Comments on the National Toxicology Program (NTP) Draft Report on Carcinogens Background Document for Glass Wool Fibers

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By way of background, I have extensive education and training in toxicology and experience in assessing the toxic and carcinogenic potential of natural and synthetic fibers (see attached list of fiber-related publications). I have a Bachelor's and a Master's degree in biology from the University of California at Los Angeles and a PhD in toxicology and pharmacology from the University of California at Davis. I conducted in vitro toxicity, neoplastic transformation, and genetic toxicology research on asbestos and synthetic vitreous fibers (SVFs)[1] while completing my postdoctoral studies at the National Institute of Environmental Health Sciences (NIEHS). I continued these fiber studies using human bronchial epithelial cells grown in culture at the Chemical Industry Institute of Toxicology (CIIT—now the Hamner Institute). After my work at CIIT, I took a position with Johns Manville Corporation, where I spent 13 years overseeing a series of chronic inhalation rodent bioassays and biopersistence studies of asbestos and SVFs, sponsored by Johns Manville and other fiber manufacturers, which provided a sound scientific basis for understanding the differences in pathogenicities of these diverse fiber types. I am currently employed by Navistar, Inc., a truck and engine manufacturing company, where my focus is on understanding the potential health risks of the exhaust from diesel engines.

I have been asked by NAIMA Canada to review and provide comments on the "Draft Report on Carcinogens Background Document for Glass Wool Fibers." Given my expertise in the areas of chronic fiber inhalation bioassays and biopersistence studies, I have focused my comments on the sections of this Draft Background Document covering "Studies of Cancer in

[1] Synthetic vitreous fibers is a generic name used to describe inorganic fibers manufactured primarily from glass, rock, minerals, slag, and processed oxides.

Experimental Animals" (Section 4, pp. 117-166) and "Other Relevant Studies" (Section 5, pp. 167-253).

Executive Summary

The Draft Background Document on the carcinogenicity of glass wool fibers is well-written; however it did not provide an adequate critical evaluation of the validity of the different animal models used to assess the risk of cancer to humans from SVF exposure, i.e., the intraperitoneal (IP) test compared to a chronic inhalation bioassay.

Although the Draft Background Document provides an overview of the 2002 International Agency for Research on Cancer (IARC) reevaluation of glass wool fibers, it is also important to include information on the earlier 1988 IARC evaluation of glass wool fibers and explain why IARC differentiated insulation glass wool fibers from special purpose fibers and changed the classification of insulation glass wool fibers from "possibly carcinogenic to humans (Group 2B)" in 1988 (IARC, 1988, p. 152) to "not classifiable as to their carcinogenicity to humans (Group 3)" in the 2002 Monograph (IARC, 2002, p. 333).

The Draft provides only very limited discussions of well-conducted chronic inhalation bioassays of Synthetic Vitreous Fibers (SVFs), other than those studies that evaluated the carcinogenicity of insulation glass wools or special purpose fibers. This gives the impression that only a few fiber types have been assessed using the more-advanced chronic inhalation bioassay, when in fact many fiber types, including 3 types of asbestos and 10 different SVFs have been evaluated using this technology. It is important to review this entire series of inhalation studies in the Draft Background Document, because they show how this methodology can detect known human carcinogens, such as asbestos, and also differentiate biosoluble SFVs from biopersistent SVFs.

Some of the same fibers that were evaluated in the series of studies using the chronic inhalation methodology were also tested in animal biopersistence studies. SVFs that produced tumors in the chronic bioassays were the more biopersistent fibers, while the non-pathogenic fibers showed low biopersistence. This provides a rational mechanism by which fibers cause pathogenicity and explains the differences in carcinogenic potential of the different SVF types. These biopersistence studies demonstrate the validity and importance of using the inhalation

route of exposure to evaluate the potential hazard and risk of exposure to SVFs, and therefore need further discussion in the Draft Background Document.

The concept of Maximum Tolerated Dose (MTD) also needs more discussion in the Draft Background Document. When lung clearance is impaired, even innocuous particles induce chronic inflammation, which results in lung injury, fibrosis, and cancer. Having a high enough dose to thoroughly test the toxicity of an SVF, while avoiding tissue "overload" from doses above the MTD, are major issues that need to be addressed when conducting animal chronic carcinogenicity studies. These issues regarding MTD have not been addressed using the IP test.

A number of problems have been identified with the use of the IP test to evaluate the potential carcinogenicity of SVFs. The shortcomings of the IP test include: lack of relevance to humans, no standardization of methodology, no validation of the test, the dose and dimensions of injected fibers do not reflect what would reach the mesothelium after inhalation, and there is no assessment of MTD.

For these reasons, critical evaluations of animal bioassay methodologies used to assess SVF risk, which have been sponsored over the last 30-plus years by eight different independent regulatory and scientific organizations, including U.S. Environmental Protection Agency (U.S. EPA), the National Institute of Environmental Health Sciences (NIEHS), the National Institute for Occupational Safety and Health (NIOSH), the Occupational Safety and Health Administration (OSHA), the International Programme on Chemical Safety (IPCS), the World Health Organization (WHO), the U.S. Agency for Toxic Substances and Disease Registry (U.S. ATSDR), and the National Academy of Sciences (NAS), all specifically pointed out the shortcomings of the IP test and recommended a well-conduct chronic inhalation bioassay to assess the potential hazard and risk of SVFs.

Therefore, much more discussion of the deficiencies of the IP test, which make it inappropriate for hazard or risk assessment of SVFs, including evaluations of the methodology by independent scientific and regulatory bodies, needs to be included in the Draft Background Document. The current version of the Document gives the impression that this non-physiological route of administration should be given equal weight to a well-conducted chronic inhalation bioassay, when in fact most world experts in fiber toxicology would give much more weight to chronic bioassays using the inhalation route of exposure.

A Critical Evaluation of the Animal Testing Methodology Used to Assess the Hazard and Risk to Humans from Exposure to Synthetic Vitreous Fibers (SVFs)

In general, I thought the Draft Background Document was a well-written review of a very large body of research reported in the scientific literature. However, I was disappointed that the report, in most cases, only discussed the results of studies without critically evaluating the validity of the various experimental models for use in assessing the risk of cancer to humans from exposure to glass wool fibers. In the commentary below, the focus will be on comparing and contrasting the two primary animal testing methodologies used to assess the potential cancer risk of SVFs, the IP injection test and a well-conducted chronic inhalation bioassay.

IARC Evaluations of the Potential Carcinogenicity of Glass Wool Fibers

Although the Draft Background Document provides information on the 2002 International Agency for Research on Cancer (IARC) reevaluation of glass wool fibers, it is important to also include some information on the 1988 IARC evaluation of glass wool fibers and explain why IARC changed the classification of insulation glass wool fibers. In 1988, IARC published the original Monograph on the potential carcinogenicity of synthetic vitreous fibers (SVFs) (IARC, 1988). This IARC Monograph did not subcategorize glass wools into insulation glass wool fibers and special purpose fibers, as was done in the subsequent IARC Monograph in 2002 (IARC, 2002), primarily because there was not enough cancer data or biopersistence data on which to base a differentiation of the two glass wool types.. The 1988 IARC Monograph classified glass wool as "possibly carcinogenic to humans (Group 2B)," based on "sufficient" animal evidence" and "inadequate human evidence" (IARC, 1988, p. 152). In this earlier evaluation, the intraperitoneal (IP) injection test results were not balanced by results of inhalation bioassays, because the inhalation studies conducted before that time were not well-designed (IARC, 1988).

In 2002, IARC published a reevaluation of the carcinogenic risk of SVFs (IARC, 2002). This time the IARC panel decided to differentiate the glass wool category into two separate SVF categories, insulation glass wool and special purpose fibers, the same two fiber categories being

evaluated by the NTP in the Draft Background Document. The IARC decision to divide glass wool into two categories was based on the large number of well-conducted animal inhalation carcinogenicity and biopersistence studies that had been completed between 1987 and 2001. These studies clearly showed that special purpose fibers were carcinogenic, while insulation glass wools were not, which was readily explained by differences in the biopersistence in the lung of these two fiber types.

The 2002 IARC Monograph made some important changes to the classification of glass wool compared to the 1988 Monograph. These changes resulted primarily because the induction of tumors by insulation glass wool fibers using the intraperitoneal (IP) test and other intracavity administration methods were balanced by the negative tumor findings in well-conducted chronic inhalation bioassays, resulting in a conclusion of only "limited" evidence for carcinogenicity of insulation glass wool fibers in animals. On the other hand, induction of cancer by inhalation of special purpose fibers in a well-conducted animal inhalation study, coupled with the finding of tumors in IP tests was considered "sufficient" animal evidence for the carcinogenicity of special purpose fibers. (The components of a well-conducted animal inhalation study will be outlined in the next section of these comments, and the reasons why IP studies were not considered as relevant as well-conducted chronic inhalation bioassays will be discussed in the final sections of these comments.) The 2002 IARC Monograph concluded that insulation glass wool was "not classifiable as to their carcinogenicity to humans (Group 3)" (IARC, 2002, p. 339), which was based on "limited" animal evidence and "inadequate" human evidence (Id., p. 338). The panel concluded that special purpose fibers were "possibly carcinogenic to humans (Group 2B)" (Id., p. 339), based on "sufficient" animal evidence and "inadequate" human evidence (Id., p. 338).

Why the 2002 IARC Monograph Gave More Weight to the Results from Well-Conducted Chronic Inhalation Bioassays

In the NTP Draft Background Document, there was understandably a lot of discussion of the results of chronic inhalation bioassays of insulation wool and special purpose fibers. However, there was very little mention of well-conducted chronic inhalation studies of other SVFs. This left the reader with the impression that only a few studies have been conducted using the new chronic bioassay model, when in fact more than 13 different fiber types, including 3

asbestos types and 10 different SVFs, have been evaluated using this new methodology. It is important to review this entire series of chronic inhalation studies in the Draft Background Document, because it shows how this more-advanced methodology can detect known human carcinogens, such as asbestos, and also differentiate carcinogenic SFVs from the non-carcinogenic SVFs. Focusing only on the results of glass wool fiber studies does not fully show why this methodology is much more appropriate than the IP test for assessing the potential hazard and risk of SVFs. As discussed below, the Draft Background Document should also point out that IARC and many other scientific and regulatory organizations, in their evaluations of methodologies to assess the potential cancer hazard and risk of SVFs, gave more weight to the results of well-conducted inhalation bioassays than the IP test.

Characteristics of a Well-Conducted Chronic Inhalation Bioassay

In 1987, a new generation of rodent inhalation studies of SVFs was initiated to thoroughly test the biological effects and lung biopersistence of representatives of each class of SVF. At least eight chronic inhalation studies and a number of short-term studies examined the potential pathogenicity and carcinogenicity of 13 different fiber types (Hesterberg et al., 1993, 1997, 1998a, 1998b, and 1999; McConnell et al., 1994 and 1999; Mast et al., 1995a and 1995b; Bernstein et al., 1996 and 1997; Hesterberg and Hart, 2001).

Several important features distinguish the new methodology used for chronic inhalation studies conducted after 1988 from the earlier rodent inhalation bioassays. The fibers in the exposure aerosols were highly rat respirable (geometric mean diameters were about 1 μm or less), had a large proportion of long fibers (approximate arithmetic mean length of 20 μm), and were representative of the fiber dimensions in occupational exposure settings (Hesterberg and Hart, 1994). An aerosolization system was used that created uniform, high concentrations of airborne fibers without destroying the biologically important long-thin fiber geometry. A nose-only inhalation exposure system was used, in which each rodent inhaled fresh fiber aerosol that had not been affected by the exhalation of other animals (Bernstein et al., 1995). To simulate occupational exposure, animals were exposed 6 hours per day, 5 days per week (Hesterberg and Hart, 1994). Fibers in the exposure aerosol and fibers recovered from low-temperature ashed lungs were counted and measured (and in some cases chemically and morphologically analyzed)

at intervals throughout the exposure and recovery periods using optical and/or scanning electron microscopy (Hesterberg and Hart, 2001; Bernstein et al., 1996 and 1997). In both the aerosols and the lungs, the concentrations of WHO fibers and fibers longer than 20 μm were determined. Fibers longer than 5 μm (F >5 μm) were reported because the World Health Organization has identified them as biologically relevant (WHO, 1985, 1988). Fibers longer than 20 μm (F >20μm), were reported because, by virtue of their greater potential for biopersistence and bioreactivity, they may be more significant for lung pathology (Bernstein et al., 1995). In the chronic studies, pathology was evaluated at regular intervals by veterinary pathologists during the exposure period and after several post-exposure recovery periods (Hesterberg and Hart, 2001).

As can be seen in the table below, asbestos fibers, refractory ceramic fibers (RCF), and special purpose fibers (E glass and 475 glass fibers) produced increases in lung fibrosis and lung cancer, whereas other fiber types did not (Hesterberg et al., 1993, 1997, 1998a, 1998b, and 1999; McConnell et al., 1994 and 1999; Mast et al., 1995a and 1995b; Bernstein et al., 1996 and 1997; Hesterberg and Hart, 2001). In contrast, in the IP studies the dose of fibers to the peritoneum is raised to such high levels that all fibers cause tumors (Roller et. al., 1997; Roller and Pott, 1998). There is little or no differentiation of different compositions of fibers in the IP test, even though, as will be discussed below, there are large differences in the biopersistence of different fiber types (Hesterberg et al., 1996b and 1996c; Hesterberg et al., 1998a and 1998b). These are important findings in terms of validating the new chronic inhalation bioassay model as more appropriate than the IP test to assess the potential carcinogenicity of fibers and should be discussed in much more detail in the Draft Background Document.

Lung Deposition, Biopersistence, and In Vitro Dissolution of SVFs Correlated with Lung Pathogenicity (From Hesterberg and Hart, 2001 and Hesterberg et al., 1998b)

Fiber	Type	Lung Deposition F/L x 10^6 ± st. dev. F/L >5 μm	Lung Deposition F/L >20 μm	Lung Clearance F >20 μm $WT_{1/2}$ Days	In Vitro Dissolution *pH 7.4* K_{dis}[a]	In Vitro Dissolution pH 4.5 k_{leach}[b]	Pathogenicity Chronic Inhalation Fibrosis	Tumors	Reference
Amosite	Asbestos	10.9 ± 1.0	1.6 ±0.3	418	<1	Nd	+	+	McConnell et al., 1994
Crocidolite	Asbestos	29.8 ±7.1	1.0 ±1.0	817[f]	<1	Nd	+	+	McConnell et al., 1994
MMVF32	Special Purpose E Glass	5.7 ±1.3	1.3 ±0.3	79	9	7	+	+	Davis et al., 1996
RCF1a[c]	Refractory	8.3 ±2.0	1.5 ±0.2	55	3	Nd	+	+	Mast et al., 1995
MMVF33	Special Purpose 475 Glass	7.1 ± 0.6	1.4 ±0.3	49	12	13	+	+/-[d]	McConnell et al., 1999
MMVF21	Rock Wool	7.7 ±1.0	1.1 ±0.1	67	20	72	+	-	McConnell et al., 1994
MMVF10	Insulation Glass Wool	8.6 ± 1.6	1.0 ± 0.2	14.5	300	329	-	-	Hesterberg et al., 1993
X607[e]	Hybrid SVF	3.6	nd	9.8	990	Nd	-	-	Hesterberg et al., 1998b[f]
MMVF11	Insulation Glass Wool	5.6 ±1.2	1.0 ±0.2	9	100	25	-	-	Hesterberg et al., 1993
MMVF22	Slag Wool	3.4 ± 0.6	0.4 ±0.1	9	400	459	-	-	McConnell et al., 1994
MMVF34	Stonewool	9.1 ± 1.7	1.5 ±0.4	6	59	1010	-	-	Kamstrup et al., 1998

Notes for the Previous Table:

Abbreviations: $WT_{1/2}$ = weighted clearance half-time; k_{dis} = ng/cm^2 hr, Nd = Not done

[a] k_{dis} (dissolution rate) values for MMVF34 from Kamstrup, et al., 1998; others from Eastes et al., 1996. K_{dis} values may differ from those published elsewhere due to varying methodologies.

[b] k_{leach} Guldberg et al., 1998. Dissolution rate constant of leaching elements represented by Ca and Mg at pH 4.5 (rounded up to whole numbers). Shaded cells indicate sharp difference in ranking of k_{dis} for pH 4.5 v. pH 7.4.

[c] RCF1 was used in pathogenicity studies. RCF1a was modified from RCF1 to contain fewer non-fibrous particles.

[d] +/- indicates tumorigenicity in hamsters (one mesothelioma in 83 animals) but not in rats; McConnell et al., 1999.

[e] Clearance half-time of 14.5 d was determined using a modified MMVF10 test fiber that had been size-selected to have longer and thinner average dimensions than the original MMVF10.

Biopersistence Studies

Some of the same fibers that were evaluated in the series of studies using the new chronic inhalation methodology were also tested in animal inhalation biopersistence studies (Hesterberg and Hart, 2001; Hesterberg et al., 1998a and1998b, Hesterberg et al., 1996b and 1996c). In animal biopersistence studies, rats were exposed by inhalation to fiber aerosols for 5 days and then held for 3-6 months. During the non-exposure holding period, animals were periodically sacrificed, their lungs low-temperature ashed, and the fibers recovered, counted and sized (length and diameter), using scanning electron microscopy. The weighted half time of clearance (WT1/2) of long fibers (> 20 um long) from the lung was then calculated and used as a measure of the biopersistence of fibers in the lung.

As can be seen in the table above, the fibers that produced fibrosis and tumors in the well-designed chronic bioassays were more the biopersistent fibers (WT1/2 > 49 days), while the non-pathogenic fibers showed low biopersistence (WT1/2 < 14.5 days). This provides additional scientific validation of the inhalation model by documenting a mechanism by which some of the fibers cause pathogenicity, while others do not. Thus biopersistence studies provide even more of a rationale for the use of chronic inhalation studies to evaluate the potential hazard and risk of exposure to SVFs

Reaching the Maximum Tolerated Dose (MTD) Without Creating Lung Overload

The importance of Maximum Tolerated Dose is not sufficiently discussed in the Draft Background Document. The highest dose used in most carcinogenicity bioassays often vastly exceeds possible or likely human exposure. The use of these high exposures is justified by the high degree of extrapolation (from groups of 50 animals to millions of humans) (Haseman et al., 1986). On the other hand, using excessively high exposure levels of particles in a carcinogenicity bioassay could result in non-specific injury and false positives (Lewis et al., 1989; Hesterberg et al., 1996a; McConnell, 1996; Oberdorster, 1995). At very high doses, “Normal physiology, homeostasis and detoxification or repair mechanisms may be overwhelmed and cancer, which otherwise might not have occurred, is induced or promoted” (U.S. OSTP, 1985, 50 Fed. Reg. 10,372, 10,415). Since, as discussed in more detail below, the IP tests use

very large doses of fibers that tend to be concentrated at the injection site in the peritoneum, it is likely the MTD is exceeded in these studies. Thus any normal clearance or repair mechanisms are likely overwhelmed in IP tests.

When lung clearance is impaired, even inhalation of innocuous particles can cause chronic inflammation, resulting in lung injury, fibrosis, and neoplasms (Morrow et al., 1996). Lung inflammation and cell proliferation have been induced in rats by high exposures to even relatively innocuous non-fibrous dusts such as TiO_2, black carbon, tack, and carbonyl iron, which at real-world exposure levels, would be cleared from the lung before they were able to accumulate sufficiently to induce pathogenicity (Warheit et al., 1997). Having a high enough dose to thoroughly test the toxicity of a substance while avoiding tissue overload are major issues that need to be addressed when conducting animal chronic toxicity studies. They have never been even looked at in the IP test.

To avoid the problem of non-specific injury due to tissue overload, the concept of a maximum tolerated dose (MTD) was developed. The maximum dose in a carcinogenicity bioassay must achieve but not exceed the maximum dose tolerated in the target organ. A special panel convened by the National Toxicology Program (NTP) concluded, "In general, the highest concentration of test material used should produce only minimal interference with the lung defense mechanisms as judged by impaired particulate clearance" (Lewis et al., 1989, p. 379). The MTD for particles has also been defined as the maximum dose that does not impair alveolar macrophage-mediated lung clearance (Oberdöster, 1995). A matrix of MTD indicators have been recommended (McConnell et al., 1996; Hesterberg et al., 1996a), which include: non-linear lung dose (loss of linearity of particle accumulation in the lung burden over time during chronic exposure); elevated lung weight; sustained elevations in lung cell proliferation; sustained elevations in inflammatory cell numbers and inflammatory chemicals in lung or thoracic fluids; histopathological changes; and retardation of clearance of innocuous microspheres. If some or all of the indicators are present, it is generally accepted that the MTD has been achieved or exceeded (McConnell et al., 1996; Hesterberg et al., 1996a).

For the well-conducted chronic inhalation bioassays discussed above, the maximum tolerated lung dose of respirable fibers for rodents was determined using these indicators. The exposure that achieves the MTD was thus determined to be 200 respirable fibers/cc, including approximately 100 fibers/cc >20 μm long (Hesterberg et al., 1996a; Hesterberg et al., 1999).

This is a relatively high aerosol concentration compared to concentrations of <1 fiber/cc that are typical of SVFs in the workplace (Hesterberg and Hart, 1994).

Detailed Discussion of the Shortcomings of the IP Test

The results of the IP test may be useful for determining the relative potency of one fiber compared to another and possibly can also be used to understand mechanisms of mesothelial carcinogenesis, at least at the molecular level (Kane, 1998). However, there are a number of reasons the 2002 IARC Monograph and evaluations sponsored by other regulatory bodies did not give the results of the IP test equal weight to the results of a well-conducted chronic inhalation bioassay. The shortcomings of the IP test have been discussed in detail elsewhere (McConnell, 1995; Rossiter, 1991; Hesterberg et al., 1991; Collier et al., 1995; and Eastes and Hadley, 1994). These shortcomings should also be discussed in the Draft Background Document on glass wool. Below, I provide an overview of the key criticisms of the IP test.

- Relevance to Humans
- Standardization of Method
- Validation of Method
- Fiber Number and Dimensions
- Maximum Tolerated Dose

Relevance to Humans: There are serious questions regarding the relevance of IP test results for cancer risk assessment in humans (Rossiter et al., 1991; McConnell 1995; Hesterberg et al., 1991). For the IP method to be relevant, one has to assume that exposure concentrations in the peritoneal tissues at the fiber injection site are comparable to tissue concentrations in the peritoneum after inhalation exposure. One would also have to assume that the inhaled fibers are unaltered by the lung and reach the peritoneal cavity in the exact form that was inhaled. In addition, one would have to assume that the normal physiological routes of removing the fibers, e.g. respiratory tract filtration, mucocilliary escalator- and macrophage-mediated clearance, are not functioning. As will be discussed later, none of these assumptions are valid.

Another important physiological issue that is not often appreciated when considering the relevance of the IP method is that it results in an exposure scenario that could never occur in humans. The "bolus dose" injected in IP studies produces a non-uniform distribution of fibers in the peritoneal cavity, i.e. massive concentrations are found at the site of injection and little or no fiber is found in other parts of the mesothelium (McConnell, 1995) . This results in "clumping" of the fibers which are then "walling off" by an inflammatory reaction. The encased fibers are then not available for removal or dissolution and breakage. This was confirmed by Collier et al., 1995, who showed long fibers (> 20 um in length) were much more biopersistent when injected into the peritoneal cavity than when inhaled into the lung. According to McConnell, 1995, the lesions seen on histopathological examination of the mesothelium after IP injection are never observed in the lung or mesothelium after inhalation exposure.

The issue of fibrosis is also a concern for establishing the potential carcinogenic potential of a fiber. Although there is no evidence that fibrosis causes lung cancer it is highly unlikely that fiber-related lung tumors will occur unless pulmonary fibrosis is also present (McConnell, 1995). This is likely a dose phenomenon, i.e. if the dose is sufficient to produce fibrosis, it is probably sufficient to cause lung cancer. Therefore, if a fiber does not produce pulmonary fibrosis it is unlikely to be a carcinogenic hazard to the lung, at least at the exposure level. A major problem with the IP method is that this endpoint (fibrosis) is not even evaluated (McConnell, 1995).

Standardization of Method: There is no "standard" way of conducting the IP test (McConnell, 1995). The dose levels to be used have never been defined. Another overriding concern is there is no specific standard for how the tissues are to be sampled and evaluated. It is uncertain whether one can rely on the "numbers" reported in the literature, because most of the original studies relied entirely on gross observations for determining whether a mesothelioma was or was not present. In fact, if there were no gross lesions, no histopathology was conducted on that animal (Pott et al., 1989). However, it would be difficult to design a standardized method of sampling the peritoneum for histopathology evaluation. One would have to devise a standard method of sampling the diaphragm, mesentery, surface of abdominal organs, peritoneal lining, etc. This has never been done in any of the IP studies (McConnell, 1995). The importance of this fact is that it has been clearly shown in well-conducted inhalation bioassays of asbestos and durable SVFs in rodents that many of the mesotheliomas were only found with microscopic examination. Adding to this analysis is that a dissecting microscope was instrumental at

necropsy for identifying many suspect lesions in the inhalation bioassays. A dissecting microscope is not normally used in IP tests (McConnell, 1995).

Finally, there is a high incidence (>10%) of mesotheliomas in some of the reported saline control groups in reported IP test results (Pott et al., 1987). Does this mean that physiological saline (the most common diluent for human and animal injectables) is a carcinogen? Saline has never, to my knowledge, caused neoplasms by any other route of exposure and it surely would have been recognized as such considering that it is one of the most common vehicles used in experimental studies. One cannot ascribe this high incidence of mesotheliomas in the saline control groups to spontaneous tumor development because the incidence of mesotheliomas in non-injected rats in these and other studies in this strain of rat (Wistar) is near zero (McConnell, 1995).

Validation of method: For any method to be used for human hazard and risk assessment, it needs to be validated (Rossiter, 1991; Hesterberg et al., 1991; McClellan et al., 1992; McConnell, 1995; Vu et al., 1996). With most methods this is typically done by using a set of "standard" materials, those known to be a human hazard for the endpoint of interest and those known not to be a hazard. This has not been done in any systematic way with the IP method. However, we can arrive at a reasonable conclusion based on the results reported in the literature. The results of greatest concern in this regard are the apparent carcinogenic activity of saline (noted above) and the tumors produced by non-fibrous inert particles. e.g. TiO2, black carbon, talc, and certain types of biosoluble glass fibers, e.g. insulation glass wool fibers (Pott et al., 1987), which have not shown a carcinogenic response at the MTD in well-conducted inhalation bioassays or in human epidemiological studies (Hesterberg and Hart, 2001). Thus, although the inhalation bioassay of SVFs is recognized as a valid method of hazard identification and risk assessment by internationally recognized scientists, the IP test is considered of only marginal utility for these assessments (IPCS, 1988; Hesterberg et al., 1991; WHO, 1992; McClellan et al., 1992; WHO, 1992; McConnell, 1995; Vu et al., 1996; NAS, 2000; U.S. ATSDR, 2004).

Fiber Number and Dimensions: It is likely that the number, dimensions (length and diameter), and chemistry of fibers that reach the peritoneum after inhalation exposure would be much different, especially for biosoluble fibers, than for fibers injected directly into the peritoneum in the IP test (McConnell, 1995). Dramatic changes in the dimensions and chemistry of inhaled biosoluble fibers have been seen in SVF inhalation studies (Hesterberg and Hart,

2001; Hesterberg et al., 1996b and 1996c). Furthermore, it has been shown that very few fibers actually reach the lung pleural mesothelium after inhalation exposure and that those fibers found in the pleural mesothelium are much shorter than those that were in the exposure aerosol (Bermudez et al., 2003). Even fewer fibers would be expected to reach the peritoneal mesothelium after inhalation, because of the long distance from the lung. Thus both the numbers and dimensions of fibers that reach the mesothelium after IP injection are vastly different than after inhalation exposure. These differences are related to the selective deposition and clearance of fibers in the lung airways during inhalation exposure, as well as the dissolution, breakage, and clearance of fibers that occur as fibers translocate to the pleural mesothelium (Hesterberg and Hart, 2001; Hesterberg et al., 1996b and 1996c). Because of the massive instantaneous doses of fibers that are achieved by IP injections, the dissolution, breakage, and clearance of fibers that are observed after inhalation exposure, do not have the opportunity to modulate the potential tumorigenicity of fibers administered by the IP route. Supporting this conclusion, Eastes and Hadley, 1994, developed a mathematical model based on fiber dissolution rates, which showed that that "all fibers regardless of their dissolution rate could be made to produce tumors in an IP experiment, if a high enough dose were administered" (Eastes and Hadley, 1994, p. S111).

Maximum Tolerated Dose (MTD): This is also a major problem with the IP method. As discussed earlier, a well accepted tenant when conducting inhalation carcinogenesis for fibers using bioassays is that the MTD should not be exceeded or if it is, the results at doses which exceed the MTD should be discounted or at least viewed with suspicion for determining human hazard (Lewis et al., 1989; Hesterberg et al., 1996a; McConnell, 1996; Oberdorster, 1995). As discussed previously, there are a number of ways to determine whether the MTD has been reached or exceeded in a chronic inhalation bioassay.

It is difficult to envision how one would establish an MTD for an IP test. Because massive doses of fibers are deposited instantaneously into the peritoneum, there is almost by definition an "overload" of clearance mechanisms using this methodology. This overload phenomenon is one way to define the MTD (Lewis et al., 1989; Oberdorster, 1995; McConnell, 1996; Hesterberg et al., 1996a). The clumping of fibers and formation of walled-off fibrogranulomas in the mesothelium after IP injection, is also considered to show that the MTD was exceeded in IP tests (McConnell, 1995). However, there is no scientifically valid method

for establishing the MTD for an IP study. This is why virtually any fiber, regardless of biopersistence, can be administered at a high enough dose to produce tumors in the IP test.

These are some of the problems encountered with use of the IP test, which factored into why the 2002 IARC Monograph did not give the results from this test equal weight to the results from well-conducted inhalation bioassays. The 2002 Monograph considered tumors resulting from SVFs administered by the IP route to be only "limited" evidence for carcinogenicity in animals, while they considered tumors found after SVF exposure in a well-conducted animal inhalation bioassay to be "sufficient" animal evidence for carcinogenicity.

Critical Assessment of the IP Test by International Regulatory and Scientific Agencies

The use of intracavitary routes of administration, including intratracheal (IT) and intraperitoneal (IP) injection, of fibers for hazard and risk assessment has been evaluated by a number of regulatory and scientific organizations. As discussed below, these agencies were generally critical of the use of the IP test for cancer hazard or risk assessment of SVFs. For example, more than 30 years ago, the National Institute for Occupational Safety and Health (NIOSH, 1977) compared inhalation and IP routes of exposure and concluded:

> "The routes of exposure used in many of the intrapleural and intraperitoneal experiments have been considered to be inappropriate to indicate the effects of fibrous glass after inhalation. It is not valid to extrapolate from the results from these intracavitary exposures to humans in the workplace."

In 1987, NIOSH (NIOSH, 1987) again compared inhalation and intracavitary routes of exposure and concluded:

> "Carcinogenic responses in animals, particularly rodents, following intrapleural or intraperitoneal fibrous glass administration is similar to the responses found after implantation of any foreign material such as polyethylene, asbestos, nylon, cellophane or Teflon [5 references given]. Tumor development in laboratory animals following pleural or intraperitoneal administration of fibrous glass

> material probably represents a non-specific foreign body response. The response depends on the physical characteristics of the fibrous glass, the most important being the size and shape; certain characteristics of the animal; and the length of time the fibrous glass is present in the animal (a critical factor). On the basis of present information, fibrous glass cannot be considered a carcinogenic agent."

The following year, the International Programme on Chemical Safety (IPCS, 1988), a division of the World Health Organization, reviewed the SVF animal study literature and warned against the over-interpretation of the results of injection and implantation studies:

> "The need for caution in the extrapolation of the results of studies involving injection or implantation in body cavities to predict the potency of various fibre samples, even with respect to the induction of mesotheliomas, cannot be overemphasized. The relevance of these types of studies to other types of cancer, such as lung cancer, has not been established." (Id., p. 97.)

In 1992, the World Health Organization (WHO, 1992) European Programme for Occupational Health, published a document titled "Validity of methods for assessing the carcinogenicity of man-made fibres." In this document, the WHO stated:

> "[T]he IP model is a non-physiological route of administration, bypassing all the lung defense and metabolic systems. Additionally, the dose in the IP test is administered as a single or small number of boli early in the life of the experimental animal, rather than through long-term incremental exposure (as occurs in humans)." (Id., p. 4.)

Later in this document, the WHO went on to state:

> "The IP model cannot be used for quantitative risk assessment, nor can the IP model be recommended at the present time for estimating the relative hazards of

> different fibres. There was no agreement as to whether or not the IP model could be used for hazard identification." (Id., p. 7.)

In 2000, the National Academy of Sciences published a report titled "Review of the U.S. Navy's Exposure Standard for Manufactured Vitreous Fibers" (NAS, 2000). In that review it was stated:

> "Intracavitary exposures, via either intraperitoneal or intrapleural injections, can produce a high incidence of mesotheliomas. Such exposures have been advocated as relatively inexpensive and highly sensitive tests to predict the carcinogenicity of inhaled fibers (Pott et al. 1989). However, this route of administration bypasses all natural pulmonary defenses, and the single dose (or a few repeated doses) is not physiologically based and can create an overload in the peritoneal or pleural cavity. Intracavitary tests can also yield false-positive results, for the assessment of lung cancer and mesothelioma risks. The WHO consultation (WHO 1992) concluded that the intracavitary model should not be used for quantitative risk assessment or for hazard evaluation of fibers." (Id., pp. 39-40.)

On May 8–10, 1995, a workshop on chronic inhalation toxicity and carcinogenicity testing of respirable fibrous particles was held in Chapel Hill, North Carolina. The workshop was sponsored by the U.S. Environmental Protection Agency (EPA), in collaboration with the National Institute of Environmental Health Sciences (NIEHS), the National Institute for Occupational Safety and Health (NIOSH), and the Occupational Safety and Health Administration (OSHA). The outcome of the workshop was published in Regulatory Toxicology and Pharmacology (Vu et al., 1996) and concluded that:

> "After extensive discussion and debate of the workshop issues, the general consensus of the expert panel is that chronic inhalation studies of fibers in the rat are the most appropriate tests for predicting inhalation hazard and risk of fibers to humans." (Id., p. 202.)

In 2002, IARC published a Monograph on the evaluation of the carcinogenic risk to humans of SVFs (IARC, 2002), which stated:

> "The intraperitoneal test, in which fibres are injected directly into the intraperitoneal cavity, bypasses the natural route of exposure. Because the lung is bypassed, the natural mechanisms by which the lung removes, dissolves or breaks fibres, thereby reducing or eliminating potential exposure of the pleural cavity, do not operate. Therefore the intraperitoneal test has no physiologically imposed maximum dose to which the animals can be exposed. (p. 38.)"

The IARC document further stated that the IP test could be used to provide information on the potential carcinogenicity of fibers if the proper positive control is used, but clearly the results of well-conducted inhalation bioassay were given more weight than the results of an IP test. For example, IARC concluded that the evidence for the carcinogenicity of insulation glass wool fibers in animals was "limited," because although insulation glass wool fibers produced tumors in IP tests, they did not produce tumors in well-conducted chronic inhalation bioassays. On the other hand, IARC concluded that the evidence for the carcinogenicity of special purpose fibers in animals was "sufficient," because these fibers produced tumors in well-conducted chronic inhalation bioassays, in addition to IP tests.

In 2004, the U.S. Agency for Toxic Substances and Disease Registry (U.S. ATSDR, 2004) of the U.S. Department of Human Health Services, published a review titled "Toxicological profile for synthetic vitreous fibers," which stated:

> "Although many animal studies administering various synthetic vitreous fibers by injection or implantation into the intrapleural or intraperitoneal cavities have reported the development of administration site non-neoplastic and neoplastic lesions (see Section 2.2.4, Other Routes of Exposure), these results are of limited usefulness for predicting health hazards in humans exposed by inhalation. Studies that exposed animals by inhalation to well-measured levels of respirable fibers are considered more appropriate for assessing potential risk to human health." (U.S. ATSDR, 2004, p. 85.)

Later in this same review document, ATSDR stated:

> "Intratracheal instillation, intrapleural implantation, and intraperitoneal injection studies with synthetic vitreous fibers have been performed. Most have been acute-duration studies (single administration followed by observation periods up to 2 years). The relevance of these studies to human inhalation exposure is unclear because of the high doses and rapid dose rates used, the bypassing of the natural defense systems of the nasal and upper respiratory system, and the overloading or lack (for intraperitoneal studies) of pulmonary clearance mechanisms." (Id., pp. 109-110.)

Recognition of the shortcomings of the IP test, including evaluations of the methodology by independent scientific and regulatory bodies, should be included in the Draft Background Document on glass wool fibers. The current version of the Document gives the false impression that this non-physiological route of administration should be given equal weight to a well-conducted chronic inhalation bioassay.

Summary and Conclusions

It is important to include in the Draft Background Document on the carcinogenicity of glass wool fibers information on the earlier 1988 IARC evaluation of glass wool fibers and explain why IARC differentiated insulation glass wool from special purpose fibers and downgraded the classification of insulation glass wool fibers from "possibly carcinogenic to humans (Group 2B)" in 1988 (IARC, 1988, p. 152) to "not classifiable as to their carcinogenicity to humans (Group 3)" in the 2002 Monograph (IARC, 2002, p. 339). Since this downgrading of the classification of insulation glass wool fibers involved a different perspective by IARC regarding the weight given to the intraperitoneal (IP) injection test compared to the chronic inhalation bioassay, the Draft Background Document should provide a more adequate critical evaluation of the validity of these two testing methodologies.

There was little discussion in the Draft Background Document of well-conducted chronic inhalation bioassays of Synthetic Vitreous Fibers (SVFs), which gives the impression that only a few fiber types have been assessed using the new chronic study model, when in fact many fiber types have been evaluated using this technology. The entire series of SVF inhalation studies should be summarized in the Draft Background Document, because this shows how this methodology can detect known human carcinogens, such as asbestos, and also differentiate biosoluble SFVs from biopersistent SVFs.

SVFs that produced tumors in the chronic bioassays were the more biopersistent fibers, while the non-pathogenic fibers had low biopersistence. This provides a rational mechanism by which fibers cause pathogenicity and adds weight to the validity of using the inhalation route of exposure to evaluate the potential hazard and risk of SVFs. Thus, discussion of biopersistence of all SVFs, not just glass wool fibers, is needed in the Draft Background Document.

The concept of Maximum Tolerated Dose (MTD) also needs more discussion in the Draft Background Document. Having a sufficiently high dose to thoroughly test the toxicity of an SVF, while avoiding the tissue overloading that results from doses above the MTD, are major issues that need to be addressed when conducting animal chronic carcinogenicity studies, including the IP test.

A number of serious issues have been identified with the use of the IP test to evaluate the potential carcinogenicity of SVFs. For these reasons, critical evaluations of the inhalation bioassay and IP test, which were sponsored by eight different regulatory and scientific organizations over the last 30 years, concluded that a well-conducted chronic inhalation bioassay is the most appropriate way to assess the potential hazard or risk from exposure to SVFs. The current version of the Draft Background Document gives the impression that the results of an IP test can be given equal weight to those of a well-conducted chronic inhalation bioassay, which is not the consensus of the world experts in the area of fiber carcinogenesis.

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Attachment:

List of Fiber Related Publications Co-Authored by Thomas W. Hesterberg, PhD, MBA.

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