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April 1, 1980

To: SPI-PVC Safety Group

Ladies and Gentlemen:

Enclosed herewith is your company's copy of a first draft of the transcript from the Vinyl Chloride and Polyvinyl Chloride Symposium held at the National Institutes of Health on March 20-21, 1980. We will, of course, send you a finalized copy with photographs of the slides presented as soon as it is available.

As you will be able to tell from a quick review of the transcript, nothing very new or startling was presented at the Symposium but the comments of a number of the presenters will provide considerable meat for comment. In this regard, the PVC Health Committee will be meeting on April 10, 1980 to formulate comments on that which transpired at the Symposium for submission and response to the Occupational Safety and Health Administration's Request for Information on Vinyl Chloride and Polyvinyl Chloride.

Cordially yours,

Joseph E. Hadley Jr./sb

Joseph E. Hadley, Jr.

Enclosure

ORIGINAL

Transcript of Proceedings

CONFERENCE TO REEVALUATE THE TOXICITY OF VINYL CHLORIDE, POLYVINYL CHLORIDE AND STRUCTURAL ANALOGUES

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peculiar toxicities of vinyl choloride, polyvinyl chloride polymer, and various analogues, and I think most particularly to review and keep up to date on the ongoing, continuing epidemiological studies.

I would like to thank very specially the program planning committee which did the large amount of work necessary for putting on such a meeting: Dr. Everson, Dr. Infante, Dr. Landrigan, Dr. Shapiro, and Dr. Waxweiler. I think they have done well, indeed.

Therefore, without further ado, let me thank you again for coming, and introduce Dr. Umberto Saffiotti of the National Cancer Institute who will chair the first session.

Dr. Saffiotti.

DR. SAFFIOTTI: We would like to have the speakers for the first session come to the podium here.

The first session is devoted to carcinogenicity bioassays of vinyl chloride monomer. The first speaker is Professor Maltoni from the Institute of Oncology at Bologna, who will speak on the carcinogenicity bioassays of vinyl chloride monomer, a model of risk assessment on an experimental basis.

Dr. Maltoni.

XXX

DR. MALTONI: Mr. Chairman, colleagues, ladies and gentlemen.

The present record deals with the representation of

rmg 3

our projects of long-term carcinogenicity bioassays on vinyl chloride. To our knowledge, this project is the most extensive experimental study performed on one industrial compound by a single institution.

The experiments of the project were applied with the following aims:

One, to test the carcinogenicity of the compound;

To obtain information on the site and type of tumors;

To evaluate the possible effects of the route of administration, with particular regard to the ones reproduced in potential human exposure;

And finally, to assess in quantitative terms the risk.

The planning of the experiment was aimed at the achievement of these finalities. The compound was tested on animals of different species, strains, and sexes.

Slide, please.

(Slide.)

In that case, rats, mice, and hamster. And two strains of rats, the Sprague-Dawley rat and the Wistar rat; Swiss mice; and golden hamster of both sexes. And we tested adult from 10 to 21 weeks old; newborn; and on embryos.

This was done, since it is known that these factors may modify in qualitative and quantitative terms the neoplastic responses.

rmg 4

Choice of the animal was made with the intention of having an integrated system of complementary models which could express the range as large as possible of neoplastic responses.

VC was administered by three different routes, encompassing inhalation and ~~digestion~~ injection, which are the two means of potential exposure. The monomer was administered in different concentrations, namely, 14 by inhalation and 6 by ingestion for various periods of time, by continuous and intermittent treatment.

(Slide.)

The route was ingestion by inhalation, ingestion by stomach tube, intraperitoneal injection, and subcutaneous injection.

Next slide, please.

(Slide.)

As I told you already, by inhalation obtaining from those tested, 4 times daily, 5 days weekly, for 52 weeks, ranging from 30,000 ppm down to 1 ppm.

Another schedule was chosen, and the range of doses by inhalation was tested 4 hours daily, 5 days weekly, for 17 weeks. Then 2 doses, 10,000, 6000, were tested for 4 hours daily, 5 days weekly, for 5 weeks; or for 4 hours daily 4 days weekly for 25 weeks; or for 1 hour daily 4 days weekly for 25 weeks; or for 4 hours daily for 7 weeks.

rmg 7

0.01 milligram per kilogram. In this second experiment, the animals were treated 4 times weekly for 52 weeks. And in the second, 5 times weekly for 52 weeks, except for a few animals the experiment was prolonged for 59 weeks and then stopped because of some intolerance of the animal.

The next slide, please.

(Slide.)

And then, as I told, the compound had been tested by injection, intraperitoneal and subcutaneous.

VC was always supplied by the same source, and it contained a very low amount of impurities.

Next slide, please.

(Slide.)

The oil which was employed as a vehicle for ingestion and ingestion studies was pure virgin olive oil from Tuscany. The animal, apart from the golden hamsters, were types which have been routinely employed for many years and they undergo periodic examination and complete autopsies, giving us extensive information concerning their basic pathology.

For inhalation exposures, they are built basically on stainless steel and glass.

Next.

(Slide.)

To control the level of exposure in the inhalation

rmg 8

experiment, an automatic gas chromatography system was used.

Next slide.

(Slide.)

The fallout of the chamber atmosphere is decontaminated --

Next slide.

(Slide.)

-- before it is reintroduced into the atmosphere in order to avoid polluted air.

Next slide, please.

(Slide.)

For the experiment on VC, as well as for any other experimental bioassays performed in our laboratory, the procedure has been always the same: highly standardized and controlled. In particular, the following points in our laboratory are standard procedures, should be emphasized:

First, dealing with the compound.

All the supplies of the compound used were examined in order to determine whether they met the required standards.

Two, concentration. The concentration, particularly when the compound was given by inhalation, was controlled by continuous gas chromatography monitoring.

Three, modality of treatment. Treatment was always performed by the same people. This is particularly important for gavage, since the animals become accustomed to the same

rmg 9

concentration.

Four, control of the animals. The control of the animals status was performed at least three times daily. Every two weeks the animals were submitted to examination for the detection of any gross change.

Five, weight of the animals. The animals were weighed every two weeks during treatment, and eight weeks after the end of the treatment.

Six, end of treatment. In the VC project, as in any long-term bioassay performed in our laboratory, the animals are kept alive until spontaneous death.

Seven Autopsies. Full autopsies were performed on each animal. All of the different parts of the body were explored, including the central nervous systems; specimens for histology included the brain, Zymbal glands, in trascapular brown fat, salivary glands, tongue, lungs, liver, kidneys, adrenals, spleen, pancreas, stomach, intestines, bladder, uterus, gonads, and any other organ with pathological changes.

Eight, histology. Spacing it with three is the standard way.

All the special techniques. For many specimens, serial cellular sections have been made. This was particularly useful in the case of lung in mice, and for the brain in rats, where supergross microscopic tumors could have been detected only by serial sections.

rmg 10

Classification of data. All the gross and macroscopic observations were classified and coded following our laboratory code. For each animal an individual file card was finally prepared which included data on experimental factors, survival, weight at 6, 12, 18, and 54 months, and any gross and macroscopic lesions.

Next slide, please.

(Slide.)

This is a prototype, a model of our card. These data are given on the animal: type of exposure, concentration, protocol of treatment, species, strain, sex, then the weight at different times, the age at death, the period from the start of the treatment, and weight.

Next, please.

(Slide.)

On the other side, all macroscopic and microscopic changes coded. This has been done for all our 7000 animals of the experiment.

The result of all the VC experiments, as well as the ones of any other experiment performed in our laboratories, will be presented in the final report now in press with the same type of tables in the same sequence. This type of presentation has been made possible by the knowledge of the basic pathology of the animal used, which enables us to make an approximate census of the suspected lesions.

rmg 11

Such a procedure permits a quick comparison among the results of different experiments of the same project, and possibly of the results of projects studying different compounds, enabling us to make a comparative index of the degree of risk presented by different industrial products.

For the interpretation of the data, they have been submitted for statistical analysis, some of them, at least. The one dealing with the seven basic experiments -- and I use this term to design the five experiments with Sprague-Dawley rats studying continuous inhalation for 52 weeks from 30,000 ppm down to 1 ppm; and the two experimental studies studying the ingestion exposure, studying the effect of a range of doses from 50 milligrams per kilogram of body weight down to 0.3 per kilogram of body weight.

Although statistical analysis supplies a very important tool for interpreting the results of long-term bioassays, it should be stressed -- and I wish to stress here -- that there may be smaller differences between exposed and controlled groups which do not reach the statistical significance while this difference still has a meaning from the oncological point of view, particularly in the cases of tumors which are very infrequent in our animals.

Therefore, the most important data should be commented upon in light of the statistical analysis performed and with the biological approach. The methodology protocol

rmg 12

adopted meets the requirement of the recent Good Laboratory Practice Act.

Now we come to the results. Part of the results I am presenting, namely, the one dealing with the seven basic experiments studying the effect of long-term exposure to a range of 14 doses by inhalation and six by ingestion in Sprague-Dawley rats, were presented November 10, '79 at the meeting of the Club de Cancerogenie Chimique at the Institut Curie in Paris, France. The abstract is now in press and will be available in a few weeks.

As an extension of the result of the whole project with the table, it will appear soon published.

We are presenting only tables summarizing only the most outstanding results and information on what we believe to be the integrated documentation and the strictly necessary comments.

In the following table we are presenting data on the incidence of the total malignant and benign tumors, and the tumor which has been considered as dependent or possibly correlated more specifically to this exposure in the 17 different experiments which study the effects that we see in the different types of systems by different dose levels.

Next slide, please.

(Slide.)

This is experiment BT-1, studying the effect of the compound for 52 weeks, from 10 down to 50 ppm. It was a very

rmg 13

fast experiment, and I pointed out here malignant tumors, total malignant tumors refer to 100 animals, liver angiosarcomas, liver angiomas, extrahepatic liver angiosarcomas, extrahepatic liver angioma, hepatomas, nephroblastoma, neuroblastoma, Zymbal gland carcinoma, skin epitheliomas, prestomach papillomas and acanthomas, mammary malignant tumors. And the tumor will be always designed in the same way.

So, as you see, we have the onset of liver angiosarcoma down at the 50 ppm. We have also fewer, the onset of extrahepatic angiosarcomas, the appearance of extrahepatic angioma.

Also, in untreated controls, we had a few cases of hepatomas. We had quite a striking prominent onset of nephroblastoma. We had neural blastoma, up to 2500 ppm. Zymbal carcinoma down to 500 ppm. We had some cases of skin epitheliomas, some results in control. And we had a few cases of mammary malignant tumor.

Next slide.

(Slide.)

In this experiment, the dose was 200, 150, and 100, with this control. And again, here we got enhancement of total malignant tumors. And when compared to control, the appearance of liver angiosarcoma, of extrahepatic angiosarcoma, of liver angioma, of nephroblastoma, fewer Zymbal gland carcinoma compared to control, fewer skin epitheliomas in the higher doses, fewer mammary malignant tumors.

rmg 14

Next slide, please.

(Slide.)

This comes out at 30,000 ppm, which was the high doses tested. And we got-liver angiosarcoma in 30 percent of the animals. We did not get nephroblastoma. We got very little neuroblastoma. We got many Zymbal gland carcinoma, very early response of Zymbal gland made unpossible for other tumor to rise. These animals had been dead after 68 weeks, all because of Zymbal gland carcinoma or because of liver angiosarcoma. Next slide.

(Slide.)

This is an integrity experiment studying on the larger group 50 ppm, at least in 1973-74, seems to be a crucial level. Here we got near 5 percent of liver angiosarcoma. We got liver angiomas, extrapatic liver angiomas, very few other tumors. And we got quite an amount of mammary malignant tumors.

Next slide.

(Slide.)

This is a slide 1815, showing the effect of 25, 10, 5, and 1 ppm, where we have a few cases of liver angiosarcoma at 25, and one at 10. One liver angiosarcoma at 25 ppm. We have few vascular tumors, extrahepatic vascular tumors. We didn't get very much Zymbal gland carcinoma. Some incidence at the higher dose, but an enhancement of mammary tumor in the four treated groups versus the control, are some not

rmg 15

dose-correlated.

Next slide, please.

(Slide.)

With 33, we studied initial doses from 10,000 to 50 ppm, and appeared immediately, but delivered only for 17 weeks instead of 52 weeks, and immediately the drop of liver angiosarcomas, reducing 1/3 the time of treatment. We got few hepatomas. We got still nephroblastoma, although with a lower incidence.

We got Zymbal gland carcinoma at an incidence, it seems, more near to the group treated for 52 weeks. I think there was a dichotomy. Some tumors are affected by the end of the treatment more than others. Liver angiosarcomas seem to be much more affected than Zymbal gland carcinomas.

And we got an announcement of skin epitheliomas and as you see, a high percent of neuroblastomas.

end #1

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DAV/whd-1
2

(Slide)

This slide, the 100 hours of treatment scattered in different ways. The different ways are the first two groups aretreated five days a week for five weeks, four hours daily, five days weekly for five weeks; the second group, one hour daily four days weekly for 25 weeks. The third, four hours daily once weekly for 25 weeks.

10,000, 6,000 on these different scales, and once again there is an onset. There is a group of very few liver angiosarcomas. When compared with continuous long-lasting treatment, we got many of the tumor types here observed with the long-lasting treatment, but to a very low percent, so feeling that the length of treatment is affecting very much the onset of VC.

The most responsive also to this short treatment is the Zymbal gland, as it has appeared already in the experiment in which four modes of treatment were studied. Also there is a slight increase in malignant mammary tumors.

Next slide, please.

(Slide)

This studies the effect of transplacental exposure. These are the models treated with 10,000-6,000's, and these are the offspring of models treated with 10,000 and 6,000. We are observing offspring nephroblastomas, and Zymbal gland carcinomas are the most important tumors. At 6,000 again, a

whd-2

high incidence of Zymbal gland carcinomas, a few skin epitheliomas, a few hepatomas and mammary tumors, but the most important data is nephroblastomas and Zymbal gland carcinomas.

This treatment was for seven days. It seems to be enough to produce the first perenctile.

Next slide, please.

(Slide)

DR. MALTONI: This is an experiment studying the effect of age. Mothers and offspring, newborn, were exposed for five weeks, four hours daily, five days weekly; these two doses -- 10,000 and 6,000 ppm, and it appears that several of the tumors which we see dependent arise from this condition, but what was highly impressive is the extremely high onset of liver angiosarcomas. They arise in 74 percent, and of hepatocarcinomas, and 45 and 47 percent.

It seems that the liver of newborn mice exposed is highly responsive.

Next slide, please.

(Slide)

DR. MALTONI: Then we come to Sprague-Dawley rats, only males treated for 52 weeks. Here again we have liver angiosarcoma. This strain responded like Sprague-Dawley, positively. We got a few cases of liver angiomas, a few cases of extraliver angiosarcomas, a few cases of hepatomas, we have said nephroblastomas, again neuroblastomas, and again a lower

whd-3

percent of Zymbal gland carcinoma and a few cases of skin epithelioma.

Next slide.

(Slide)

Then we studied the effect of 1 ppm on Wistar rats. We didn't get -- we got more Zymbal gland tumor, no effect on neuroblastoma, but we got some cases of extrahepatic angiomas and angiosarcomas, which are more difficult since these tumors are not rare in the rats we used.

Next slide, please.

(Slide)

These are the data dealing with the effect of 30 weeks of exposure at a range of six doses on Swiss mice. And here again we observe, as in the two previous strains of rats, liver angiosarcoma, liver angiomas, extrahepatic angiosarcomas, extrahepatic angiomas, a very high percent of lung adenomas, down to 250.

At 50 ppm we already had figured there to the untreated control, but down from 10,000 to 250, we get a very high percent of these tumors. We're very surprised, and we have got this figure of our animals. Of course, it's a liver section in treated and controlled ones; we got an enhancement of memory carcinoma down to 50 ppm, we got a few cases of skin epitheliomas in at least three treated groups versus control, and we got a few cases of pre-stomach papillomas and angiomas.

whd-4

The control group is 150 animals.

Next slide.

(Slide)

After we studied the effect on the golden hamster, again male animals for each group -- 30 male animals for this group and 30 male animals in the control group. We saw a few cases of liver angiosarcomas, few cases of liver angiomas, few cases of extrahepatic angiosarcoma; we got also some cases of cholangiocarcinoma.

We didn't get variants; we get more, as a matter of fact, cholangiomas than in the treated groups, but we didn't get as many in the control group as we did in the treated ones.

We got several tumors of the acoustic duct, which is not the Zymbal gland, but they are tumors of the squamus epithelium lining the duct. We got enhancement of the skin tumors.

We have seen in the little group some cases of mellanomas, but an enhancement of stomach papillomas, and this slide announces an enhancement of leukemias, but at this point it should be stressed that in the treated group it is much lower than in the control group. Time of latency is much lower in the treated group than in the control group. 16, 27, 10, 19, 22 in the treated group; 75 weeks in the lower group and 76 weeks in the control group.

whd-5

Next, please.

(Slide)

Here we bring the data by ingestion, always with the Sprague-Dawley rats, which is our most studied system. We got an enhancement of total malignant tumors in the group at 50 mgs and at 16 mgs, versus the other group, the lower dose group and olive oil.

We got liver angiosarcomas in the two groups with the highest doses. We observed liver angiosarcomas in the group treated with the highest, and in the lower doses, and we got some of -- a very loose number of all sorts of tumors that we had observed in inhalation, at least, at the higher doses.

Next slide, please.

(Slide)

Then in the second experiment, three lower doses were studied, the one mg, 0.3 mgs and 0.03 mgs, and still we observed in this experiment, using the latter group, two cases of liver angiosarcomas at 0.3mg per kg as well as scanty cases of other tumors correlated more or less to technique.

No angiosarcomas, no nephroblastomas, neuroblastomas, Zymbal gland carcinomas, hepatomas, were observed in the 0.03 mg per kg of body weight.

Next slide, please.

(Slide)

whd-6

Finally, we have studied the effect of intraperitoneal injection in these cases; again, we got few vascular type tumors, extrahepatic vascular type of tumors, but the very small figure, and the meaning of this figure, is very questionable.

Howver, I think that the fact that we have seen this tumor in the treated group and not in the control group may mean something.

Next slide, please.

(Slide)

This is by subcutaneous injection, where the only data is the onset of one nephroblastoma, which is extremely rate as a spontaneous tumor in the animal treated with one subcutaneous injection.

I would like very briefly to deal with pathology, to show some examples of the tumors we got.

Next slide, please.

(Slide)

This is a very well-differentiated angiosarcoma in the Sprague-Dawley rats.

Next slide, please.

(Slide)

This is a really well-developed angiosarcoma in Sprague-Dawley rats' liver.

The next one?

whd-7

(Slide)

This is a metastasis, lung metastasis of the previous angiosarcoma --

(Slide)

-- again a magnification in the metastasis.

Next?

(Slide)

This is a more differentiated liver angiosarcoma, always in Sprague-Dawley rats.

Next.

(Slide)

This is only a lung metastasis.

Next.

(Slide)

This is a very well-differentiated liver angiosarcoma in a Wistar rate.

Next.

(Slide)

With this lung metastasis.

Next.

(Slide)

This is a poorly differentiated angio -- liver angiosarcoma in a Wistar rat.

Next.

(Slide)

whd-8

This is lung metastasis.

Next.

(Slide)

This is liver angiosarcoma in mice, in the mouse.

Next.

(Slide)

This is, again, another angiosarcoma in the mouse, undifferentiated.

Next.

(Slide)

This is a lung metastasis.

Next.

(Slide)

Another metastasis.

Next.

(Slide)

This is a liver angiosarcoma in a golden hamster.

Next.

(Slide)

This is a lung metastasis.

Next.

(Slide)

This is an angioma, characteristic angioma, in a Sprague-Dawley rat.

Next.

whd-9

(Slide)

This is an angioma in a Swiss mouse.

Next.

(Slide)

This is what we call -- and I comment a few minutes -- what we call a dysplastic proliferation of lighter cells. You see the sub-sinusoidal is prominent over the liver.

Next.

(Slide)

This is still a more clear type of this dysplastic lesion of sinusoidal cell.

Next .

(Slide)

This is a peritoneal angiosarcoma in a rat.

Next.

(Slide)

Magnification.

Next.

(Slide)

This is a lung metastasis of this tumor.

Next.

(Slide)

This is an intracardiac angiosarcoma.

Next.

(Slide)

whd-10

This is lung metastasis.

Next .

(Slide)

This is a kidney angiosarcoma.

Next.

(Slide)

Next slide.

(Slide)

This is a spleen angiosarcoma.

Next.

(Slide)

This is a peritoneal angiosarcoma in the mouse.

Next.

(Slide)

This is a hepatocarcinoma of the type we see in the adult animal.

Next.

(Slide)

This is magnification.

Next.

(Slide)

This is a liver metastasis.

Next.

(Slide)

This is a hepatocarcinoma of the type we most often

whd-11

see in newborn animals.

Next.

(Slide)

This is a lung metastasis.

Next.

(Slide)

Another case, more differentiated.

Next.

(Slide)

Again, this is all lung metastasis.

Next.

(Slide)

Is all metastasis.

Next.

(Slide)

This is a case of a combined tumor -- hepatocarcinoma and angiosarcoma, both associated.

Next.

(Slide)

This is all lung metastasis.

Next.

(Slide)

This is a case in which the hepatocarcinoma and the liver angiosarcoma are associated but not in the same place; are near one to the other.

whd-12

Next.

(Slide)

But we got this liver metastasis. This is angiosarcoma, and hepatoma.

Next.

(Slide)

This is a typical kidney tumor, often observed in our animals.

Next.

(Slide)

Magnification.

Next.

(Slide)

Again another type of incidence of this type of kidney tumor.

Next.

(Slide)

Lung pleural metastasis.

Next.

(Slide)

This is a neuroblastoma in the liver.

Next.

(Slide)

Magnification.

Next.

whd-13

(Slide)

Typical case of neuroblastoma, again.

Next.

(Slide)

This is a metastasis of this neuroblastoma, lung metastasis. We got three cases of this metastasis.

Next.

(Slide)

This is a Zymbal gland carcinoma.

Next.

(Slide)

Magnification.

Next.

(Slide)

With lung metastasis.

Next.

(Slide)

Squamous carcinoma, Zymbal gland.

Next.

(Slide)

With lung metastasis again.

Next.

(Slide)

This is again a Zymbal gland carcinoma, squamous type.

whd-14

Next.

(Slide)

With brain metastasis.

Next.

(Slide)

These are the adenomas of a usual type we saw in other animals, but some of these adenomas have early materialization.

Next.

(Slide)

This is a detail of this adenoma.

Next.

(Slide)

This is an adenoma pseudo invading the bronchus.

Next.

(Slide)

This is one of these adenomas undergoing early malignant changes. Next.

(Slide.)

This is a mammary carcinoma in rats of the usual type. Next.

(Slide)

With a lung metastasis.

Next.

(Slide)

This is a type of adenocarcinoma that we saw in

whd-15

mice, often with squamous hyperplasia.

Next.

(Slide)

Magnification.

Next.

(Slide)

These are repeated in the lung metastases .

Next.

(Slide)

Which you may see in detail of the liver metastasis.

Next.

(Slide)

This is a squamous carcinoma of the usual type we see,

(Slide)

but we also often see sebaceous gland carcinomas in skin of this type.

Next.

(Slide)

This is squamous carcinoma in mouse.

Next.

(Slide)

Again, another squamous carcinoma in the mouse.

Next.

(Slide)

whd-16

This is the usual type of tumor we got in hamsters.

Next.

(Slide)

This is a particular feature.

Next.

(Slide)

This particular feature --

Next.

(Slide)

This is a type of melanoma we got in the hamster.

Next.

(Slide)

Magnification.

Next.

(Slide)

Or an obscure malignant type like this one.

Next.

(Slide)

This is a type of papilloma of the stomach we have said, in rats.

Next.

(Slide)

The type of papilloma we observed in mice.

Next.

(Slide)

whd-17

The type of papilloma we observe in hamsters.

Next.

(Slide)

Acantoma of the stomach.

Next.

(Slide)

This is a type of leukemia that we saw in the hamster.

Next.

(Slide)

A liver localization.

(Slide)

A renal localization.

Next.

(Slide)

I think that's all.

The data on those exposed to long-term treatment by inhalation and by ingestion is the basis of the experiment on Sprague-Dawley rats, which is the most important experiment we have performed, the one giving us the more precise information on dose response relations.

Here in this table we try to summarize, starting from 30,000ppm down to 1ppm the incidence of liver angiosarcomas, of liver angiomas, and also of pre-cursor lesions, such as dysplasia of the sinusoidal lining cells, and what is for

d-18

us of some interest is to show that at 10ppm we have a sort of extinction of liver in our system, at least of liver angiosarcomas, of liver angiomas, but also of dysplastic and neoplastic lesions.

It was interesting for us to see this beautiful type of correspondence between tumors, between pre-cursor lesions.

Next slide, please.

(Slide)

The same evaluation we have done for liver extrahepatic angiosarcomas, and again we obtained this line, extrahepatic liver angiomas.

Next.

(Slide)

When we go to hepatocarcinomas, again, we monitor hepatocarcinomas, neoplastic or dysplastic nodules, nodular hyperplasia, and diffuse hyperplasia, and again, we see at 10ppm we have no more lesion, at 10ppm no tumor, at 50ppm, no tumor for this type of dysplasia; also at 10ppm we can say there is some stopping of the incidence of nodular hyperplasia and diffuse hyperplasia.

At 5 and 1 ppm we got more or less the same value as we have gotten in controls.

Next slide.

(Slide)

whd-19

And again, here are exposed in sequence by doses, the incidence of nephroblastoma, male, female, and total.

Next.

(Slide)

Here is neuroblastoma, which stopped at 2,500.

Next.

(Slide)

Here we have the Zymbal gland carcinonmas, the incidence which, at 5ppm and 1ppm is of the same value as our controls.

Next.

(Slide)

Here we have pre-stomach papillomas, which in Sprague rats are siqnificant only at the high doses of 10,000 ppm.

Next .

(Slide)

Here we have the incidence of liver angiosarcomas and liver a ngiomas in dysplastic and neoplastic lesions, when the compound is delivered by ingestion. Here again, we are sort of stopping at 0.3.

Next .

(Slide)

Here is the rat extrahepatic angiosarcoma and angiomas in solution.

whd-20

Next.

(Slide)

Here we have, by ingestion, the incidence of hepatoma neoplastic nodular hyperplasia and diffuse hyperplasia, and we see that the neoplastic nodules seem to stop at 0.3.

Next.

(Slide)

Here is a rat, the incidence of nephroblastoma in the rat by ingestion, stopping at 16.65.

Next.

(Slide)

We have no neuroblastoma by ingestion.

Next.

(Slide)

This is incidence of Zymbal gland carcinoma. There is no more of this tumor at 0.3.

Next.

(Slide)

Here we have the distribution, which is not significant at all, of pre-neoplastic papillomas.

Next.

(Slide)

Here we have the incidence of liver angiosarcoma and Zymbal gland carcinoma. Two tumors which behave in a different way when the compound is even at the same dose,

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10,000 and 6,000, but at different times. 52 weeks, 17 weeks, and 100 hours, with different schedules, and we see a drop of liver angiosarcomas with a reduction of the type of exposure from 11 down to 0.8, from 22 down to 0.8.

Meanwhile, we have, of course, a drop in the Zymbal gland carcinomas, but less evidence than in the liver angiosarcomas.

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(Slide.)

We wanted also to compare with this table the incidence of liver angiosarcoma in different types of animals. One may see that in the Wistar rats, Sprague-Dawley and Swiss mice — the Swiss mice are the more senstive ones, while the golden hamsters are the least sensitive of the species. Next —

(Slide.)

Here, there is the effect of strain, for example, in Zymbal gland carcinoma, they may be very —Sprague-Dawley mice are much more responsive than Wistar rats. Next —

(Slide.)

And here, there is the effect of age. As I told before — and in comparing newborn animals and one-week-old rats, one may see how striking is the difference in the onset from 74 to 0.8 and 14. Next slide.

(Slide.)

In conclusion, we see dependent tumors have been identified in our experiment on the basis of one or more of the following parameters: sharp peak in incidence, rare or restricted occurrence in the colony of animal use, dose response relationship, association of the dose relations. From the presented data, before a conclusion may be drawn, we see tumors in all the different animal systems tested. We see the multipotential carcinogens. Since it causes

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tumors on different types and different sizes. Slide, please.

(Slide.)

You may see that all that sites in one or more of the systems tested, we got tumors. We show carcinogenic effects both given by inhalation and ingestion in the rat, some indication of VC correlated tumors by injection. Some kinds of tumors are seen in all the animals studied, like for example, angiosarcoma. Others were found only in one animal system.

The degree of evidence of correlation between VC treatment and the tumors that we see are VC-dependent varies from tumor to tumor. Throughout inhalation and injestion experiments, a dose-response relationship has been observed. The duration of the treatment greatly affected neoplastic response.

The neoplastic response in qualititative and quantitative terms is greatly affected by the species, the strain and the sex and the age of the animal studied. Newborn animals appear to be extremely responsive, and easily develop liver tumors, both hepatocarcincmas and angiosarcoma. We have now going on a new project to assess the relative responsiveness of newborn rats to several monomers, encompassing an extension of our VC studies.

VC produces carcinogenic effects on

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embryos via placenta. We have set criteria for the VC-dependent tumors. We see it show carcinogenic effect also at low doses, namely down to 50 ppm and even less. The result of the seven basic experiments studying the effect of the dose of VC when given by inhalation and by ingestion has been submitted to statistical analysis following the Fischer exact probability test.

The total cancerogenic animals and the tumors significantly in excess in this experiment in relation to dose are given in the following table. Next slide, please.

(Slide.)

All animals bearing cancer, in males, by inhalation — there is, at the Fisher exact probability test 0.05 significant enhancement down to 50 ppm and 50 mg per kg body weight; in females, by 50 mm per kg of body weight, when given by ingestion.

(Slide.)

And they are given this — as we study this significance of the incidence of different tumors at different dose levels. The Fischer exact probability test at 95 percent confidence is in relation — not sensitive enough in our experimental condition. Biologically, in our opinion, the following result, although not statistically significant, according to the tests used, should be given proper attention for what extent extrahepatic angiosarcomas of different sizes these

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tumors are observed at a very low incidence dose in Sprague-Dawley rats in our colony.

The results of experments BT-1 and particularly BT-9 strongly suggest a relationship between this tumor and VC exposure. This relationship is reported a successive incidents in extrahepatic tumors in mice, as we see. For one percent hepatomas there are few cases when hepatomas have been observed in treated groups, particularly in BT-1. This tumor is exceptionally rare in our colony of animals and none have been observed in the control group of the 17 experiments.

Moreover, the relationship with treatment is supported by the fact that the high incidence of hepatomas has been observed in Sprague-Dawley rats following neonatal exposure to high doses for short periods.

For the Zymbal gland carcinoma, an excess of this tumor is observed, down to 50 and 25 ppm. For angiosarcomas this tumor is extremely rare in our colony. We have only seen four cases over several thousand treated animals; therefore, one must consider the onset of this tumor as important even at doses not shown by statistical analysis, in particular below 50, where we have five liver angiosarcomas at 55 ppm, one liver angiosarcoma out of 150 animals at 10 ppm, and at one mg per kg body weight, three liver angiosarcoma out of 150 animals and one liver

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angiosarcoma out of 150 animals.

The meaning at the results of the lowest doses may be better evaluated in considering not singly but together the tumors found to be VC-dependent. Next slide, please.

(Slide.)

In this table, we may see at 25/120 animals, we have five liver angiosarcomas, four Zymbal gland carcinomas and one nephroblastoma. At 10 we have 120 animals, one liver angiosarcoma, two extrahepatic angiosarcomas and two Zymbal gland carcinomas. At one mg per kg body weight, for over 150 animals, we have three liver angiosarcomas, one extrahepatic angiosarcoma, one hepatoma and five Zymbal gland carcinomas. At 0.3/150 animals, one liver angiosarcoma and one hepatoma.

No increase of specifically VC-treated tumor, shown in this slide, was shown in the seven basic experiments to Sprague-Dawley rats at doses below 10 ppm, when given by inhalation, and 0.3 ppm when given at 1.3 mg per kg body weight, when given by injestion.

The VC long-term experimental studies led to a discovery of VC carcinogenicity and that has a direct consequence to what has probably been the greatest effort ever made at controlling exposure to industrial carcinogens in work place. Moreover, long-term carcinogenicity by VC is a crucial step in the field of environmental carcinogenesis,

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which I am sure are among the most important areas in public health nowadays.

This study has demonstrated that long-term carcinogenicity bioassays (1) may predict carcinogenic risk for humans ; (2) may give indication of the level of risk in relation to dose; (3) may provide information on the possible target organs, and in general terms on the quality of neoplastic response; (4) may represent a tool for obtaining information on the relative risk represented by different compounds, provided that they are tested under the same sort of conditions and with the same protocol.

Next slide, please.

(Slide.)

You may see here the data on rats and mice when given by inhalation, compared with the effect of vinyl dichloride and often ethylene dichloride, just performed in our institute under the same experimental condition, by the same people, with the same properties. You see in vinyl chloride we got the effect on this organ — in this, we found no response in right and only response on two sites in mice and no response in this system with ethylene dichloride. This, for us, is a very important type of information on relative potency of compound in animal systems.

Finally, the bioassay of vinyl chloride reveals the need to identify animal systems more equivalent to human

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neoplastic response, which in turn depends on partly known factors, such as basic spontaneous tumorigrams and enzymatic profiles.

To conclude, the most important goal at present in evaluating carcinogenesis is, in our view, the extrapolation of results from animals to humans, both in qualitative and quantitative terms. VC carcinogenicity may provide an important tool to watch on this problem. We know now a lot about the effect of VC in experimental animal systems, both in qualitative — but also in quantitative terms.

On the other hand, epidemiological investigation on occupationally exposed populations will be made, and are being carried out, in different parts of the world, particularly Western Europe and the USA, with reference to general pathology and neoplasia.

If this epidemiological investigation provides precise figures on the whole we consider, including figures on the length of the exposure so as to define the homogeneous exposed group of workers and collect all possible available data on pathology, we see an opportunity — unique at present — to compare animal and human data both in qualitative and quantitative terms, and to help to find a possible key for extrapolating from animal to human. Thank you.

(Applause.)

DR. SAFFIOTTI: I wish to congratulate

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Professor Maltoni for the detail, accuracy and monumental extent of his study on vinyl chloride effects in several species of experimental animals. We have about seven minutes before the scheduled start of the next speaker and I would suggest that we use this time to have discussion addressed to specific methodological questions or clarifications of Dr. Maltoni's data.

Then we have a discussion period at 10:00 o'clock, which we can use for more general discussions, after Dr. Suzuki's paper. We'd like to have the people who are going to participate in discussion give their name and affiliation for the record, and speak into the microphones. Are there any questions to Dr. Maltoni?

DR. TAMBURRO: Dr. Maltoni, the same premalignant — if I may use that word — kind of focal nongeneral hyperplasia, focal areas such as the liver, has also been seen in man. Have you seen with your animals other histological lesions that would adhere to that interpretation? For instance, in man we have problems of fat and other evidence of injury that give the same kind of histological lesion.

DR. MALTONI: The series of changes to which we paid attention were parenchymal. The parenchymal was, of course, hepatocarcinomas, what we call dysplastic or dinodule, clear-cut nodular hyperplasia, basophilic

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clear cell hyperplasia and diffuse hyperplasia, particularly the hygienophilic (?) and basophilic hyperplasia. And of course, for neoplastic lesion, of course angiosarcomas, angiomas, hyperplasia associated with nuclear dysplasia, and hyperplasia. Our study was done not as much to build up the natural history of the tumor, which was already being suggested and done in a group of work which we produced in the past on human beings treated by Dr. Popper, but it was mainly to try to assess if a dose is very low or further tumors appeared -- we could still get some indication that there was an effect.

And we were extremely interested to know that, as I showed to you before, at least for the dysplastic lesions where the tumors start to appear, it was a good line. So to say to us in our experimental system, at five and one ppm we do not see this type of change, based only on the observation of two clear-cut tumors, would end up being a sensitive parameter.

If we just wanted to go -- just to see if there wre any of the oncological type of lesions. As you see, we find just this line in between this neoplastic carcinoma and nodular hyperplasia, we see displasia of angioblastics and angiosarcomas.

We did not observe any of that type of lesion, and as a matter of fact, we observed atypias both in the parenchymal cell and in the angioblastic cell, even in the

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liver, in which no other type of changes, no fibrotic changes, took place.

DR. SAFFIOTTI: Are there any other questions? Yes, please?

DR. ANDERSON: Anderson of NIHS. I wonder if you have been able to compare the latency for your angiosarcomas to the latency for tumors at other sites?

DR. MALTONI: I beg your pardon?

DR. ANDERSON: Were you able to compare the latency of angiosarcomas with the latency of tumors in other sites?

DR. MALTONI: You know, we have all these data, and if you wish I have tables with me: I think that I already — I have took too much on the patience of the people here, to show so many tables, but of course, we have tables for all different types of tumors and for each animal we have the latency types for all the different types of tumors, as soon as we could detect them.

So if you are interested, I'll be happy to show you this data. As a matter of fact, I would like to say that some discrepancy in dose-response relationship for each single tumor may be due to the effect of competition of the tumor, since it has a multi-target effect. And so, some tumors arising before, of course, may kill the animal before the animal gets a chance to fight the tumor.

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DR. SAFFIOTTI: One more question.

DR. INFANTE: Peter Infante of OSHA. Dr. Maltoni, in your presentation here, I didn't quite follow -- I couldn't determine whether or not you had shown data on mammary carcinomas clear down to one part per million. But I have seen some data from your laboratory indicating something like seven percent mammary carcinomas of one part per million, and then higher tumor incidences, going up to 25 parts per million.

But there does not appear to be a linear dose response. I am wondering why that is. Do you think it's because of the small numbers, because here I am not sure you would expect competition from other sites at that level -- or do you think that is the reason, because of the small numbers?

DR. MALTONI: I have doubt about that, Dr. Infante. Our experiment has been done on four groups, on five groups, one of them control and four treated. Of 150 animals -- it is not an enormous amount of animals, it is not a small number, particularly for a tumor -- it is a very high incidence, and we report our figure such that in the treated group the onset, 15, 17, 18 and 12, against a mere six in the control group.

So there is a slight enhancement, but without a dose-response relationship in this series, unexpectedly, a

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finally — we have in nearly all the long-term experiments by inhalation, at low doses or with compounds not otherwise shown as being carcinogenic.

We have by inhalation, but not by ingestion, an enhancement of tumor, not dose-related. Is it a matter of concentration? We do not know. We have this enhancement, which is also clear-cut, but is not a dose-response relationship, on which we could not place any type of interpretation.

Still, we confirm our own finding of an enhancement of the dose-response relationship. Did that answer your question?

DR. INFANTE: Yes.

DR. SAFFIOTTI: Thank you very much. We should now proceed to the paper by Dr. Suzuki of Mt. Sinai Medical School. The title has been somewhat changed. The Pulmonary Effects of Vinyl Chloride in Mice.

DR. SUZUKI: Thank you, Dr. Saffiotti.

Ladies and gentlemen, hepatic angiosarcoma has been accepted as a cancer associated with vinyl chloride exposure among workers in vinyl chloride plants. In addition, the risk of lung cancer has been reported by Buckwhiler et. al. among the workers on the basis of epidemiological studies.

Although a number of studies have (been) demonstrated that tumors can be induced by vinyl chloride in

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mice, the significance of that occurrence and the nature of the tumors have not yet been appropriately explored.

Another problem is the known neoplastic human effects of vinyl chloride. Indeed, known neoplastic abnormalities have been reported on the basis of chest X-rays, the pulminary function and the sputal cytology. However, histopathological evaluation of such known neoplastic abnormalities has not been reported.

We have therefore undertaken a detailed study under electron microscopy of the mouse exposed to vinyl chloride of graduated doses, long term, to characterize the plural effects of vinyl chloride. May I have the first slide, please?

(Slide.)

The 27 mice were exposed to vinyl chloride monomer at 2500 ppm and 6000 ppm, five hours a day, five days a week for five and six months. The animals were sacrificed and their lungs were studied by electron microscopy. Next slide, please.

(Slide.)

The pulmonary tumors were observed in 26 of 27 experimental animals. None were found in 16 control mice. Next slide, please.

(Slide.)

The tumors were multiple in number, round in

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(Slide.)

This was taken from solid tumor parts. The neoplastic cells, the microvillae and oscinioseminglomerial (?) bodies, we may call these cells "tumor-initiated neoplastic cells."

Next slide, please.

(Slide.)

Recently, we have done some vinyl chloride studies of smaller doses for short-time exposure. As you can see in the table, mice received three different doses: 100 ppm, 10 ppm, one ppm, for approximately for four weeks. And three different stages. The animals were sacrificed. Between these sacrifices, some animals spontaneously died. However, when you look at this spot, even at one ppm, one out of nine mice showed production of the alveolar tumor. Five out of nine at 100 ppm and 10 ppm group had two out of nine. Of course, the numbers of animals are small, but the dose-response relation seems to be perfect.

The next slide, please.

(Slide.)

The non-neoplastic effects of vinyl chloride occurred mainly in the bronchial alveolar area of the rat. These pictures show the normal structure of the bronchiolus. Now, this is the bronchiolus to the alveolar epithelium.

Next slide, please.

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(Slide.)

This is a treated mouse, the bronchial alveolar area. And as you can see, the hyperplasia of the bronchial epithelium is very sharp.

Next slide, please.

(Slide.)

The electron microscopy, the hyperplastic bronchiolar epithelium, consisting of the siliated cell and the Kupffer cell, has an increased number. Also the rhizosomal values are increased in numbers.

The next slide, please.

(Slide.)

This is a mitochondrian, seen in the Kupffer cell cytoplasm. As you can see, the mitochondryalis were abnormal in structure.

Next slide, please.

(Slide.)

It is well known that normal cells contain well-developed full endoplasmic reticulum. The smooth-surfaced endoplastic reticulum. The transformation of the smooth-surfaced endoplasmic reticulum into the rough-surfaced endoplasmic reticulum and the dose-response relation of the endoplasmic reticulum were frequently observed in the Kupffer cells of the treated mice.

Next slide, please.

(Slide.)

This is a part of a siliated bronchial cells, the cell cytoplasm, similar to a Kupffer cell. The appearance of intercell mydochondria, well-developed Golgi complex, as well as rhizosomal granules, are shown. And the numbers of endoplasmic reticulums are increased.

Next slide, please.

(Slide.)

Alterations seen in the pulmonary alveolas are characterized by hyperplasia of the alveolar cells and organization of the alveolar macrophagias were also noticed. Occasionally, as shown in the right slide, pneumonia-type inflammation was also observed.

Next slide, please.

(Slide.)

The ultrastructure of the hypoplastic alveolar lining cells is shown in this slide. As you can see, the hyperplastic cells are also Type 2 alveolar epithelia cells.

Next slide, please.

(Slide.)

Immobilized alveolar macrophagias which contain a large numbers of rhizosomal bodies which were released by the Type 2 cell and which were absorbed by the macrophagias can be seen here.

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Next slide, please.

(Slide.)

In addition, this is part of the cytoplasm of the macrophage. Cholesterol crystals were observed in the macrophage.

Next slide, please.

(Slide.)

The ultrastructure, as shown, was a basement membrane. As you can see, the basement membrane of the alveolar capillary is thickened. Now, this thickening of the basement membrane was quite often observed in the treated mice lung.

Next slide, please.

(Slide.)

The cell debris of degenerated alveolar cell is also observed in the alveolar septum.

Next slide, please.

(Slide.)

This is a capillary endothelium. In addition to this swelling of cytoplasm, a large nucleus segmented is shown in this microgram.

Light, please.

Since Maltoni and his associates first reported a high rate of production in mice exposed to vinyl chloride in 1974, several interested groups, including us, have found

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these findings for mice.

Based on all the data available, it may be concluded that of all the tumors induced by vinyl chloride in all species, those in the lungs of mice appear earliest and that mouse lung is a sensitive organ for demonstrating the oncogenicity of vinyl chloride. The malignancy of this tumor has been disputed. Stuart emphasized that this tumor has application to the test site. As Dr. Maltoni just showed, some vinyl chloride, at least in mice, undergoes such a malignant transformation. Also, our materials did not show such a finding.

Electron microscopy of the induced tumor suggests that the precursors of the tumor cells is the Type 2 alveolar epithelium. Waxweiler and associates have reported an increased number of deaths due to lung cancer among vinyl chloride workers. Since these human lung cancers are of bronchogenic origin, it may be that the target pulmonary cells of vinyl chloride oncogenicity are different in human and mouse lung.

Beyond the difference, however, the induction of alveolar tumors in mice by vinyl chloride may be predictive of a risk of human propagating cancer from the chemical. Consistent with this is the fact that various carcinogens, such as polycyclic aromatic hydrocarbons, nitrogen mustards, and chromatic compounds, are known to induce alveolar tumors

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in mice, on the one hand; and, on the other hand, to be associated with excess broncnogenic carcinoma with workers exposed to the carcinogens. It is known that a similar relation has been suggested for vinyl chloride.

As for non-neoplastic effects of vinyl chloride, cell changes, while found in the broncheolus and pulmonary alveolus microscopically, the hypertrophy and the proliferation of the bronchial cells, the hyperplasia of alveolar rhinal cells, and the alveolar microphage commonly observed, electron microscopy, both Kupffer cells and asiliated cells of the alveolar bronchiolus show the rough and smooth surface in the endoplasmic ventriclum, and abnormally shaped mitochondria in the alveolus.

In addition to the hyperplasia of Type 2 cells, basement membrane and cholesterol destroyed in macrophage, the regeneration of septal cells and the occasional appearance of a large nucleus with sweating of the capillary epithelium were observed.

Thank you very much.

(Applause.)

DR. SAFFIOTTI: Thank you, Dr. Suzuki.

I would like now to open the discussion and first ask for questions addressed to Dr. Suzuki on his paper. Let me start with a question, myself.

And that is: you described a variety of effects

at the cellular level. Some of those might be related to nonspecific toxic effects of chlorides. Some of them may relate to the carcinogenic process. What is your evaluation of the relevance of the lesions found to the carcinogenicity of the agent?

DR. SUZUKI: In the first place, of course, the vinyl chloride finding, such as hyperplasia, is not specific in vinyl chloride. But to me, it seems to me that this vinyl chloride induced this kind of a hyperplastic change not associated one site or another. Plasma cells — in other words, without any acute deceptive proliferative change are more positive. That seems to be a unique point, I know this one, to vinyl chloride. But, as I say, individual cells are not so.

DR. SAFFIOTTI: Thank you.

Are there other questions?

DR. TAMBURRO: Dr. Suzuki, we too, in our population, have found, as has been reported by Dr. Waxweiler, an increased incidence of lung cancer among vinyl chloride workers. The problem is that when you go to correlate the exposure of the worker or when you look at pulmonary function studies done on the individuals who have been exposed, one finds neither the correlation with vinyl chloride, or even closely. Nor does one find incidence of pulmonary involvement in these cases.

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Is it possible that what we're seeing is a true increase in lung cancer, but not due to vinyl chloride, or not due to it alone, and that what we're simply seeing is a difference in species response rather than direct effect of vinyl chloride?

DR. SUZUKI: Maybe I don't have a very clear answer to you, sir. But, you know, the data which I demonstrated here, as I stated, first of all, the target cells of vinyl chloride — I am talking about oncogenesis, such as alveolar tumors in the alveolar epithelia cells, and not the bronchial epithelia cells — in the first place, I wonder, the target cells of vinyl chloride between human and mice is the same.

DR. SAFFIOTTI: I would like to ask if there are other questions and comments for discussion relating to both the presentations of Dr. Suzuki and of Dr. Maltoni. We can resume the general discussion.

Yes, Dr. Price.

DR. PRICE: NCI. Dr. Maltoni, in your studies in mice on the carcinogenicity of vinyl chloride, one of the tumors that was not correlated was exposure to vinyl chloride, was the liver cell tumor, hepatoma. The liver cell tumor in the mouse is a type of tumor that is very frequently correlated with carcinogenicity to other chlorinated types of hydrocarbons. Have you any comment at

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all on the discrepancy between your negative findings for vinyl chloride and the more general response of the mouse liver to this more general class of compounds?

DR. MALTONI: No, I am as surprised as you are. And as a matter of fact, there was a census of all the possible lesions correlated possibly to vinyl chloride exposure. I did it in rats, but I didn't do it in mice because we did not observe in mice, apart from angiosarcoma in lesions which could lead me to think that the compound had an effect on the liver cells. It surprised me, since the cell dose response, which as a matter of fact, stopped in the liver.

DR. SAFFIOTTI: Comments? Yes.

DR. HOPPER: Ken Hooper, biochemistry department, University of California.

Dr. Maltoni, I am concerned about the exposure levels that workers are still permitted to experience. Is your finding clear evidence of the carcinogenicity at 10 ppm? What do you and what do others in the audience think about the advisability of this exposure to workers?

DR. MALTONI: This is a different question, as you know. You give me a type of question which would be impossible to answer. I don't know if any one of us is able to.

What we had to do in our work was to try to make

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sort of a risk assessment in our system. It is not so much up to us to make the decision on what goes in the workplace. But these are the people who today here who have the function. What may be an experimental model, how this model can be applicable to man. If they wish, more epidemiology should be done with an experimental type of knowledge, adopting for this epidemiology the same type of rigorous approach as we have been making in experimental science.

There is a time in which we may say we can pick up this and that and that, but one is a homogenous group. Up to the moment, we don't have this type. It would only be a matter of influence, an emotional type of approach. One would then say that "No, we have to do this." Someone else may say, "No, we have to do that."

DR. SAFFIOTTI: If I could add a comment to this obviously very important issue. I think that the present criteria used in arriving at occupational standards in this area are those of feasibility, are those of trying to obtain the maximum feasible reduction in exposure, and that we would not assume an absolute, say, level, even at levels below one part per million. I think the data on animals are a strong indication of the potential level of risk in people, but could not be taken as a direct quantitative indication of the size of risk in people, certainly not with

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the enormous variation in susceptibility.

I am wondering, Dr. Hooper, would you like to comment any more on the problem of quantitative correlations, since you have done a lot of work in reviewing the whole problem of the levels of activity of the different carcinogens in the different systems.

DR. HOOPER: Just briefly. We have been looking, trying to quantify the potency of chemicals in animal systems, looking at a large part of the animal cancer tests which meet certain criteria. We have been also trying to get data from human epidemiological data and calculate also potencies in humans. This is still ongoing.

In addition, we're getting data on monkeys, cancer tests on monkeys, from Richard Adamson, here at NCI. And we're trying to take a look at these inter-species comparisons. So far, there doesn't seem to be that much difference in the amount for human potencies that we have and the potencies in animals.

I didn't mean to address that emotionally. In the sense that it's just a concern, it seems to me that if we have positive results at 10 parts per million in animal bioassay, it would seem to be consistent that it's difficult to justify scientifically exposing humans, permitting them to be exposed to doses, say, of even one-tenth of that. And I think the heterogeneity of the human body means that in a

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sense we should be more concerned.

DR. SAFFIOTTI: Thank you.

Yes.

DR. COOPER: Clark Cooper, from Berkeley.

I think the question that has just been raised is clearly — will be the main focus of this conference. And I think it's something that should be considered as we go through each of the phases of this conference, because I think the epidemiologic studies that will be presented, and some that are ongoing are very relevant to answering this question. So, I think you should keep this in mind.

My feeling today is that the human populations that have been exposed the last 25 or 30 years are not sharing this experience that one might have predicted from the animal work. So I just think that I would like for us to just keep this in mind as we proceed.

Thank you.

DR. SAFFIOTTI: Thank you.

Are there any other brief comments? Yes?

DR. CHU: Ken Chu, NCI. I would like to ask Dr. Maltoni a question. One of the obvious ones is: would you like to comment on the significance of the absence of effects in vinyl chloride at the very lowest dose levels on the concept of thresholds? I would like to know your point of view. Many people are going to interpret this data, and

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I would like to know what your views are.

DR. MALTONI: You put me in another difficult spot.

(Laughter.)

DR. MALTONI: This point I have made again and again all day. It is one of the most occurring questions today. But I may say I was speaking in this particular one experimental system, the threshold had a different meaning. You may have a theoretical threshold; you may have an experimental type of threshold; you may have a practical threshold. That's an entirely different concept.

I am not in favor to say that our data could be interpreted in a theoretical way as to proving a threshold effect. I do not think that our data do that. As a matter of fact, I am not a great believer in a threshold effect. But on a practical basis and just limited to this type of experiment, it was greatly interesting to show a drop, a sudden drop. What is the meaning we have to study about it?

But in the Sprague-Dawley rats, after 10 ppm, there is — we have studied all kinds of lesions — there is sort of a drop, and I thought that it was useful to say that. That's all there is.

DR. SAFFIOTTI: One more quick question, please.

DR. TASSIGNON: Tassignon, from Brussels.

In Europe at the November meeting, the same type

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of questions in Paris were asked to Professor Maltoni, and it is interesting to see how these problems of threshold, of dose-effect relationships, extrapolation to man, are asked in both continents.

Of course, there are different types of tumors that were in use in vinyl chloride, two of which, like Zymbal gland carcinoma and liver angiosarcoma, show a smooth rise increasing doses: on the other hand, a shortening of a latency time. This is a classically induced chemically induced tumor.

Then we have other types of tumors, like neuroblastomas, appearing at higher doses and then disappearing at still higher doses, with a kind of bell-shaped curve. And perhaps one nephoblastoma. Then we have borderline tumors difficult to interpret because statistics cannot help us very much there to define the significance. The biology is there.

And Professor Truhaut, there, remarked that for neuroblastoma we have a very long range of doses at which there is no observed effect. In fact, neuroblastomas start to occur at 2500 ppm. And I think this is an example of a target tumor that might have a kind of threshold, but again this needs more study.

DR. MALTONI: As a matter of fact, we have been surprised.

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We are surprised by the different behaviors at different doses of tumors. This has had much to do with the basic tumorigram of the animal, which is an expression of the responsiveness of the animal.

DR. SAFFIOTTI: I am afraid that we are running into a problem of schedule. We should have our break now. We reconvene at 10:45.

I would like to remind those who have not yet registered that the registration desk is open and that the name tags will be available. 10:45.

(Brief recess.)

DR. SAFFIOTTI: I would like to reconvene the session. In order to keep our schedule on time, we should resume our session, and the next speaker is Dr. Groth, but I don't seem him yet.

(Laughter.)

DR. SAFFIOTTI: We'll introduce Dr. Groth while he's not yet here. Dr. Groth is a pathologist at NIOSH. He is now coming. And David, I'm introducing you; take your time.

Dr. Groth is going to talk on the role of aging on cancer induced by vinyl chloride monomer.

DR. GROTH: It's well-known that newborn animals are very susceptible to carcinogens. Many studies have been performed to demonstrate this. The other end of the

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spectrum — that is, older aged animals -- have not been examined in this depth. It has been suggested that only older people should work in carcinogenic environments since the induction time for cancer would exceed their life expectancy.

This opinion is based on the assumption that the susceptibility of older individuals is the same as that of young adults and that the latent periods are the same.

This experiment was designed to test that hypothesis by exposing adult rats of different ages to vinyl chloride and comparing the angiosarcoma incidences in the different age groups at specific time intervals. This experiment was not designed to test the susceptibility of sexually immature animals or to determine the long-term effect of relative — particularly short-term exposures.

A total of 473 male and 478 female Sprague-Dawley rats were exposed to vinyl chloride monomer in a dynamic flow chamber. An equal number of control male and female rats kept in a similar chamber were exposed to conditioned outside air only. Exposures were for seven hours per day, five days per week, for a mean duration of 24 and a half weeks to vinyl chloride at a mean concentration of 948 parts per million.

The concentration of vinyl chloride was monitored by gas chromatography or with an ultraviolet

photo-ionization analyzer seven times each day on each exposure day.

The animals were individually housed in suspended stainless steel wire mesh cages equipped with automatic watering taps. Water was provided, but food was removed during exposures.

Four differently aged sets of rats of both sexes were used in both exposed and control groups. Their ages at initiation of exposure were 6, 17, 32, and 52 weeks. The numbers in each group and their disposition appear in the following tables — Numbers 1 and 2.

Slides, please.

(Slide.)

You don't have to memorize all these numbers. The important thing here is that there are 473 males, 478 females, and the numbers that were autopsied and had tissues examined were 470 males and 476 females. We broke these down in different groups — death and moribund sacrifices appear in this column; scheduled sacrifices at 3, 6, and 9 months appear in this column; and the animals that were left after 43 weeks — the final sacrifices — appear in this column.

Next slide.

(Slide.)

These are the controls. The primary reason for

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putting in the controls to assure that there would be no compounding factors -- that is, an elevated increase in agniosarcomas. We didn't really expect it, but we had to make sure that we didn't have any significant numbers of angiosarcomas. Consequently, we didn't autopsy all the control rats. Only 357 out of 472 males and 382 out of 477.

Because of the large numbers of animals, it was not practical to begin exposures on all rats simultaneously. Consequently, proportionate representations of each age group were assigned to their chambers each day throughout a ten week period. The original plan was to expose the rats for 12 months; however, because of an epidemic of pneumonia among the exposed animals, the exposures ended after 29 calendar weeks.

As you may expect, this produced a little concern, inasmuch as the animals had different exposure times. As I will show, however, this did not seriously affect the interpretation of the results, inasmuchas the mean exposure times for each group were either identical or very similar.

As the results will show, that particular exposure period was sufficient to demonstrate the differences in effects between groups. Between 20 and 25 animals per group were killed and autopsied after 3, 6, and 9 months of exposure. These are referred to as the interim sacrifice groups.

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Animals that died, those that were sacrificed while moribund and those that were killed at the final sacrifice, 43 weeks after initiating exposure, constituted another group referred to as the non-scheduled sacrifice group.

At the autopsies, all the organs were examined. Several sections of most of the organs were taken and processed and examined by a pathologist. At the interim sacrifices, blood was taken from each animal for hematology and clinical chemistry, including measurements for albumin, gammaglobulin, alkaline phosphatase, SGOT,SGPT, and gammaglutamil.

No clinically or statistically significant differences in hematological and clinical chemistry measurements between treated and control groups of the same age and sex were detected among any of the interim sacrifices.

The most frequently occuring tumor types in both sexes were the liver angiosarcoma, pituitary adenomas, and mammary tumors. The statistics I will show show mainly liver angiosarcomas; however, there is one in the statistics — there is one — two metastases to the lungs — the primary one, which was not identified, and also one splenic angiosarcoma.

Since the first liver angiosarcoma did not occur

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until the 16th week of the study, only those animals alive at that time were considered to be at risk for the development of angiosarcoma.

Slides, please.

(Slide.)

This slide shows the combined non-scheduled and interim sacrifices — that is, all the animals that were autopsied — exposed to vinyl chloride. In the younger age group, the incidence was 1.2 percent; the next age group, 2.2 percent; the next stage, 7.4; and in the oldest, the ones that were 52 weeks old at the beginning, the incidence of angiosarcoma was 18 percent. In the females, this trend also existed with one exception — that is, the oldest animals, the incidence dropped off.

The next slide, please.

(Slide.)

When the incidence data in the males was analyzed, the relationship to age at the onset of exposure and analyzed the regression analysis, it showed a significant quadratic fit. R-squared equalled 99.85 percent, which essentially means, that was the only variable necessary to explain the differences in incidence between these different age groups.

The next slide, please.

(Slide.)

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Now comparing, we broke this down into the non-scheduled versus interim sacrifices to see whether or not the animals were appearing in greater frequency in one group over the other. In fact, there was some greater incidence in the older aged animals in the non-sacrificed — that is, most of the animals that died over those that were sacrificed, and the specific intervals.

This was also true — we reached a maximum incidence of 47 percent angiosarcomas in females both that died and were sacrificed at the final sacrifice.

Next slide, please.

(Slide.)

This slide is cut up here to show you that the exposure durations in the several age groups were very similar and that their mean survivals were quite similar in weeks. At the same time, we show you the incidence. These are in the non-scheduled sacrifice groups. We did not see any animals with angiosarcomas in the two youngest age groups.

Metastases did occur in three animals here, all three, and the oldest age group, 11 out of 13 of the angiosarcomas metastasized, primarily to the lungs.

Next slide.

(Slide.)

This shows the same data for the female, showing

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that the durations of exposure are quite similar. The mean survivals are similar, but my exception is the youngest age group had a longer mean survival. This might account for having a higher percentage of angiosarcomas in the females in this particular age group compared to the males.

The first angiosarcoma — I failed to mention that on the other slide — but the time of the first diagnosis of angiosarcoma was shortest in the oldest aged animals — 16 weeks, 20, 28, and 30 weeks after initiating exposures.

The next slide, please.

(Slide.)

This is just a comparison of the males and the females. We put that all on one slide so that you can readily see, compare them. Also I was interested in finding out whether or not an angiosarcoma might be responsible for the death of the animal. Apparently, this was not true, inasmuch as the mean survival for the animals with the angiosarcomas was somewhat longer than the mean survival for the sacrifice groups in both these age categories.

Over here, they're about the same. In this one, the mean survival for the animals with the angiosarcomas was longer — slightly longer here, slightly shorter in this category. In general, however, the animals with the angiosarcomas appeared to live a little bit longer, whether or not the vinyl chloride — the major cause of death —

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well, one of the principal causes of death was pneumonia, and it could be argues, I suppose, that the vinyl chloride protected some of the animals from pneumonia. But it could also be argued that some of the animals that had an immunological response to prevent death from pneumonia also lived longer.

Next slide, please.

(Slide.)

This data was also analyzed to show a cumulative incidence with time in each age category. For females, in the youngest group, there is no cumulative increase in incidence, although the time after the exposure stopped — also the total time exposures — we can't really say what the long-term effect will be, say, 40 weeks after this time here. But there is no obvious increase in incidence.

There is some increase in this age group. There is not any apparent increase in this age group, and there seems to be somewhat of a decreased incidence in the oldest age group of females.

Next slide.

(Slide.)

For the pathologists in the audience — would you dim the lights? I've got to show what some of these lesions look like. This is an early angiosarcoma in the liver of one of the rats.

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Next slide, please.

(Slide.)

This is a well-developed angiosarcoma in the liver of the rat. Next slide.

(Slide.)

Higher magnification, similar to the slides you saw earlier that Dr. Maltoni showed.

Next, please.

(Slide.)

Here is a metastasis to the lung. It appears in the blood vessel and spreads out to form cystic spaces within the lung.

Next slide, please.

(Slide.)

Another metastasis in the lung which seems to take place in a large area.

Next slide.

There is no slide. I think that's the last slide. Lights, please.

The results clearly indicate that the incidence of angiosarcoma is higher and the tumors occur earlier in the older rats of both sexes and that females in at least three of the age groups are more susceptible than the males.

Therefore, it can be concluded that older rats are more susceptible to the angiosarcoma-inducing effect of

vinyl chloride than are young adult rats and that female rats are more susceptible than are males.

No published epidemiological studies have been designed to determine the role of aging and cancer susceptibility. However, the data of Mancuso has shown an extremely high risk for lung cancer for workers exposed for relatively short periods of time between the ages of 40 and 55.

Long latent period from the time of first exposure to a carcinogen until cancer is diagnosed and long exposure periods before cancer is found are common observations. Long latent periods are probably a function of increasing susceptibility with age. Long exposure periods before cancer develops are probably a function of dose as well as age, and the accumulated doses in the early adult years are probably not as important as accumulated doses in the later years.

Studies designed to test these theories in humans are needed. Thank you.

(Applause.)

DR. SAFFIOTTI: Thank you, Dr. Groth. I believe that we should proceed with the subsequent papers, and if we have a few minutes at the end for discussion, we will then have the discussion for all the presentations.

The next paper will be given by Dr. Martha Radike

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of the University of Cincinnati on carcinogenic effects of exposure to vinyl chloride monomer and ethyl alcohol on rats.

DR. RADIKE: Well, Dr. Groth just changed my experimental protocol. I decreed, because I was so old, if there was a problem, I was the one who was going to go in, so he just scared the death out of me.

(Laughter.)

DR. RADIKE: It's been well demonstrated that vinyl chloride and ethyl alcohol probably share a step in the metabolic pathway. Early work by Heffner indicated that ethanol can inhibit the uptake of vinyl chloride and that pyrozol also would inhibit the uptake. It's well know that vinyl chloride is in the microsomal system. Also ethyl alcohol is not inducible; however, it's been well shown by Lieber and many others that ethanol is oxidized via this pathway.

For these reasons and also, as you know, carbon tetracholoride and other chlorinated and other halogenated hydrocarbons have been shown to increase toxicity, have caused increased toxicity in experimental animals.

We designed this study to look at chronic ethanol consumption. For this reason, we chose to give five percent ethanol and water, rather than a high intake of alcohol. This was done ad lib. This was the entire water supply,

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kind of like a six-pack man or two or three glasses of wine with dinner.

Rats, incidentally, do clear their alcohol rather rapidly. I'd like to have the first slide, please.

(Slide.)

We started with 320 Sprague-Dawley rats, divided them into four groups -- two control groups and two vinyl chloride treated groups. The diet was just Purina Rat Chow, and the ethanol was combined with water. Exposure was to 600 parts per million in a chamber which did not have automatic water supplies. Water and food were withdrawn from the animals during exposure periods. The exposure period was four hours a day, five days a week, for one year.

The alcohol was administered for four weeks prior to the start of 600 parts per million vinyl chloride, and the alcohol ingestion was continued for the life of the animal.

Next slide, please.

(Slide.)

I might say that in the chamber the animals were put in holding cages. They were rotated in the chamber. The chamber was monitored by automatic sampling, gas chromatograph with ten leads. We had two columns, so we could look at high concentration and low concentration. We were able to monitor the operator area, the effluent, and

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the scrubbed effluent. And we chose to scrub our effluent from the chamber by incineration, which we ran around 700 degrees Centigrade.

As you can see, there really wasn't a very great effect on the body weight — average body weights of the animals until about 12 months at the end of the exposure. However, the difference really isn't significant. There's great variation in all three groups.

So the ethanol — you can see, the ethanol really didn't have much of an effect either, as far as calories. So there was four weeks of the ethanol. Then when we started to do vinyl chloride for five weeks. So these were older animals at the beginning of the vinyl chloride exposure in relation to Dr. Maltoni's work or some of the other work you've seen.

Next slide, please.

(Slide.)

Survival of rats, as you can see — the two control groups which are the two bars to the left — I guess bar was the wrong word to use —

(Laughter.)

DR. RADIKE: The alcohol group survived a little bit better up until 22 months. These two groups are the control groups. This is vinyl chloride. This is vinyl chloride and alcohol ingestion.

You can see -- well, I got that eleven o'clock yesterday, and I didn't proof it. This belongs here, and this belongs here.

(Laughter.)

DR. RADIKE: He's fired.

(Laughter.)

DR. RADIKE: At any rate, this is not due to unspecific causes. Yes, we did have some problems with murine pneumonia; however, when we saw a rise in murine pneumonia, which interestingly enough occurred more frequently in the control groups -- I think that's because they're not handled as much -- we treated the entire colony with an antibiotic. This was our choice; we wanted to keep them alive, and I treated twice during this experiement. We treated the entire colony.

You can see that the vinyl chloride group, drinking Cincinnati tap water on which a tremendous amount of work has been done, you get the picture.

Next slide, please.

(Slide.)

The incidence of neoplasms in the liver. As you can see, the vinyl chloride group -- the most often observed neoplasm was the hepatocyte or carcinoma. In the vinyl chloride group, 35 animals, the first one observed at 11.5 months. 46 hepatocytes or carcinomas, the first one

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observed at 10 months. In the ethanol group, 8 at 15 months, and one was observed in the control.

Angiosarcoma — 18 animals with angiosarcome first observed at 12 months; 40 angiosarcomas, vinyl chloride and ethanol at 9 months; none in the control groups.

This — by the way, the pathologist doing this work — he called it a "mixed tumor", such as Dr. Maltoni had shown. He didn't want to call it just one or the other, so he termed these a "mixed angiosarcoma hepatocyte or carcinoma."

These were completely separated, not mixed. Again, 6 at 14 months, and 10 animals at 11 months. These were listed simply as an indication — they're not included in any of the statistics, but as a measure of toxicity, as you can see, there wasn't a great difference.

(Slide.)

This -- I don't know whether you can see it -- is an early hepatocellular carcinoma. Here's normal tissue, here's the tumor type. I'm not going to present very many of these slides, because I'm not a pathologist. I'm sure somebody is angry, but I do have some more slides, if there is someone who would be interested in looking at them.

Next slide, please.

(Slide.)

This is an angiosarcoma that even a non-pathologist could recognize.

Next slide, please..

(Slide.)

There was a large incidence of endocrine tumors. It's well known that ethanol, ingested ethanol, does have an effect on the endocrine system, and you can see this. I don't worry too much about the pituitary adenomas, because these rats commonly have a very high incidence of pituitary adenomas.

The thyroid -- these were malignant. Again, not any real relationship. These are of interest, the adrenal tumors. By the pathologist these were termed to be malignant based upon invasiveness and cytological characteristics. However, none of the metastases were seen.

And the pancreas -- this is very, I think,

interesting -- non-malignant adenomas, however, none. But in the two ethanol-treated groups there were also fibrotis lesions, not in these animals but in other animals treated with ethanol.

This is worth mentioning because these were seminomas and were malignant.

Next slide, please.

(Slide.)

Incidence of neoplasms except in the liver and endocrine system. In general, these rats tended to have a background of these types of lesions. So that, along with the pituitary adenoma, may not carry as much weight as you might thing.

The lymphosarcoma, however, was in greater incidence in this group. The fibromas -- these were all male animals and most of these occurred in the area of the mammary gland and perhaps did not develop as they might have in the female.

And of course, of special interest is the glioma, and it's interesting that we observed one in the alcohol control. Under others, these represent kidney, epidermis, and other areas.

Next slide, please.

(Slide.)

This is a breakdown of the malignant and benign tumors, the number of rats. This wasn't planned. That poor

unfortunate beast went through the cage-washer, so we didn't include him.

(Laughter.)

DR. RADIKE: This is the number of rats with tumors, as you can see, 73 of 80, 63 of 80, and so forth. The total number of tumors obviously is much greater in the vinyl chloride and ethanol group, less in the vinyl chloride, and a large number in the alcohol-treated group.

However, as you can see, most of them or a large number of them are benign.

Malignant tumors. You can see the increase in this group is due to an increase in malignant tumors. Benign tumors in the two vinyl chloride treated groups is about the same.

This is of interest, the number of malignant tumors observed in the ethanol control.

Next slide, please.

(Slide.)

The incidence of all types of neoplasms. Again, the same kinds of figures, but this gives you the percentage of rats with neoplasms, and this is including both malignant and benign.

The next slide, please.

(Slide.)

That basically is the data that we have. Of course,

there are many more observations on toxicity. One of the things that is noted very well in both vinyl chloride treated animals and ethanol treated animals is the effect upon the mitochondria. This has been reported by others. This is very typical of what we see.

This animal was treated with both ethanol and vinyl chloride, but you can see the sick mitochondria, and you see this in both the ethanol treated animals and the vinyl chloride treated animals.

And as usual, I completely departed from my notes. I suspect my time is up anyway. However, there are a couple of things I would like to add.

One is that we did do clinical blood chemistry on those animals that we were able to observe either dying or in a moribund condition. The blood chemistry as far as SMA-12's and liver enzymes were meaningless as far as averages in groups. They can only be interpreted as related to histological and pathological picture of that particular animal.

I think there are a number of things to be observed here. One is the increased damage to the mitochondria, the undoubted change in NAD pH, in a P ratio. They have an effect on the whole picture. I feel that it certain warrants the collection of good alcohol ingestion data.

And now I'm going to cheat and say what I wanted to say right at the end of the other session, and that is, when

we were asking about threshold levels, remember that the person who is doing a study like this -- I had 80 animals in a group. How can you extrapolate and say threshold level, when you may be talking about 10,000 people? So you have to really think about the number of animals that are in these studies.

Perhaps if Dr. Maltoni had a huge place that he could expose 10,000 rats at one part per million, he could come up with something. But it's very difficult for a person in my position or an experimenter's position to say that's where it ends. That's where it ends in that experiment, with that number of animals.

Thank you.

(Applause.)

DR. SAFFIOTTI: Thank you very much, Dr. Radike.

We will proceed now with the next paper, and the discussion will come at the end of the morning session. Dr. Robert Hehir from the Consumer Products Safety Commission to speak on cancer induction following singe and multiple exposures to a constant amount of vinyl chloride monomer.

X DR. HEHIR: Thank you, Dr. Saffiotti and ladies and gentlemen.

I am going to talk about cancer induction following single and multiple exposures to a constant amount of vinyl chloride. I'd like to acknowledge, first off, the co-authors of this paper, Dr. Bernard McNamara, Dr. McLaughlin,

Dr. Don Willickon, Dr. Gerry Hardesty, and Dr. Joyce Bierbaum. We have a well-balanced study. We have three toxicologists and three pathologists.

I would also like to make this disclaimer at the outset, that the views and opinions expressed in my presentation today are totally those of the authors and do not necessarily reflect the opinion of the Commission. Our General Counsel likes that.

(Laughter.)

DR. HEHIR: Over the last decade there have been monumental strides to identify carcinogenic chemicals in the workplace. However, as we all know, carcinogenic materials are not just limited to occupational exposures. Many of these same chemicals are used in the production of consumer products. Therefore, consumers in all walks of life are exposed and they're exposed in a very insidious way. They're not aware that these particular chemicals are there.

A chemical may be completely changed. For example, in the case of vinyl chloride polymerization, it may be polymerized to polyvinyl chloride. There may be small quantities there that can be leached out, or the material can be used as a propellant because it has some unique physical or chemical properties. It may be totally inert or it may be odorless or what have you.

But it becomes in a way a part of the exposure problem.

While regulatory bodies can and do oftentimes get an estimate of what they consider to be a safe level, and then the industrial engineer, the environmental engineer, goes in and kind of works around the production facilities in such a way as to lower that level to the point where you can work in the environment, there are no such practical measures in the home.

There's another problem associated with making value judgments about consumer risks, and that is that there are really no comparable studies to low-level concentrations of any type of carcinogenic material. Therefore, our approach was simple.

We simply wanted to take laboratory animals and expose them either acutely or to very low intermittent doses over a period of time. And vinyl chloride appeared to be an ideal compound to work with because it had some unique situations here. It was widespread in both the industrial and the consumer product areas.

Because the relationship to the production of unique biological lesions and high exposure levels in animals and man had already been established by others.

May I have the first slide, please.

(Slide.)

These are some factors which have to be addressed in any normal biological assay. But they're even more

pertinent when you're considering very low dose levels. The substance may not reach the susceptible cell. It may make some type of bonding action in combination with the cell. The initiated lesion may not receive adequate promotion. Host factors may be unfavorable to carcinogenesis. You may have a biochemical repair of a DNA lesion. Morphological regression tumorigenic proliferation may occur. Carcinogenic cells may be destroyed by the body's immune system.

May I have the next slide, please.

(Slide.)

In our experimental conditions, we used Rochester type 1,000 liter dynamic chambers. We monitored the gas in the chamber using a dual flame ionization gas chromatograph with a sensitivity to 60 parts per million vinyl chloride. We observed the animals twice daily for general health, sores, masses, alertness, activity and mortality.

May I have the next slide, please.

(Slide.)

This particular sequence, we exposed Fisher 344 and ICR mice to various concentrations of 50, 500, 5,000, and 15,000 parts per million for one hour. We took another group of Fisher rats and AJ mice and we exposed them to 50 parts per million vinyl chloride for ten one-hour exposures and 50 parts per million vinyl chloride in 100 hour exposures. We had a third group, for example, which were exposed -- and

these were in reproduction studies which we did, and we studied these animals 24 months post-exposures.

What happened here is that we exposed some Sprague-Dawley Wistar rats which we obtained to 50 or 500 parts per million for 49 one-hour exposures.

May I have the next slide, please.

(Slide.)

By light microscopy we examined the controlled and exposed animals. These were the tissues we were looking at, and the single-exposure studies we monitored at 8 and 18 months. The multiple exposure studies, we monitored the animals at 8, 16, and 20 months.

Next slide, please.

(Slide.)

We also went on to do some electron microscopy, in which case we took male and female Fischer rates from each of the single exposure studies and an equal number of corresponding controls, which we sacrified at age 16 and 24 months. We also took male and females from multiple exposure, one-hour studies; we sacrificed these at 16 and 24 months.

Slide off, please.

Toxicity during exposure. Rats and mice exposed for one hour to varying concentrations of VC, from 50 to 50,000 parts per million, or repeated exposures, produce no remarkable signs of toxicity, with the exception to mice at the highest

dose level.

At the 50,000 part per million concentration, 50 percent of the male mice exhibited hyperventilation at 45 minutes, together with twitching and ataxia. Female mice became hyperactive at 40 minutes of exposure and had respiratory difficulty and ataxia in about 25 percent of the animals in about 55 minutes.

Upon removal from the test atmosphere, all animals recovered to normal within 24 hours after exposure. There was no consistent or dose-related differences between the control or exposed animals, mice or rats, in death rate, toxic signs or gain in body weight.

As far as the gross pathology is concerned, there was a suggestion of higher frequency of masses in the lungs and livers of mice exposed, once or repeatedly, to VCM at the higher dose levels -- that is, at 500, 5,000, and at 50,000 parts per million.

Can I have the next slide, please.

(Slide.)

This shows the histologic examination of ICR mice at 8 and 18 months following a single one-hour exposure to vinyl chloride. I have two important things here.

First of all, you can see, looking at the control incidence of pulmonary adenomas, that there is a dose-related response ranging from about 13 percent pulmonary adenomas at

500 parts per million to 33 percent adenomas at the highest dose level. However, the progression to carcinoma here is not really significant. It does occur; statistically, it may not be that meaningful.

The next slide, please.

(Slide.)

I apologize for this. This is kind of a last minute, put it into perspective, because here we're talking about the single inhalation exposure to vinyl chloride monomer in ICR mice. Here we're looking at the incidence of pneumonitis and adenomas and carcinomas. What we're trying to show here is, I think, that that is the control population and the incidence of about six percent pneumonitis. And when you look at the levels in here, at the 50,000 parts per million and 5,000 parts per million and the 500 parts per million, what you're getting is about a significance of 23 percent incidence of pneumonitis.

As I pointed out in the previous slide, there is a dose response relationship here to the development of adenomas. And again, there's a marginal increase in the carcinoma for these animals.

May I have the next slide, please.

(Slide.)

This represents the histological examination of the A/J mice at 8, 16 and 20 months following multiple one-hour

exposures to vinyl chloride. There are a number of interesting things here. For example, if we take this first group here, the controls, versus the ten one-hour exposures to 500 parts per million, we can see the induction of pulmonary adenomas is significant. There's at least a twofold increase, and this is statistically significant.

Then the progression to carcinoma here at the control level is 3.3 percent, while at the 500 part per million, 10 one-hour exposures, the incidence here is 13.3 percent. We consider that to be quite significant.

Now, at the lower exposure level, that is the 50 part per million, 100 for one hour, you can see that there seems to be an increase. It is not significant. It's there. However, again, in the progression to carcinoma there is an apparent increase. But it may not be statistically significant.

I think the main number here is 500 parts per million multiple exposures. We did have a significant pulmonary adenoma increase and we did get a progression to carcinoma which is quite significant.

I can say that there are other neoplasms and non-neoplastic changes, and they occurred, it seemed, in both the test and control animals. The relationship by incidence and severity to test exposure is not evident, however.

May I have the next slide, please.

(Slide.)

This again compares the incidence of pneumonitis, adenomas and carcinoma and the multiple inhalation exposures to vinyl chloride for the A/J mice. You can see right off the bad, this is your control group and the incidence of pneumonitis is about 2 percent, whereas at that particular concentration you have no incidence at all. Again, with your control group you've got a 6 percent incidence of pneumonitis and again in your test exposure you get 6 percent. So others have said, it's almost as effective, if you will, at this point to develop your own information.

There is another interesting point here, and I'm searching for it right now -- well, it's immaterial.

(Laughter.)

DR. HEHIR: I know that's not supposed to be said. But the results are even more striking when you compare the repeated exposure with the single exposure. You remember in the ICR mice the incidence of pneumonitis was about 24, 21, somewhere around 23, 24 percent, as compared to these animals, which were much, much lower.

Slide off, please.

As far as the electron microscopic results are concerned, in general these studies indicate that exposure to vinyl chloride increased organelle turnover as well as loss of body control, and increased lososomal activities in

the liver of rats. These alterations progressively decreased as recovery after exposure increased. There were some hepatocellular carcinomas and there was a lymphosarcoma noted in one male and one female rat, but this was not considered to be significant because of the circumstances. We saw nothing at the 50,000 part per million level, so we do not relate that to the vinyl chloride exposure.

Now, on the reproduction carcinogenesis studies which we take on these Sprague-Dawley and Wistar rats--these were an inbred, pathogen-free group of rats which we obtained from the Animal Resource Branch of the Veterinary Medicine Division of Edgewood Arsenal. They were 12 weeks old when we started our exposures, and the 100 rats -- the 150 were divided into two dose levels as I described on slide three. The remaining 50 were carried out as unexposed controls.

Then the parental generation of the Sprague-Dawley and Wistar rats were maintained 24 months after the final carcinogenic dose. A complete gross and microscopic examination of the tissues was performed on each of the controlled and exposed animals. Particular attention was placed on examination of the brain, lung, liver tissue and Zymbal gland. The livers of randomly selected rats were also prepared and examined by electron microscopic techniques.

Neoplastic and non-neoplastic lesions were observed in approximately equal frequency in both the control and the

mte 15

FO parent generation of Sprague-Dawley, Wistar rats. The only lesion that occurred in higher frequency in the exposed animals as compared to control rats was an eosinophillic cellular alteration which was presented as foci or areas, and these occurrances coincided with increasing doses of vinyl chloride.

The nature of these lesions are of interest, but they're certainly still controversial and we're certainly not drawing any conclusions based upon their presence.

Finally, discussion. The fact and the severity of the carcinogenic effects of vinyl chloride have been described by various investigators to coincide with the dose and length of exposure, and it implies that total dosage may be an important factor in carcinogenicity for vinyl chloride. Therefore, the inhaled dose was approximated by the Haber concept.

In its simpler form, this concept states that dosage CT is the product of C, concentrations in milligrams per cubic meter, times time in minutes. The total concentration can also be expressed in parts per million, and the time can be expressed in hours, producing a CT, if you will, in parts per million hours. Factors for breathing rate and detoxification could be added if the data is available. However, the simplified CT approximation, total inhaled dose, was relatively useful as is for comparative purposes.

May I have the next slide, please.

(Slide.)

These are the studies that were carried out by Dr. Maltoni's group and, as he explained to you, there is a series of experiments here that he worked on, and as far as these here, this is Sprague-Dawley rats. They were treated at six dose levels ranging from 50 to 10,000 parts per million, four hours a day, five days a week, 52 weeks, and we're concerned now with the lowest dose level, the 50 parts per million.

Here at 52,000 parts per million total dose, we believe the data here shows questionable carcinogenesis. Dr. Maltoni this morning convinced me that it's less questionable in his mind than it was in ours.

And the third experiment here, also with Sprague-Dawley rats for a shorter space of time, we find negative results at a total dose exposure of 17,000 parts per million. It's questionable at 85,000 parts per million, but it was positive at 170,000 parts per million and above.

e-6

SUNDAY

I jump down to the 7th experiment because this was the experiment with the Wistar rats. These are all Sprague-Dawley. This here is the Wistar group.

Here, again, the data on the rats shows a negative effect, carcinogenic effect, 52,000 parts per million and 260,000 parts per million.

It was questionable being as high as 520,000 parts per million. There were parts of the data where you're talking about the total cumulative dose at 2.6 million and above.

In the experiment with mice, the data indicate here that you had a very positive result at 30,000 parts per million and above.

May I have the next slide, please?

(Slide.)

Quickly, to summarize, these are some of the other studies. You're seeing the same type trend as before, but you're getting negative results, especially in rats at low dose levels. You're getting more consistent results in rats above 50,000 parts per million.

Here is one experiment with rabbits showing, again, a dose, the total dose of 70.2 million. Kiplinger's results show for mice a positive effect at 56,000 parts per million. Lee pretty much with mice showed a similar result at 78,000.

gshDAV

To summarize, the commission's results with ICR mice, we had a 50 and 500 negative results, 5000 borderline positive. At 50,000 we had a positive result. With repeated studies with the AJ mice, 5000 was positive. Again, that was a 500 parts per million dose. In the Fischer rates, 5500, 5000, and 50,000 parts were negative. And this basically is the Sprague-Dawley rats, the total exposure of 24,500. You had eosinophillic loci, but you had no cancers.

May I have the next slide, please?

(Slide.)

And the next.

(Slide.)

In summary, for example, with vinyl chloride, cancer seems to depend on total dose with vinyl chloride. This is especially true in repeated dose concentrations that we see with the long-term dose effects. One dose is sufficient, if the dose is high enough.

The carcinogenic total dose was 5000 parts per million hours or greater for mice, and above 50,000 parts per million hours for rats.

There were apparently non-carcinogenic doses in the study and mice would seem to be more sensitive indicators than rats for carcinogenic effects for vinyl chloride. Thank you very much.

(Applause.)

DR. SAFFIOTTI: Thank you, Dr. Hehir. I think that the time is pressing. I would like to thank all the speakers of the session and turn the session over to Dr. Infante.

DR. INFANTE: Thank you very much, Dr. Saffiotti. I think that we should get into the next seesion immediately, as we're about 10 minutes behind schedule. I will be chairing papers, discussing the toxicology of polyvinyl chloride.

Our first speaker will be Dr. David Groth, presenting a paper on pneumoconiosis in animals exposed to vinyl chloride.

DR. GROTH: We're doing our best to cut down on travel expenses, so I'm giving two papers.

Because of the interest generated by the discovery that workers in polyvinyl chloride production facilities developed liver angiosarcomas, study was initiated in 1975 to determine the chronic effects of PVC in animals.

Geon 121, the product of the Goodrich Chemical Company, was used in this experiment. Manufacturer's specification stated particle size of 0.5 to 1.5 micrometers. We looked at it by electron microscopy and 90 percent of the particles were more or less than 1.5 microns in diameter.

gshJAV

The PVC powder was packed daily, or every other day, into a dust feeder at a pressure of 3000 pounds per square inch. The generated dust was fed into the mainstream of the inhalation exposure chamber and intake air supply and through a static eliminator before entering the 160-foot chamber.

Four chamber dust samples were collected each day by drawing chamber environment air at a rate of 10 liters per minute for 20 minutes through 5 micron pore sized metricell filters with a vacuum pump.

In addition, simultaneous samples consisting of particles with an aerodynamic diameter equal or less than 7 microns were collected with a 10-plate horizontal lutreator.

All samples were weighed immediately after each sampling period and the chamber dust concentration was calculated.

Adjustments in the dust concentrations were made to maintain a mean concentration approximating 10 milligrams of respirable dust per cubic meter of air.

Daily and grand means derived from the daily means were calculated. Midway through the experiment, two 6-hour chamber air samples were collected on charcoal while PVC dust was being generated.

These two samples and the bulk PVC were analyzed for vinyl chloride by gas chromatography. Samples of dust