) is short (16 days) while in Coin study, the half life for fibres greater than 16µm is at least 114 days.

In this study Coin et al. specify: “Statistically, the clearance rate for fibres greater than 16 µm was not significantly different from zero (half-life infinity)”. This nearly stability with time can be explained by a decrease of the average diameters of the fibres and an increase of the number of long fibres due to their longitudinal cleavage. In the same study, Coin et al.

demonstrate that the clearance rate of the fibres is inversely correlated to their length. For example, the half life for fibres 0.5-4 µm is 10 days, while it is 114 days for fibres greater than 16 µm.

On the contrary, according to Bernstein et al., the half life of the fibres increases when the length decreases (16 days for fibres greater than 20 µm against 107 days for fibres smaller than 5 µm).

This increase in half lives of the short fibres is explained by Bernstein et al. by breaks of the long fibres, thus resulting in an increase of the load of small fibres in the lung and a subsequent slackening of their clearance. For Coin et al., the number of long fibres increase by longitudinal cleavage due to a rupture on the hydrogen bonds that insure the cohesion of fibrils in the fibres. The number of short fibres is thus little affected by the contribution due to breaks of long fibres, the elimination of short fibres being accelerated by phagocytosis, then transport and clearance by macrophages.

This main difference in the results from the two groups cannot be explained by a lung overload. It is generally accepted that such overload occur above 1.5 mg pulmonary burden but in the Coin study the total load is 30 µg, and an overload in Bernstein study would result in lengthening of the half life of the long fibres. Moreover this difference in results cannot be explained by a too high concentration of short fibres in the Coin study, as proposed by Bernstein. Indeed, according to Coin, one day after exposure, 32% of the fibres are shorter than 4 µm, while Bernstein et al claim that 88% of their fibres are shorter than 5 µm after the same time

The only explanation between these contradictory results lies in the density of structural defects in the fibres, defects that break off the structural continuity of long fibres, thus generating on their whole length zones of weakness that create transverse breaks as soon as the fibres are in aqueous medium.

This high incidence of transverse defects is either the result of very old geological conditions, or the results of the pre-treatment of the fibres before the formation of the aerosols.

Taking into account that the half lives of the long fibres, obtained by Bernstein et al. are always low to very low, it looks probable that these are due to the preliminary treatment of the samples which induces multiple defects and semi breaks all along the long fibres.

It must be noticed that Bernstein et al discussed the results from Coin et al. only recently (13). In this manuscript they criticised the method of grinding of Coin et al. : "The products was ground three times using a hurricane pulveriser which is a commercial device designed to grind materials under steel (…) Although not stated in the publication, due to extensive grinding of the Plastibest-20, many more short fibres would be expected". If this was the case, the opposite result must have been obtained, since such a treatment would have increased the number of structural defects, rendering the fibres more brittle thus increasing the number of short fibres. If a too severe grinding can be evoked, this can only apply to Bernstein method which causes a great fragility of the long fibres..

Thus, Bernstein et al. are not in a position to justify their results in regard to those of Coin, Roggli and Brody, a research team known for its work in the field of asbestos since 20 years.

AN OBVIOUS CONCLUSION

The results from Bernstein et al. do not support their conclusion (13):

- That in vivo chrysotile do not act as a fibre but as a particle
- The chrysotile can only induce cancer in case of lung overload which implies very important exposures.

Thus, the only possible conclusion is an absence of credibility of those results from Bernstein et al.

This being said, if the chemical composition, the surface reactivity and the dimensional characteristics of the fibres allow an oxidative stress on biological macromolecules, including DNA, in certain target organs, the biopersistence can have its role on the duration of the aggression. It is not necessary that this half life be several months or even years, especially when the exposure is repeated daily. In addition, it is not known if the biopersistent fibres keep their activity for a long time in the pulmonary medium. Depending on their composition and their structure, certain fibres will quickly loose part to all of their activity while others, on the contrary may acquire a new surface activity due to interactions with endogenous entities, such as iron ions.

In conclusion the results from Bernstein et al. bring no new scientific input. But it is evident that they are abusively exploited by the international lobby of asbestos producers to substantiate the thesis of the absence of risk due to the chrysotile. This criminal attitude will lead to sacrifice the life of numerous workers from emerging countries where the living and working conditions do not allow epidemiological studies.

IV – ABOUT THE SUBSTITUTES OF CHRYSOTILE

The experts from the WHO working group decided to classify them in 3 classes of risk: high, medium and low. For some of the material, the data being sparse, they have thus not been able to perform an evaluation. In this case the human health hazard potential was considered to be indeterminate.

The results of these evaluations are summarised in the following table. It must be noted that the experts have not provisioned a category of “no existing risk” and that there is a large number of classification “indeterminate”, due to the absence of relevant data!

Nature of fibres	Risk Evaluation
Aramide and para aramide fibres	Medium
Attapulgite	High for long fibres Low for short fibres
Carbon fibres	Low
Cellulose fibres	Indeterminate
Graphite whiskers	Indeterminate
Magnesium sulphate whiskers	Low or indeterminate
Fibres from polyethylene, polyvinyl chloride, polyvinyl alcohol or polypropylene	Indeterminate
Potassium octotitanate fibres	High
Synthetic vitreous fibres	High for long biopersistence Low for short biopersistence
Wollastonite	Low
Xonotlite	Low

(1) http: //www.who.int/ipcs/publications/new issues/summary report.pdf

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