IN THE CIRCUIT COURT
TWENTIETH JUDICIAL CIRCUIT OF ILLINOIS
ST. CLAIR COUNTY

FRANCES E. KEMNER, et al.)
)
Plaintiff,)
)
vs.) No. 80-L-970
)
MONSANTO COMPANY,)
)
Defendant.)

Before the HON. RICHARD P. GOLDENHERSH, Judge

REPORT OF PROCEEDINGS

JURY TRIAL

November 4, 1985

APPEARANCES:

MR. REX CARR & MR. JERRY SEIGFREID, Attorneys at Law
Appeared on Behalf of the Plaintiff.

MR. KENNETH R. HEINEMAN & MR. JOSEPH NASSIF,
Attorneys at Law
Appeared on Behalf of the Defendant.

MARSHA SCHNIPPER
Official Court Reporter

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BE IT REMEMBERED AND CERTIFIED that heretofore, on to-wit: Monday, November 4, 1985, being one of the regular judicial days of this Court, the matter as hereinbefore set forth came on for hearing before the Honorable Richard P. Goldenhersh, a Judge in and for the Twentieth Judicial Circuit of the State of Illinois, Belleville, St. Clair County, Illinois, and the following was had of record, to-wit:

* * * * * * *

(The following proceedings were had in open Court.)

MR. HEINEMAN: Your Honor, if the Court please, I think the last thing we did was the Court admitted Exhibit No. 1269A.

THE COURT: Right.

MR. HEINEMAN: And I'd like to pass copies to the jury.

THE COURT: Fine, go right ahead.

FRANK DOST,

resuming the witness stand, having been previously sworn, testified further as follows:

DIRECT EXAMINATION (Continued)

BY MR. HEINEMAN:

Q. Dr. Dost, when we left off Friday afternoon, we were talking about this Exhibit 1269A and the dose response curves. Is this a test in the intact animal?

A. This is a study in the intact animal, yes.

Q. All right. And what is the significance of the upper and lower levels of response in connection with the frequency or the intensity of the effect. How do those -- how are those reflected there?

A. In biological systems there will be an upper limit that in this case is expressed at the top where the effect no longer is increasing with increasing dose. At the bottom there is a point at which no effect occurs. In other words, we find a point whereas the dose decreases, we reach a point where there is no response, there is a no effect dose or no effect level.

Q. And did I understand you to say that -- well what was the figure on this curve that you attributed as the no effect level?

A. In the case of relatively-- the mouse strain of

limited responsivity, the no effect level is clearly at about -- this is a logarithmic scale, keep in mind, and this is a value of about 2.3, so that the no effect level for this strain of mice is at about 3 tenths of a microgram per kilogram for this. The nature of the curve, this is very nearly down at zero effect, and the no effect level here is at about tenfold lower, at about.03 micrograms per kilogram.

Q. Now, the no effect level that you've stated there is expressed in terms of an effect or impact on the body, is it not?

A. Well, it's expressed in terms of whichever parameter we're -- we're examining, because if we're looking at enzyme induction, that does not tell us about other parameters. We have to examine a large number of different parameters to get some idea of what's going in the rest of body, because some effect would be perhaps less sensitive, some more sensitive, just as we have two strains of animals, two separate strains, one of which is sensitive and one of which is less sensitive.

Q. What I'm trying to get at, and I guess I expressed my question pretty inarticulately. Is this dose down here or exposure?

A. This is dose. In other words, you recall in the -- well, here. I drew a dotted line that bypassed the

interaction with the environment when we were talking about some earlier principles.

Q. Would you like to put that up here?

A. Well, perhaps. And what we have done is administer this directly to the animal, and actually we could have drawn that line down here. The exposure and the dose, in effect, are the same in a situation where we inject the material into the animal or if we put it into the digestive tract so what we have done here is administer the dose directly to the animal without the--

MR. CARR: Your Honor, I will object. I think the witness is saying that we have done that, and I thought this was a study by somebody else?

A. I beg your pardon. Dr. Kouri's group has injected it directly into the animal, and the environment, so to speak, has not intervened and done anything to the chemical.

Q. All right, sir. Now, so this does this figure in this particular type of experiment, does the dose figure equate with the exposure?

A. In this situation, yes, because we have -- the nature of the exposure is injection of the material, and it is all -- it all goes into the animal, whereas in -- if the material was on the skin, only a very, very small fraction of it would be -- would go into the animal and so the exposure

would be much, much larger than the dose.

Q. All right. Now, are there experiments in which this type of phenomenon has been observed in an in vitro situation?

A. Oh, yes.

Q. This is what you would call what?

A. This is in vivo, in the animal, in the animal. In vitro is the other term. It means literally in glass.

Q. Okay. Have you had a chart prepared of an in vitro experiment?

A. I do exactly, yes.

Q. Would this assist you in your explanation of the matter to the jury?

A. Yes.

Q. Let me have this marked, if I may.

(At this time Defendant's Exhibit 1270 was marked for identification.)

Q. Dr. Dost, let me show you what's been marked as Defendant's Exhibit No. 1270, and ask you to examine that and identify it for me please.

MR. CARR: Counsel, may I see it please?

MR. HEINEMAN: Certainly.

MR. CARR: I'm through with it, Counsel.

MR. HEINEMAN: Okay.

Q. Again, sir, this is Defendant's Exhibit 1270. Would you examine that and identify it for me please?

A. This is a graph that has been drawn from numerical data in an article by Niwa, who was at that time working in the laboratory of Dr. Daniel Nieburg at the National Institutes of Health, and it's published in Molecular Pharmacology, Volume 11 in 1975. I'd say it's taken from Table 1.

Q. All right. This is a graph prepared by whom?

A. I prepared the draft of the graph.

Q. All right. Now would you explain what this demonstrates to the jury, please?

A. Again, this is a dose response curve that is -- that is information quite similar to what was shown in the last graph. It is an experiment in which TCDD, 2,3,7,8-TCDD, was put into an-- into a culture medium. These are cultured cells taken from -- from rat, and what we see here rather than a dose is a concentration, because we have a homogenous system here, and what we are interested in is the concentration of the chemical in the medium in micrograms per liter.

Very crudely you might relate that to an intact animal, because one liter weighs one kilogram, and the animal -- the animal is not homogenous, the material doesn't

distribute uniformly in the animal as it is in this-- in this culture, in this solution, but a microgram per liter is very, very crudely equivalent to a microgram per kilogram if we tried to compare it. It's very difficult to compare a culture system with an intact animal, but the biochemistry is the same.

Q. By a culture system what do you mean, how do they do this?

A. Well, these cells were derived from a rat tumor. This is a very well established tumor or cell line, and cells in culture after they have been changed, can by either entering into the tumor process or other biochemical changes can be cultured. They will reproduce, and we can -- in the laboratory cell biologists can grow cells, mammalian cells in the same fashion that we would grow bacteria, for example, not quite the same process, because it requires different kinds of culturing conditions, but it is possible to maintain a cell line like this indefinitely, and the practice around the country is that people who have established these lines generally maintain the culture and make them available to other researchers.

Q. Hence a name of some sort, they'll be identified as something?

A. It's the identity of that particular line. In this

particular case, we have a typical dose response relationship. The concentration down here where the activity is at zero, and I should point out that in a culture system many biochemical systems are actually resting at zero rather than at some level. In the -- in the last graph you will notice that these level out at some activity; in other words, where there is no induction by TCDD, there is a base line of activity. And the reason for this is that in an intact animal that system is under at least some demand all the time, and almost all kinds of activity in a normal cell inside the animal, when the animal is all intact, they have some resting level. It's very much like, for example, your resting respiratory rate when you're asleep, which is quite low relative to the rate when you're awake and around doing a lot of things. That's the way it is in an intact animal.

However, in the cell, the isolated cell, there is not all of the other activity in the animal going on, and so at rest oftentimes that enzyme level is essentially zero, because there is no need for it. The cells are in a totally protected environment, they aren't coming in contact with agents that will induce them. We're in contact with things that will induce enzyme induction all of the time.

What we see here is a number of points, and then interestingly, here is a point. One of the reasons that I

chose this particular example is because we can rely so heavily on the dose response relationship and the orderliness of this system that when we see a piece of information like this, we report it. When I find something in my laboratory that is like that, it is reported just as they reported it, but finding that it does not fit into an orderly dose response indicates very strongly that in that particular group of cells something went wrong, something went wrong, whether it was an error in recording, in the process of bringing the data together, whether it was an error in actually conducting an experiment would be impossible to tell from here, but it is clearly an erroneous data point, and I put that in there deliberately, because it tells us the level of confidence that we have in the dose response in general.

In any case there is a no effect level here at.003 micrograms per liter of medium. Now in this system there isn't much metabolism. The material goes in, there's no circulation around the body, there's no excretion so when that material is put in, it stays there.

Q. Now, what is -- what is the figure when you created this graph, what was the figure that caused you to put the "X" there.

A. My recollection is that it was -- was an activity of 94 units.

Q. All right. If you inverted those numbers, what would the figure be?

A. It happens. You recall I just mentioned that it's possible that an error in recording data could imaginably be the case. If you were to make 49 out of that 94, it would fall on the curve. I would not presume to judge that that's what happened. That may just be a coincidence.

Q. All right. Now would you talk to us about the no observable effect level. The jury has heard the expression NOEL, N-O-E-L. What is that and what's its significance?

A. No observed effect level and the "O", the observed is very important, because it depends on what we can observe. We cannot take this prior graph and say we have a no effect level and assume that that represents everything else that's happening in the animal. It is what we are observing here. Now the idea of the no observed effect level is that it is the -- it is the highest dose that does not produce an effect.

Q. All right. Now, what if you have a situation in which there are a number of effects going on in the animal. How do you select an N-O-E-L, how do you find one?

A. Well, it depends on how much information we have, but if we are seeking a no effect level or a no observed effect level that we would like to relate to the whole

animal, we would find the lowest. We wouldn't take in the case of -- in this situation here, let's say we wanted a no effect level for the mouse, we certainly would not take the highest of these two numbers, we would take the lowest, the most conservative. We would not -- we require a substantial amount of information to represent a no effect or a no observed effect level for a whole animal. A single experiment is not going to give us that much information.

Q. How does one go about determining what a no effect level is? Does it require a series of doses, one dose, how do you go about doing that?

A. It requires -- it requires a series of doses. If we have -- if we had only these two doses, we would know that somewhere down in here there is probably a no effect level. I won't say that there is going to be one. I certainly won't say that it's going to be here or here or some other place. We need a sufficient number of doses. There has to be a full range of dosages to show particularly the no effect level.

Sometimes we can't find the point where the effect tops off, because we may be reaching a dose so high that the animal fails before that particular function fails, and then, of course, we don't know anything, but down at the low level we have to have enough doses so that we can tell, a couple of doses or one dose, and many studies are done for the purpose

of identifying the mechanism by which the chemical is working, and they are only interested in one dose, a dose that will cause the effect. That tells us nothing about the dose response.

Q. All right. Now I'm not sure I'm understanding this. You say there may be a test where they only use one dose?

A. Well, many experiments only use one dose. They want to produce the effect and having done that, they want to evaluate the characteristics of the system and see how it has been changed from the normal.

Q. All right.

A. That's a perfectly valid kind of experiment for the kind of determination that they are making.

Q. But that doesn't tell you anything about a no effect level?

A. That's correct.

Q. Because the dose was selected to produce an effect?

A. That's right.

Q. So a one shot deal like that, will that tell you anything about a no effect level?

A. No, it wouldn't tell us anything about it even if it were down here at a point where it caused no effect, because we don't know for any given chemical. It maybe that

the no effect dose is done here, and that's, of course, a useless experiment, but the no effect dose may be here or here or here. There would be no information.

Q. All right. So how many -- for example, the Kociba experiments of Dow Chemical that the jury has heard a lot about. In the Kociba experiments have they attempted to find a no effect level?

A. Yes.

Q. All right. How did they go about doing that?

A. Well, that was -- Those where long term experiments in which the material was administered, in this case TCDD, 2,3,7,8-TCDD, in the diet over a lifetime, and so it was a chronic dose, a chronic experiment, and they used three doses in their two-year experiment. They used four doses in their shortrange finding experiment. Those doses were.001 micrograms per kilogram per day,.01 and.1 in the long-term experiment, and in the shorter one they started at one microgram per kilogram per day, and that was-- caused the animals, they carried it through the experiment, but it caused a lot of damage, so quite a bit of toxicity.

Q. What did the Kociba experiments find to be the no effect level?

A. They did find a no effect level at a daily dose of .001 micrograms per kilogram.

Q. Which would correspond to what, what's .001 micrograms?

A. That's one nanogram per kilogram.

Q. Now, why is it that AAH activity is studied in so many of these experiments?

A. AHH activity is one of the -- one of the enzyme -- one of the enzymes that is induced in animals that are responsive to the -- with the AH locus. In other words, there are -- there is a process in the cell that responds to a wide variety of polycyclic hydrocarbons 3-methylcholanthrene, benzpyrene, dimethylbenzanthrozene, TCDD, and other, other generally related compounds. And this particular enzyme is often used as an index of -- that an effect has taken place when researchers are looking for other effects. That's the reason why -- why in Kouri's experiments they did this, because they were working on a general problem, and they wanted an idea of how much of an induction response these animals had experienced with the doses they were using.

Q. Well, does AHH induction have anything to do with toxicity?

A. It has to do with the fact that a foreign chemical has come into the body. It is not a toxic response as such. It is something that we are doing all of the time, you and I, everyone here, we have that capacity, all mammals have that

capacity. In fact, lots of non-mammals have similar capacity, and when we encounter chemicals of this class, we go into -- we make a biochemical change that the purpose of which is to reduce, detoxify, and reduce the amount of that chemical that is in the body.

Q. Okay. What is it that AHH does?

A. AHH is one of the enzymes that detoxifies, if you will, aromatic hydrocarbons. In fact, that's the name of it, aromatic hyrdocarbon hydroxylase. By that I mean that it attaches -- I believe that on Friday I talked about the hydroxyl group, and this enzyme attaches a hydroxyl group to foreign compounds, relatively non-soluble, relatively fat soluble compounds, makes them more water soluble, and makes them less fat soluble, and much more easily excreted.

Q. What is the difference between a threshhold and a no observed effect level?

A. Well it's partly a semantics problem, just what words mean. It's also somewhat of a philosophical problem. I mentioned that a no effect level is the highest dose that doesn't produce a effect, and a threshhold often is defined as the lowest dose that does produce a effect. It's very difficult to sort those two out, particularly since there is always statistical variation in any experiment, and so there's a sort of blurry area here, and generally in -- just

in the common discussion the two are used interchangeably even though they don't exactly mean the same thing.

Q. Now, how is a no observed effect level used in determining a safe level for a human exposure?

A. Well, experimentally, after we have gathered a great deal of information about a chemical, any chemical, and we can relate -- we find a number of no effect levels, we gathered a great deal of toxicological information, identified no effect levels not only for specific functions like this, but for much more general functions. In the Kociba study that you mentioned a little while ago these animals were observed throughout their lifetime, a lot of clinical chemistry, the same kind of tests that are run if you go into the hospital for lab tests, the same kind of information obtained, pathology, microscopic examination of tissues, blood values, every bit of information that can be extracted from the animal, so that there is a good index of the general condition of the animal, or a test may be a whole animal test on some kind of reproductive function again, which is a general test. That coupled with specific kinds of studies that look for certain biochemical changes, eventually give us a number that we can consider is, in fact, a no effect level for -- for the animal.

Now your question was how does this relate to

humans? And when we are trying to identify what might be a safe level of a chemical for the human, we would take a small fraction of the no effect level in animals and make the assumption, and this is a very common practice in toxicology that we would drop, depending on how much information we have, tenfold lower, a hundred fold lower, a thousand fold lower. Generally, we would want to have a human exposure at least a hundred fold lower than the no effect level.

Q. All right. How does that relate to the amount of data that's available in the human population?

A. Well, the amount of data in the human population and the animal population are both of importance. If we have lots and lots of data in the animal population, it's possible to make -- to work with a narrower margin, because the biochemistry of the animal and the human are, of course, are alike. If there is a lot of data in the human population, epidemiological studies, actual clinical studies of people known to have been exposed to some chemical. The more data of that sort that is available obviously the more data there is that of direct applicability to the human for making a judgment about what levels will have no effect.

Q. All right. Now, when you say the biochemistry is alike, isn't it a fact -- well, with respect to TCDD, are there species variances?

A. Yes, there are species variances for every chemical.

Q. So what kind of problems, if there are any problems, do you have in extrapolating animal data to humans?

A. We have in the case of -- in the case of TCDD or any substance? In the case of TCDD. We have to look at the reasons why there are variabilities among the animals species and in terms of -- there are two problems. One is predicting effects one is judging effects that might take place, that might have -- on the basis of an existing exposure as differentiated from a general, potential situation.

In the CDC study a whole group of calculations were made based on exposure, absorption, and using a no effect level of.001 micrograms per kilogram and extending that into a-- into a calculation of acceptable, what is presumed to be acceptable exposure in humans. So the process is rather involved, and it really is a matter of judging risks.

Q. All right. Now, do all symptoms have no observed effect levels?

A. With respect to TCDD?

Q. Yes.

A. In every experiment that I have been able to find, in every experiment in which there has been an adequate range of doses, a dose, a no effect dose has emerged, and there is

no -- in many of them, of course, perhaps the curve may stop here. They may have not used sufficient number of doses. The curve is certainly aiming toward a no effect dose, but I can't say where it is. But in every case where there is a sufficient amount of data, where the points come down to or even below the zero effect level, it is very clear that a no effect level has emerged with TCDD in every parameter that has been studied.

Q. Now, Doctor, you're familiar -- well, have you read the testimony of Dr. Ellen Silbergeld?

A. Yes.

Q. You have? Do you recall Dr. Silbergeld referring to the work by a Dr. Kaminsky?

A. Yes.

Q. All right. Do you recall, sir, that she testified that based upon the experiments that Dr. Kaminsky has done there is no threshhold, there is no dose that does not cause an effect on the liver. Do you recall that in her testimony?

A. Yes, I do.

Q. Okay. Now, are you familiar with the tests that she's referring to, the studies that she's referring to?

A. Yes, I am.

Q. Okay. Is Dr. Kaminsky is he the first named author in those tests?

A. Dr. Kaminsky is in a -- works with a rather large group of investigators in New York, and I believe he is senior author of one of those papers. I believe J. Silkworth is senior author of one of those papers. There are three actually that he has published on this subject and and though DeCaprio -- yes, he is the senior author of the third paper.

Q. Now, what incident were those papers studying?

A. These papers -- that research was the outgrowth of the -- there was a fire in an office, of a state office building in Binghamton, New York, in which an PCB transformer blew up and contaminated, I believe, pretty much the entire building with soot that as it turned out had a substantial amount dibenzofurans and chlorinated dibenzofurans, and dibenzodioxins, including apparently TCDD, 2,3,7,8-TCDD.

Q. All right. Now, based upon your examination of these studies, do you agree with Dr. Silbergeld's assessment of their result?

A. No, no.

Q. Why not?

A. Well, each of those studies did show clearly a no effect dose. Two of them involved parallel studies of the soot, and they were well-done experiments. Parallel studies of soot and TCDD, and in one case they were studying guinea pigs, which is a very sensitive species, and they also used a

model, a chick embryo model, in which the material is actually injected into a fertile egg and following through the incubation period. This is a sensitive system, because TCDD cannot escape. That experiment showed a threshhold. They were looking at mortality in that case.

The guinea pig study showed a threshhold and did not show appreciable liver effects, which is not surprising, because the guinea pig is not very sensitive to liver damage. Oftentimes guinea pigs will succumb to TCDD without a great deal of liver damage. They are peculiar.

Q. Now, let me just-- may I interrupt you a minute? We have heard that the sensitive measure of TCDD exposure is measured in a guinea pig, that it's a very, very sensitive animal.

A. The thymus weight is very sensitive.

Q. All right. And its ability to live?

A. Excuse me.

Q. And its ability to live?

A. Well, yes, the guinea pig -- the guinea pig is very sensitive to the lethal effects of TCDD, and the weight of the thymus is also a very sensitive indicator that -- it's also more sensitive in the guinea pig than in the -- in other species. And in all of the indexes that they looked at they clearly showed a no effect dose. In the third paper, which

was the one authored by DeCaprio their objective, their stated objective in the paper was to find a no effect dose, and they did a chronic, a subchronic experiment so-called. They fed various concentrations of the soot over a 90-day period to guinea pigs, and they found a no effect level. They stated that as their objective, and they stated it in their conclusions.

Q. All right. Now, is there -- is there other evidence of the fact that there is a no observed effect level in -- in liver experiments or other experiments?

A. Every experiment, as I said earlier, every experiment that I know of in which a sufficient number of doses has been used, there has been no effect. We talked about -- we talked about Kociba's studies a moment ago, and they clearly showed no effect, both with respect to all of the biochemistry and with respect to liver changes, pathology, and so forth.

Q. Now when were these DeCaprio, Silkworth and Kaminsky papers published, sir?

A. They were published in, I think, 1983, 1984, 1983, I believe.

Q. All right. Now, let me ask you a bit about species variability. What is the variability with respect to TCDD among species, how does it appear to vary?

A. Well, one index is the lethality, how much does it take to kill an animal, and the guinea pig is very sensitive. Something around a microgram per kilogram, some sex difference, some strain difference, but a microgram per kilogram, the lowest figure that has been found is 6 tenths of a microgram per kilogram, which is really not very much.

The hamster the median lethal dose, that is, the dose that is required to kill half of the animals in the experiment -- That's a kind of experiment we don't do very often, believe me, but it is necessary in some circumstances, and that, the LD-50 for the hamster is about 5,000 micrograms per kilogram.

Q. So, how many times more than the guinea pig?

A. Roughly five thousand.

Q. All right. Are there any studies which have looked into this variability of species susceptibility to dioxin?

A. A great number, because one of -- this enormous difference causes a good deal of confusion when trying to identify the mechanism by which TCDD actually exerts its lethal effect, which we still do not understand, so efforts have been made to find out whether the biochemical sensitivities follow the whole animal sensitivity, and whether the effects on reproductive tracts or reproductive systems or other systems follow the lethality, and they tend

not to.

We don't see the enormous differen[illegible] hamster and guinea pig, for example, in the metabolism of TCDD or in the sensitivity to induction. There are also differences -- I mentioned that the guinea pig does not, is not highly susceptible to the liver injury that is so common with TCDD while the rat is, so there are a lot of mysteries there, but where similar functions take place the range is nowhere nearly as narrow as the lethality.

Q. Now with respect to the -- with respect to the human being, where does the human seem to fit into this spectrum?

A. Noone has ever known to have died as a result of TCDD intoxication. There have been events that have taken place in which many animals of various kinds have died. In Seveso, Italy when that accident occurred, there was a substantial mortality in particularly small animals, rabbits, dogs, cats, and so on. The impact on the human population, there was chloracne, there were some effects observed, but the overall impact was apparently not that severe, and noone died of the exposure certainly.

The Missouri horse arena episode that has been so well-known, where the waste oil containing TCDD was mixed in with the arena soil, and the children playing in that and so

forth, there were a great number of deaths of horses, because horses will pull the material up apparently through their hooves, songbirds that are around the area, small rodents, apparently cats and dogs, a substantial number of animals died, and there was human intoxication, there was human toxicity, but those individuals, according to the information I have, have recovered. None died.

Q. The -- one of the things that you mentioned Friday was in relation to metabolism of chemicals. Would you tell us about the metabolism of chemicals within the animal's body and what is the effect of that metabolism on toxicity?

A. Well, the whole idea of metabolism of foreign chemicals, and I will use the term metabolism very narrowly to refer only to what happens to foreign substances once in the body rather than all of the other multitude of metabolic activities that are taking place, animals have to find a way of disposing of foreign chemicals if they aren't easily removed.

I think I mentioned earlier that if a substantial is water soluble, obviously it's going to stay in the body water, and it will be processed by the kidney, and it will be removed, and that's a relatively easy process, provided that the chemical is not causing some kind of damage while it is there, and of course, any chemical in sufficient dose is

going to produce toxicity, but the idea here is to find a way of getting it out of the body.

So in the case of substances like -- that are insoluble or relatively insoluble in water, it's very difficult to move them. The amount that goes out in through the kidneys is very, very small, because that kind of material, not only is it insoluble, but it would tend to bind to the proteins into the plasma, and that would keep it from going into the urine, and it would -- a number of things would keep those kind of substances in the body. Their fat solubility would help to keep them in the body. So the whole idea is to make it more soluble and make it less toxic, and often that can be accomplished in the same operation, which is fortunate.

In other words, we don't have to undertake two processes, one of which makes it less toxic and other of which makes it more soluble. Most of the time it is a relative simply process, and it would have to be because of the thousands and thousands of different kinds of chemicals that we are continually exposed to. If you walk through a forest, there are enormous number of aromatic hydrocarbons that volatilize from trees. In fact, in Oregon on a hot summer day if you look across the valley -- if anyone's been in the Smoky Mountains in the east, you can see a haze out

over these valleys. There isn't a bit of industrial activity, hardly any vehicles, and yet there is this haze out there, and in large measure that is material, organic material that is volatilized from the vegatation. So not only do we have synthetic substances, but there's lots of natural materials out there that we've got to do something with, because they are going to come into the body.

Q. In terms of actual exposure to human beings, what part does -- and those things that have to be metabolized, what part does synthetic material play in connection with the overall numbers of naturally occurring things that the body is exposed to?

A. Probably pretty small, pretty small Bruce Ames published a paper in Science a couple of years ago that described very vividly the enormous number of natural--

MR. CARR: Object to this line of testimony, clearly hearsay, objectionable, counsel knows it.

THE COURT: Objection sustained. It is hearsay.

Q. Well, Doctor, are there -- what is the relationship between the exposure levels that the body -- not levels, the numbers of items that the body is exposed to that occur naturally versus the number of items that are exposed to that occur as a result of synthesis?

A. Probably far, far more natural substances of

toxicological significance.

Q. Now, when you were talking about the forests, for example, what specifically are the materials that trees give off?

A. Turpenes, particularly, and this is a very wide range of compounds, but a number of what we have called polyaromatic hydrocarbons, organic materials with a number of aromatic rings.

Q. Is that where the name turpentine comes from?

A. Yeah, yes, it is.

Q. Okay. Now, what is it that the body does with these materials when they -- when they come into body through inhalation or dermal exposure or whatever?

A. As I just said, obviously we can't devise a whole separate system for every single kind of chemical that's going to come into the body. Fortunately, in spite of the enormous number of combinations that can -- that can and -- and the enormous number of different kinds of organic molecules, they have a lot of common characteristics, and so we don't necessarily have to devise a separate system. The AHH will attack a vast variety of compounds that have some common moiety, namely this benzene ring that has-- that has-- that is part of the molecule, and so the whole idea is to make it more soluble, as I've said many times, by attaching a

hydroxyl group to it, and then quite possibly on that hydroxyl group attaching another even more soluble substance, and then the material is excreted very rapidly.

Q. Now, in order for the body to attach this hydroxyl group biochemically, does it have to do anything to the chemical itself, may it necessarily knock some atom off?

A. Two general processes, one of which involves an intermediate in which -- that is very reactive, and then the hydroxyl group goes on. What has to happen is we have two chlorines, for example, side by side. One of them will be-- may be driven off and -- or even both, and an oxygen, a very active oxygen attached, and sometimes that can get us into trouble. That's what is known as a reactive intermediate, and that's the kind of -- that's what happens with oh, benzanthracene, for example, and that reactive intermediate if it isn't changed quickly enough to put another hydrogen on and make a hydroxyl out of it could very well react with DNA. Other organic rings simply have an exchange of the chlorine for a hydrogen, but usually in a biological system they exchange for a hydroxyl group, and there is no reactive intermediate in the process. So there's two general steps that can take place there.

Q. How does the body metabolize TCDD?

A. It clearly metabolizes it, and it metabolizes it

apparently by the latter process, one in which a chlorine is removed and a hydroxyl group is substituted.

Q. Would it be helpful for you to use an easel and show the jury at this point.

A. It would be much easier, I think.

Q. I guess the best thing to do is to start with the TCDD molecule that I suspect you have seen on a number of occasions. There are also, by the way, hydrogens in each of these corners. We never -- organic chemists never show the hydrogens, and they never show the carbons. Each of these intersections there's a carbon molecule, so one of these corners would look something like this.

What we're interested in doing with this substance, which is really much, much more fat soluble than it is water soluble, is to find a way of getting it out of the body, and it doesn't react very well, it's hard to get at because of shape of the molecule, but what happens is essentially this. A chlorine will be taken off, and a hydroxyl group will be put on. Now, that could happen at any of these corners. There is also some evidence that a hydroxyl group might be attached here either after removing a chlorine or even leaving it intact, but in general it is very clear that there is a hydroxyl and sometimes two hydroxyls attached.

Now, it's interesting that by making that very,

very minor change that that molecule, number one, will lose its toxicity, a large part of its toxicity. The other thing is that it will become quite soluble. Then the other thing that can happen in the next step if this molecule as it stands is not -- is not carried out of the body, that there may be attached, the hydrogen will be taken off, and there may be attached a relatively large molecule -- I think that Friday I mentioned a molecule that's something like glucose, and it's a 6 carbon glucuronide that attaches, and that's got a lot of hydroxyls on it, and it makes it extremely soluble.

So this is the process in general that would be undertaken. So all we have done is really-- all we have done is change a corner of that molecule. That's the only change that's been made, and the material then becomes soluble enough to be removed, and that takes place almost entirely in the liver.

Q. Now, Dr. Dost, are there -- when TCDD comes into the body, does it tend to store in certain places?

A. Well, because of its fat solubility, it tends to store in adipose tissue. It also tends to store in the fat deposits that are in every organ, so it would -- and then it also tends to store very vigorously in the liver. Early on more of it will store in the liver or a higher concentration will store in the liver than in fat and at higher

concentrations there is greater amounts in the liver. The reason for this is because the proteins of the liver have a nonspecific affinity for TCDD, they tend to bind to TCDD, and as I think I've mentioned, this is different from the binding to the receptor that we've talked about a number of times. This is a nonspecific binding, and it stores a lot of TCDD in that form. Relatively small amounts in the rest of the tissues.

Now for a normal human about ten percent of the body weight is fat and maybe two and a half percent of the body weight is liver, so the total amount stored would be factored by the size of the organ or the storage site.

Q. Now, how does that storage affect the ability of the body to metabolize the compound?

A. It doesn't affect it appreciably. In other words, it's not locked into those places. There is -- there is a equilibrium. TCDD or any other substance in the body is continually moving back and forth even from a site like fat where it's stored in substantial amounts, it's moving back and forth. I guess a good -- a good analogy would be the movement of -- you could think of water, a vessel of water, where there are molecules of water moving into the vapor phase in atmosphere. This cup, even though it's cold water, there is a little water coming into the atmosphere, and there

is also water going back in, but in spite of the fact that the tendency is to stay in the water phase, there is always some coming out, and the same thing is true in fat with TCDD. Even though it is soluble in fat, has an affinity for fat, there's always movement out as well as movement in.

Q. So that does its storage in fat affect metabolism? Is the body still able to metabolize the material?

A. The body is still able to metabolize the material.

Q. Now, if the body metabolizes some of the material, and it is excreted, for example, in the urine, what then happens to that which remains in the fat and has not yet been metabolized?

A. Well, let me make another illustration. Let's -- we'll just draw some compartments. Biologists love to talk about compartments, we make compartments into everything. But we're going to have, we have a fat compartment here, and and we have connecting the fat compartment with the liver, and for the moment we'll ignore the TCDD that is just stored in the liver, we'll only think about the TCDD that is being altered in the liver for excretion, and we have the liver here, and the liver is removing now some of this material. Now, TCDD does not metabolize as rapidly as many other chemicals. It's faster than DDT perhaps, but it's not as fast as a number of other substances, so we have -- going to

the kidney, and we'll ignore bile for the moment, because it's the same idea, we've got ultimately some TCDD coming out. This is a slow process here. What happens in the liver is not going on really very fast, at least when we relate it to the ability of the kidney to get rid of the product. The liver is making this material. The kidney has got lots and lots of capacity to get rid of it, so anything that comes out of here is gone.

If we have in the fat a lot of material, and it's bound there pretty tightly, and the amount that can be in the blood at any time is very slight, because it doesn't really bind to very much in the blood, and it's not soluble in water to any great extent. So there is a -- there is what we would describe as an equilibrium here. There is -- if the material has just come into the body, we could draw a large arrow here pointing into the fat showing that it's moving into the fat rather rapidly. At the same time there is always a little coming back out. It may turn around and go right back in, but there's always an exchange here, and that's carried by the blood, and even though the blood can't hold very much it's being removed so fast, and the blood is moving so fast that it can actually transmit, it can transport quite a bit over time.

Well let's get to the point where it is in the body

and it is -- we've come to a steady state so to speak. The liver is continually receiving some, because there's a little in the blood, always, because of this exchange here, there's always a little, and we'll get rid of this arrow, and we'll make these two-- almost these two arrows almost the same so it's coming in about the same rate as it's going out, except since there's no more coming in it's slightly faster coming out, because the liver is taking it out of the blood, and in its slow fashion is getting rid of it. So there is a continual drain on the body burden of TCDD.

So what happens is that over time, even though the material is sort of stuck in the fat, it's not stuck permanently, and it moves out slowly, eventually gets to the liver, and once the liver has it, it is disposed of so in time the levels in fat will slowly come down, the levels in all the other and tissues will come down similarly, and sooner or later the material will be down to some level that would probably depend on how much we're just taking in adventitiously in our daily everyday life.

Q. What do you mean by adventitiously in our everyday life?

A. Well, we know that a lot of people, most everyone who has been evaluated has some TCDD in their fat, and it -- so most of us must have some kind of an -- of a low level

exposure out in prairies, anywhere.

Q. Now, you mentioned something about a reactive intermediate. You've read the testimony of Dr. Zahalsky?

A. Yes.

Q. Who testified before here?

A. Yes.

Q. Do you recall, sir, that Dr. Zahalsky testified that dioxin when it's metabolized is changed into a highly reactive metabolite?

A. I remember reading that.

Q. You remember that, sir? Do you agree with that?

A. No, I do not.

Q. All right. Have there been any studies to -- that demonstrate whether or not it's -- the dioxin is turned into a highly reactive metabolite?

A. Yes, there have.

Q. What are those studies?

A. Well, they have shown just the opposite, and the evidence, there's a variety of evidence. To begin with if dioxin was made more reactive and more toxic by -- by the intermediate, through a reactive intermediate or if it was made more toxic in its metabolism, animals with a high AHH level and a high level of all of the P-450 enzymes would suffer greater toxicity, and some very early studies that

were done in Robert Neal's lab by Patrick Beatty showed that in animals with normally higher AHH levels, higher P-450 levels as a function of sex, had lower, were less responsive to TCDD.

Later work that's been done has shown the nature of the metabolite that I just illustrated. That's been very clearly shown. The Swiss workers, Poiger and Schlatter, have done experiments in dogs, in which they have taken the TCDD metabolites from dogs. The dog is a big animal, and it's easier to work with, and have found that those metabolites of TCDD are at least a hundred fold less toxic in the guinea pig. So they have taken the metabolites from one species and introduced them to a species that is much more sensitive and shown that they are, in fact, less toxic.

Q. If -- what would be the effect, if any, on DNA if dioxin were metabolized into a reactive intermediate.

A. If it were metabolized into a reactive intermediate, particularly where this is taking place in the liver, it would be possible to find on DNA, it would be possible to identify DNA damage, and it would be possible to find the binding of TCDD or its metabolite to DNA, and efforts to identify such binding have simply disclosed that it doesn't happen. Allen Poland has done this and Gunther in the National Institute of Health has also shown that the

binding of TCDD either with or without induction of microsomal drug metabolizing enzyme just is not there. The level are so low that they cannot imaginably account for any binding.

There is also a wealth of mutagenesis studies in which the -- in these test systems it is possible to identify biological changes that result from DNA damage in the cell, and they have all shown no response that could possibly account for any kind of interaction with DNA.

Q. Has -- has your position on this matter and what you have described found any support in the Environmental Protection Agency?

A. Environmental Protection Agency states very clearly that the metabolites of TCDD are less toxic than TCDD itself.

Q. Now, if I can deal briefly again with the storage question, how is it that the dioxin or other chemicals that enter the body get distributed around to various organs?

A. When a material comes into the body by whatever route, by whatever -- whether it's taken in by mouth or whether it's absorbed through the the skin, by whatever route, it's going to get into the bloodstream sooner or later, and once it's in the bloodstream -- I mentioned in the case of TCDD that it's really not very soluble, and there's

been some speculation that some of the lipids in the blood may actually have some influence in transporting. This is not clearly shown, but in any case the TCDD will distribute to all organs. It has to because it's in the blood, and blood goes to all of the organs, and it goes to a number of organs in rather large amounts because of their great activity, the heart muscle, the kidney, because it processes all of the blood, and the liver particularly have a very high rate of supply of blood, and so there would be presumably a opportunity for a lot of contact of those organs.

Then the other factor is what organs and tissues have an affinity for the substance and would tend to draw it out of the blood, and that would include the fat. Fat has a substantial circulation. All of the fat cells are supplied by blood vessels ultimately, so there is an opportunity and once it's in the fat it tends, has a much lesser tendency to come out again, whereas in many cells it would have just as great a tendency to come back into the circulation as it did to go into the cell. That's the reason, for example, that the brain has very, very little TCDD in -- in distribution studies. So what happens is that the material moves through the the body and incites -- where it has some affinity, it will tend to store.

THE COURT: Mr. Heineman, is this a good point for a

short break?

MR. HEINEMAN: Yes, it is.

THE COURT: Ladies and gentlemen, we'll take a short break at this time. We'll resume testimony after that. I would remind you that you're not to discuss this matter among yourselves or with anyone outside the jury panel or as of yet form any opinions or conclusions about the matters on trial. Court's in a short recess.

(At this time a short recess was taken.)

Q. Dr. Dost, I have had the first drawing that you made today marked Defendant's Exhibit 1271. Is that -- what is that drawing again?

A. That drawing is a general description of the metabolism of TCDD.

Q. All right.

THE COURT: What was that number? I'm sorry.

MR. HEINEMAN: 1271, Your Honor.

THE COURT: Thank you.

Q. And the second drawing I have had labeled Defendant's Exhibit 1272. Would you tell us briefly that is again?

A. It's a very simple representation of the equilibrium between TCDD stored in fat and moving to the liver for metabolism and out through the kidney.

MR. HEINEMAN: Your Honor, at this time defendants would move the admission of Exhibits 1271 and 1272.

MR. CARR: We have no objection, Your Honor.

THE COURT: They're both admitted without objection.

MR. HEINEMAN: And I'm told that I neglected to offer Defendant's Exhibit 1270, which is the Niwa chart as well.

THE COURT: Any objection to that?

MR. CARR: I thought the witness said that he made that chart.

MR.HEINEMAN: Yes.

MR. CARR: If the witness made the chart, I don't object to it. If it's a Niwa chart I do object to it, it's obviously hearsay.

MR.HEINEMAN: The witness made the chart based upon data from the Niwa--

MR. CARR: I would object to it if it's based upon some data that's not in evidence and can't be put in evidence. I thought this witness did this work.

THE COURT: Do you have that article?

MR. HEINEMAN: Yes, I do, Your Honor.

THE COURT: Could you approach the bench with it please.

(At this time a conference was had at the bench out

of the hearing of the jury.)

THE COURT: What data was that graph made from? You indicated it was, that the graph was drawn from data in this article.

MR. HEINEMAN: Table 1.

THE COURT: Table 1? Have you seen this?

MR. CARR: Just now. I object to it, Your Honor. It's clearly based upon data that is not in evidence and can't be put into evidence. I understood the man was testifying he made the chart, but he's apparently made it from some data from some other study. We have no absolutely way of knowing whether this data is correct or not.

THE COURT: I think we went through this the other day, the arguments on it.

MR. HEINEMAN: Yes, Your Honor, the question was at that time whether or not the witness was going to testify as to the data itself.

THE COURT: Umhm.

MR. HEINEMAN: Whether he was going to sit there and talk about everything that the study showed, and the -- and at that time what we did was the Court permitted in a table which was taken from the paper itself.

THE COURT: Right.

MR. HEINEMAN: Which is Kouri exhibit, which he is

referring to here. This is very little different from that.

THE COURT: I think it's different enough. The objection is sustained, it's not admitted.

MR. CARR: I did not object to the other one, because it was for illustrative purposes. It was not for the purpose of giving any date, simply to illustrate his testimony.

MR. HEINEMAN: That's all this has been used for.

MR. CARR: This is clearly using data from an inadmissible document.

THE COURT: It is. Objection is sustained.

(The following proceedings were had in open Court.)

THE COURT: The objection to the admission of Defendant's Exhibit 1270 is sustained.

MR. CARR: Your Honor, I know it's a little late, but could the jury be instructed to disregard the information that's apparently on this exhibit that they have seen?

MR. HEINEMAN: Your Honor, the witness used this chart to illustrate his opinion and testimony regarding the dose response relationship, and it's exactly the same kind of thing that Dr. Ellen Silbergeld was permitted to do.

THE COURT: No objection -- that is not an accurate statement of what happened, and in no part in Dr.

Silbergeld's testimony was data transformed in the manner in which it is in 1270. The jury is so instructed to disregard the testimony by Dr. Dost that pertains to and uses Defendant's Exhibit 1270. The objection is sustained.

MR. HEINEMAN: Your Honor, may I address it further at this moment.

THE COURT: No, not at this moment.

Q. Dr. Dost, with respect to the fate of TCDD in the body, now, you talked about what happens to TCDD when it gets actually into the bloodstream, but let's assume that the -- that food which has TCDD put in it for experimental purposes is consumed by the rat or the mouse, all right?

A. Yes.

Q. Now, what happens to that as it goes down the alimentary canal?

A. Some of the TCDD would be absorbed, some of the TCDD would remain bound to the particulate material in the food, and there will also -- that's the -- those are the two things that will happen first obviously. Those are the only things that can happen. It will either be absorbed or not absorbed.

THE COURT: Excuse me just a minute, Doctor. Could the two of you approach the bench for a minute, please.

(At this time a conference was had at the bench out

of the hearing of the jury.)

THE COURT: Is that 1270 that's up on the thing? Turn it around or put it against the rail or something. It's standing up.

MR. CARR: The chart.

THE COURT: The chart that I've just objected, that I've just sustained.

MR. HEINEMAN: All right.

(The following proceedings were had in open Court.)

THE COURT: I'm sorry, Doctor, go right ahead.

A. The -- as I said, obviously only two things can happen, and one is that it is absorbed and the other is that it is not absorbed. The material that's in the digestive tract will have a significant effect on the amount of material that is absorbed. In other words, if in the experimental context, and this has been demonstrated, I must say, if the TCDD is introduced into the digestive tract in a solution of methanol, in which it is reasonably soluble, anywhere from perhaps 60 to 80 percent of it will be absorbed, because -- and this is in an animal with an empty digestive tract. If it is introduced in some other medium, there will be enough binding that this amount will be reduced. If it were on soil, the data indicate that that absorption rate will be --

MR. CARR: Your Honor, I would object to this unless the witness is testifying from his own experiments, and it's my understanding that he has never done any work, any research work with TCDD whatsoever.

MR. HEINEMAN: Your Honor, may I address that point.

THE COURT: Sure.

MR. HEINEMAN: Your Honor, witnesses who have testified for the plaintiffs in this case, notably Dr. Silbergeld and Dr. Zahalsky, made a extremely large amount of reference to the work of others. Dr. Silbergeld testified with respect to the Eisen chart, the Eisen study. Dr. Zahalsky testified with respect to the data from the Borman study, which was used in his testimony, and there's a Plantiff's Exhibit on the point, and this witness is doing nothing more than giving his own opinion and then basing it upon literature which he's read, and Mr. Carr previously took the position with his experts that, and the Court ruled that they were permitted to rely upon literature that they had read, and that's what this witness is doing.

MR. CARR: Your Honor, if I might respond to the argument counsel has made in front of the Court, and he knows it was improperly made. The Eisen chart that was referred to by Dr. Silbergeld was referred to without any objection by

counsel at the time. Any other data that was referred to that could be considered hearsay was done so without an objection by counsel. They are the ones that cross-examined had Dr. Silbergeld and Dr. Zahalsky and Dr. Carnow about the great body of literature dealing with this. There is 30 or 40 articles made Monsanto exhibits, none of which were made plaintiff's exhibits dealing with the literature. All of the literature that counsel refers to was literature that Monsanto introduced into this case. Counsel knows that the -- all experts may testify to opinions that they've arrived at based upon the scientific studies done by others. Counsel knows that, and that's the way science progresses. What counsel is doing with this witness is making exhibits appear as if this witness has done the work himself, and when he hasn't done the work himself, he is not permitted to cite the data produced by others. We can't cross-examine these others, we can't go into this man's brain and see if he is accurately giving this data forth, and as counsel knows, this is an improper way. He is an expert, but his expertise has to be based upon his knowledge and not upon the knowledge of others.

THE COURT: Objection is sustained.

MR. HEINEMAN: Your Honor, we have never represented through this witness that he prepared any of the

data. He specifically testified to the contrary, and Mr. Carr has copies of every article he is referring to and has been cross-examining him on.

THE COURT: In the course of your examination he has adopted that data in the manner that Mr. Carr suggested is improper and I agree is improper. The objection is sustained. You're going to have to rephrase your questions to stay in the permissible scope of examining your expert witness.

Q. Dr. Dost, how much -- how much of the dioxin that would be attached to soil if it were fed to an animal is absorbed through the gut?

A. About 30 percent.

Q. Now, what happens to the other 70 percent?

A. It remains in the digestive tract and continues and is lost in the feces.

Q. All right. Now, with respect to the 30 percent that enters the body through the digestive tract, is that the TCDD that is acted upon in the way you have described in Defendant's Exhibit 1271?

A. Ultimately, yes.

Q. Now, how much of the TCDD that actually gets into the body through the digestive tract, the 30 percent, is disposed of in the way you have described in that exhibit?

A. Eventually all of it. It is a slow process.

Q. How quickly does it occur?

A. The half time is in various animal species on the order of 30 days, that is, half of it will have been disposed of in 30 days, half of what remains will be disposed of in 30 days and so on.

Q. All right. Now, you recall, sir, do you not, that Dr. Silbergeld testified that a person who was exposed to dioxin at low levels for years could accumulate ten thousand to forty thousand times the exposure level; do you recall that testimony?

A. I do recall that.

Q. I'm sorry?

A. I do recall that.

Q. Do you agree with that statement?

A. No, I do not.

Q. Why not, sir?

A. There is no evidence of any kind that suggests that. The existing evidence on concentration of TCDD from the environment provides numbers much, much lower than that. The best example that I can suggest is the -- are the long term feeding studies.

MR. CARR: Your Honor, we object to this. Obviously this is not work this witness has done. I object

to it. It's hearsay.

THE COURT: Objection is sustained.

MR. CARR: Unless this witness has done this work.

MR.HEINEMAN: You object to it even if he's referring to it as literature?

MR. CARR: Yes, I do indeed, counsel. Anybody can read the literature.

THE COURT: Rephrase your question please.

MR. HEINEMAN: This is the same way that Dr. Silbergeld testified and--

THE COURT: I have ruled. You don't have to argue among yourselves. I have made my ruling. Now rephrase the question or go to another point, Mr. Heineman.

Q. Dr. Dost, why do you believe that a person who was exposed to dioxin at low levels for years would not accumulate ten thousand to forty thousand times the exposure level?

A. Because I know that the concentration in animals doesn't exceed 25 times the concentration in the diet. That is probably the best evidence. They also -- I know of evidence in environmental studies in which other species that have--

MR. CARR: Your Honor, the witness is doing it again.

THE COURT: Objection is sustained.

Q. Doctor, is there any evidence to demonstrate that such an accumulation can occur in human beings?

A. No.

Q. Ten thousand to forty thousand?

A. No.

Q. Now, you recall, do you not, in Dr. Silbergeld's testimony that she referred in determining that ten thousand to forty thousand times to certain data relating to fish. Do you remember that?

A. Yes.

Q. Now, what is that information?

A. EPA made an estimate based on -- not on data in the environment not on experimental data, made a estimate based on what is known as the partition coefficient for TCDD. By the partition coefficient, I mean the relationship between the amount of TCDD that is in solution in water and the amount that is in solution in a fat solvent, and that represents oftentimes at least a model of the distribution of a chemical between the fat and the water compartment in an organism. TCDD is not really very fat soluble, but is more fat soluble than water, and when you have a substance that is soluble in water only to the extent of perhaps ten parts per trillion, it doesn't take a very large multiplier in the

other phase, in the lipid soluble phase, to create a very large differential and the partition coefficient between water and a solvent for TCDD is about a 40 thousand fold difference.

There was apparently a leap of faith made because the simile between water surrounding a fish -- this data was originally related to the concentration of TCDD that might arise in fish that are in a water body that is contaminated with TCDD. The data on fish don't reflect this. There is no such concentration in fish. The -- so there was a direct extrapolation from a laboratory bench test of the respective solubility of TCDD in water and a solvent called octanol, and that was applied directly in this case, as nearly as I can see, because the numbers are the same, it was applied directly to some relationship in the human, and I don't know how it was arrived at, but it's not a proper deduction.

Q. Now, I'd like to ask you about the mechanism of action of TCDD in the animal. What -- would you explain the concept, first of all, of mechanism of action, what does that mean?

A. Mechanism of action refers to the ultimate biochemical events that take place when a chemical is exerting its toxicological or pharmacological effect. What is really happening in the cell as a result of the

introduction of that chemical, and that really -- sometimes mechanism is referred to in more superficial terms on a whole organ basis, but really to provide valid information the mechanism is the biochemical interaction that the chemical causes in the cell.

Q. Does -- does dioxin by means of this mechanism of action interfere with energy metabolism?

A. No, it does not.

Q. Is its mechanism of action dependent on a depressed immune function?

A. There is no evidence that the mechanism of action, the mechanism of lethal action has any relation to immune function.

Q. Now you said the mechanism of lethal action. What are you talking about there?

A. The mechanism by which it causes animals to die.

Q. All right. Now, does that have anything to do with the immune function?

A. Animals that are maintained for very long periods of time on TCDD at levels that cause substantial pathology do not show any evidence of increased infectivity. There is no increased instance in respiratory infections and so forth. Respiratory infections are particularly important because laboratory animal colonies have a great deal of problem with

respiratory infection.

Animals that die of TCDD intoxication do not show any evidence of increased infection. They don't die of infections. Animals that are maintained in a germ free status -- it's possible to breed laboratory animals in a completely germ free environment, even -- it's possible to exclude even the bacteria that normally inhabit the digestive tract. It's very difficult to do, but it can be done. Animals in that condition are no more -- have no better resistance to TCDD than animals that are grown under normal circumstances. To be sure, TCDD does have an impact on the immune system, but it is not the means by which animals' lives are shortened or by which they die.

Q. Now what thyroid or endocrin effects?

A. Again, there are a number of endocrin effects caused by TCDD. None of them is responsible for the lethal effect of TCDD.

Q. Does -- do toxicologists know, have they been able to determine exactly what it is that kills animals exposed to 2,3,7,8-TCDD?

A. No, sir, this is still not known.

Q. With respect to enzyme induction and its connection with mechanism of action, how are you -- how do enzymes and enzyme induction relate to the mechanism of action of TCDD?

A. The induction of enzymes by TCDD was found to be part of a, if you will, a genetic battery. There are -- the stimulus that causes induction is associated with -- with a number of other effects that relate to the so-called AH locus, the -- which is a section of the -- of the gene -- it's a gene, a collection of genes in the -- in the animal that respond in a characteristic way to TCDD. One component of that characteristic response is the induction of aryl hydrocarbon hydroxylase, which we discussed earlier, and one of reasons that these two strains of mice behaved differently is because they are less responsive to TCDD interaction with that particular group of genes.

Because many of other effects were associated with -- with the responsiveness to the induction and other -- other effects that are related to this particular collection of genes. For example, in mice of different sensitivities the highly sensitive animals are also more sensitive apparently to the teratogenic effects of TCDD, its ability to cause birth defects, and for a while there was a great interest in -- many people were convinced that this function was really responsible for all of the major toxicity of TCDD and responsible for its lethal impact. It turns out that while there are a number of related -- genetisists would call this coordinated functions that are affected by TCDD in this

-- this system. They are not responsible either for the lethal effect of TCDD.

Q. The -- is enzyme induction itself a toxic response?

A. No, it is not. It is a response, it's a protective response to an intoxicant.

Q. Is it an adverse reaction?

A. No, it is not.

Q. Now, is -- let me -- I'd like to talk to you about the limitations that exist on the levels of enzymes induced.

MR. HEINEMAN: Your Honor, may counsel approach the bench for a minute?

THE COURT: Sure, come on up.

(At this time a conference was had at the bench out of the hearing of the jury.)

MR. HEINEMAN: Now, am I to understand that, and I want to be sure I understand this. We are prepared to go through with this witness a series of diagrams that are prepared either by him or taken from a study, the purpose of which is not to put in evidence the data in the study or the data that's reflected in the diagram, but to demonstrate the principle that he is talking about, limitation of effect of TCDD on producing enzyme induction or the dose response curve.

THE COURT: Are these diagrams from the study or are

these diagrams that he prepared from data in the study?

MR. HEINEMAN: In some respects -- well, some are both -- some are one or the other.

THE COURT: So they're both kinds?

MR. HEINEMAN: Both kinds. Now do I understand the Court is going to exclude his testimony or exclude the admission of either of those types of diagrams based upon the objection that Mr. Carr has made to this point?

MR. CARR: If I might respond to that, your Honor? My understanding of the rule that we have been confronted with here is that this witness may not testify to data from hearsay sources. He can testify to data from works that he has done himself. I do not object to him drawing a graph based upon his theory of what's done. I do object to a graph drawn from data or a graph drawn from some -- taken from other hearsay source that has not been introduced and cannot be introduced into evidence, and so if he has a graph from a hearsay source that's not admissible in evidence or if he has a graph from data based upon a hearsay source that's not admissible in evidence, I will object to both of those, either of those kind of graphs.

THE COURT: Have you seen these?

MR. CARR: No, I haven't seen them.

THE COURT: How many of them are there?

MR. HEINEMAN: There aren't that many here.

THE COURT: Do you have some small copies of them?

MR. HEINEMAN: Umhm.

THE COURT: Why don't you bring those up, and we'll talk about them.

MR. HEINEMAN: All right. Here are the ones that we're talking about.

MR. CARR: All right. Clearly shows it's apparently it's -- clearly shows it's from this work.

THE COURT: Now, is that from this work or constructed from the work, this one?

MR. HEINEMAN: I'm not -- I'd have to look at the paper and see. I'm not sure in this instance. I can find that out and let you know in each one.

MR. CARR: This one is also cited as a source from some work. It's a source from somewhere.

MR. HEINEMAN: Here's another one.

MR. CARR: They've all got.

THE COURT: They're all in that position, but that doesn't tell us whether it's constructed from it or comes from it.

MR. HEINEMAN: This one I know. This one comes right out. This is -- this is a figure in the paper.

THE COURT: Okay.

MR. HEINEMAN: This S-L-A-G-A on two-stage carcinogenesis.

THE COURT: Fine.

MR. HEINEMAN: This comes right out of the paper.

MR. CARR: In either event, we object to it on the basis of hearsay.

MR. HEINEMAN: I know that Thigpen, T-H-I-G-P-E-N, this mortality of mice. This is one that he has created from data from a data table. On the -- on the Smith chart relating to dose response on porphyrins and on the Poland and Glover chart relating to response for induction of hepatic aryl hydrocarbon hydroxylase, I don't know as I stand here whether these were created by him or whether they come out directly from the paper. I'd have to pull the paper to look.

MR. CARR: Our position is the same, Your Honor, that these are all hearsay documents. They're either a graph from a hearsay document or its data is a graph that he prepared from data in a hearsay document. It's not admitted or admissible into evidence, and he cannot testify to hearsay. He can testify to his opinions, but he can't testify to hearsay unless I don't know the hearsay rule.

MR. HEINEMAN: Your Honor, I think that -- that he is certainly entitled to use illustrations of this kind to

explain his opinions and the basis for his opinions. He is not offering the data itself into evidence to prove anything. He is just trying to demonstrate that there -- that these things exist.

THE COURT: I am sustaining the objection. I think they are hearsay. He is using them in a manner which as a practical matter gets them before the jury in the context which violates the hearsay rule. He is not foreclosed in his testimony of making original drawings illustrative of the same type of points in a visual nature with a visual aid in order to illustrate graphically in front of jury the point that he is making. This is sort of a back door way of getting this data in, and I don't think it would properly be used in this context. I am sustaining the objection to these, which probably should be marked so that we can refer to them. Could you just mark these in sequence, and I will read into the record that these--

MR. CARR: Another one.

MR. HEINEMAN: This one is actual data taken from a table.

THE COURT: Again to be used as illustrative purposes.

MR. HEINEMAN: Yes.

THE COURT: Marsha, could you mark all these.

(Defendant's Exhibits 1273 to 1277 were marked for identification.)

THE COURT: On the record this -- the objections are sustained to Defendant's Exhibit 1273, 1274, 1275, 1276, and 1277, which are the smaller copies of blowups.

(The following proceedings were had in open Court.)

Q. Dr. Dost, is there a limitation on the level of enzymes that can be induced in this way?

A. Yes, there is a very clear limitation.

Q. All right. And how does that come about?

A. It comes about because of the capacity of the cell to produce the enzyme, and the location of the limitation is known, has been worked out biochemically, but in essence the cell has a limited ability to respond to the stimulus to produce the enzyme.

Q. And is that true regardless of what the chemical is that's stimulating the induction?

A. That's correct. The chemical that -- that will induce -- and as a matter of fact, this has been very clearly with aryl hydrocarbon hydroxylase by Allen Poland.

MR. CARR: Your Honor, the witness insists upon doing that which he may not do. I object to it.

THE COURT: Objection is sustained.

MR. CARR: Ask the jury be instructed to disregard.

Counsel knows it, the witness knows it. I don't understand this.

THE COURT: Objection is sustained. Jury is ordered to disregard it. It's about five minutes to noon. We are going to break for lunch at this time, and, Mr. Heineman, I'd like you to explain exactly what the problem is to this witness so that we can avoid it in testimony after lunch.

We'll break for lunch now. We'll resu;me at 1:15. The admonishments that I've given you earlier will apply during this lunch break. Court's in recess for lunch.

(At this time a short recess was taken.)

(The following proceedings were had in open Court.)

MR. CARR: Your Honor, at this time the plaintiffs would like to withdraw the objection to Exhibit 1271 that we made this morning, and in addition, advise counsel and the Court that we are withdrawing other objections that we made to the testimony of Dr. Dost as long as the exhibits that they use identify the source and as long as the witness identifies the source of the data upon which he may or may not -- that he is basing his opinion, and we will, therefore, withdraw those objections so that counsel can show what he wants to show in these areas.

THE COURT: Now 1271, 1271 was admitted without

objection.

MR. CARR: 1270 then, Your Honor, would be the exhibit that we withdraw the objection to.

THE COURT: Okay.

MR. CARR: And, of course, the Court, I think, instructed the jury to disregard that exhibit, and the Court should then, since I have withdrawn the objection, if we've withdrawn the objection, the jury should be instructed that they may consider that.

THE COURT: The jury is so instructed, so you can disregard my admonition to disregard the testimony.

Q. Dr. Dost, with respect to the limitations that exist on the level of enzymes induced, is there a chart that you have had prepared that would assist you in the description?

A. That is correct.

Q. --of this subject matter to the jury?

A. Yes.

(Defendant's Exhibit 1278 marked for identification.)

Q. Let me hand you, sir, what's been marked as Defendant's Exhibit 1278. Would you examine that and identify it for me, please?

A. That is a--

MR. CARR: May I see it first, counsel?

MR. HEINEMAN: Certainly.

MR. CARR: Do you have a little one for us?

MR. HEINEMAN: Yes.

Q. Go ahead, sir.

A. That is a graph, the graphic portion of which has been copied directly from an article by Allen Poland and Edmund Glover in Molecular Pharmacology, the Journal of Molecular Pharmacology, Volume 10. I believe that was in 1976, but we don't have the date on it, I'm sorry. The caption was moved from below the graph up on top, but the graph itself was copied directly from the article.

Q. All right, sir. I wonder if you would be so kind as to come down here and discuss this exhibit with the jury.

A. I had earlier shown a graph that showed two curves, and one of them was what I called a responsive mouse, and the other was in a strain of mouse that -- this graph right here, the responsive and the nonresponsive, and the difference is that the dose required to produce this effect is greater in one than in the other.

The reason that this work was done was because it was known for some time that this strain of mouse did not respond with induction to a compound called 3-methylcholanthrene, which is a fairly effective inducer.

Dr. Poland in his group did an experiment in which they compared the actual doses required to produce a dose response curve with TCDD on this enzyme aryl hydrocarbon hydroxylase and with 3-methylcholanthrene. The reason that they did that was because they wanted to learn whether the mechanism essentially was the same. Now all of the details of the mechanism would not emerge, but at the very least if the dose curves were parallel, it would be reasonable to assume that there at least was strong similarity in the -- in the mechanism. What they found was that there was a clear upper limit in the ability of TCDD to induce this enzyme, and the upper limit is the same as it is with 3-methylcholanthrene. Now, the reason that this curve doesn't go up any farther is because, as I explained earlier, this is a very toxic substance, and when we get up to high doses like this, the animal simply cannot tolerate them.

What is interesting is that with 3-methylcholanthrene, which has always been thought to be a very potent inducer, you see we have a logarithmic expression of dosage here, and we have almost one, two, three, four, five orders of magnitude difference. There's actually a 30 thousand fold difference in the potency of these two compounds. Now, when I say potency, I mean the amount that it takes the number of molecules really that it takes to

accomplish a specific amount of activity.

So that TCDD is, in fact, a exceedingly potent substance, when we consider it in terms of the number of molecules it takes to induce the effect. But the capacity for the cell to respond is the same in both cases. In other words, TCDD doesn't make 30 thousand times more enzymes, for example. It causes the same response, and this response limits -- even though we don't have the data up here, from the shape of the curve here and through here compared with this, we can be quite certain that 3-methylcholanthrene is not going to produce any more. Certainly at this level you see the comparison is the same at this level, at this level, so the relative potency of the two compound is constant throughout the dose response curve.

What this demonstrates is clearly there is a limit to the capacity of the system to respond, and it also is strongly suggestive that the two are acting by the same mechanism.

Q. Now, Dr. Dost, you recall -- I'll ask you if you do recall the testimony of Dr. Silbergeld in court here on April 12th when she told the jury that AHH can be induced by ten thousand times. Do you recall that testimony, sir?

A. Yes, I do.

Q. Do you agree with that?

A. No, I do not.

Q. Why not, sir?

MR. CARR: What page would that be on, counsel?

MR. HEINEMAN: I think it's Page 137?

A. The reason that I don't agree is because in all studies of this enzyme and related enzymes for that matter there is a clear upper limit to the ability of the cell to respond to induction, even to TCDD, and that limit -- partly when we say so many, you know, times, in this case we are talking about something on the order of 25 times, you can see that if the resting level was only half, which it could have very well have been in another experiment, it would have been a fifty-fold difference, but we don't get massive differences. In many situations the maximum induction is only two or three-fold. It depends in part on the organ -- this is in liver, and it depends in part on the organ and to some extent the species, but there is a very clear upper limit.

Q. And that upper limit is approximately what?

A. On the order of 25 fold. There is difference from organ to organ in its ability to synthesize this enzymes, but these are not large numbers.

Q. Does that -- is that limit reached by other chemicals besides TCDD?

A. Well, yes, surely. We have that situation here with 3-methylcholanthrene. Other inducers such as phenobarbital, which induces somewhat different pattern of enzymes than TCDD. Also with all the enzymes that it induces there is a clear upper limit to the-- there is just so much capacity to make the enzyme.

MR. CARR: Your Honor, I'd object to the question asked the witness, because it's -- Dr. Silbergeld did not say that dioxin makes ten thousand times. The reference that counsel gave me was she illustrated that the dioxin receptor gets on the line and says I'm going to give you a modification of the message, don't make ten units of AHH, make ten thousand.

Now that doesn't say make ten thousand times, it says the message -- just modifies the message, don't make ten units of AHH, make ten thousand units of AHH. That's not at all what counsel gave to the witness.

THE COURT: Gentlemen, could you approach the bench.

MR.HEINEMAN: That's exactly what it says.

MR. CARR: You said ten thousand times.

THE COURT: Let me see that.

(At this time a conference was had at the bench out of the hearing of the jury.)

MR. HEINEMAN: Your Honor, may I respond to the

objection? I know exactly what you're talking about. Your Honor, if in fact scientific evidence is that it goes up 25 times, what she is implying here is that the -- it can make ten thousand units.

THE COURT: Not at all. All she is saying is that message is delivered, she in no place in that language implies that that in fact is what happens, and that's what you represented to the witness in the question. Your objection is sustained, and I will order the jury to disregard it.

(The following proceedings were had in open Court.)

THE COURT: Ladies and gentlemen, the objection is sustained. The question to Dr. Dost and the contents of that question as far as Dr. Silbergeld testifying as to ten thousand times and the response that it brought from Dr. Dost is to be disregarded by the jury. You're so ordered. You may continue, Mr. Heineman.

MR. HEINEMAN: Very well, your Honor.

Q. Dr. Dost, let me read to you what Dr. Silbergeld said, and I would ask you if you agree with this statement.

So using that analogy, what the dioxin receptor complex does is gets on that telephone line. DNA has called up RNA and said would you please make a certain amount of this enzyme, AHH we been talking about, but dioxin receptor

gets on the line and says, don't listen to that message. I'm going to give you a modification of the message. Don't make ten units of AHH, make ten thousand. And what happens is you get an overriding of this message, and that is indeed what goes on, what we call enzyme induction.

Now, do you agree with that statement sir?

A. I think that's incorrect.

Q. And for what reason?

A. Well, the implication in that statement is that TCDD acts differently from other substances that induce AHH. The only difference is that TCDD is more potent. The message is the same, the message is the same, and it depends on the amount of the inducing chemical that binds to the receptor in the cell.

Now, it takes a much higher concentration of 3-methylchlolanthrene to bind sufficient numbers of receptors to produce the induction, but once that binding to receptor is accomplished, the complex of the receptor and the ligon, the chemical that binds to it, accomplishes the same thing either way.

Q. And that limit is what, sir?

A. The limit is on the order of, depending on the organ and the tissue and immediate circumstances, it's going to be around 25 fold. It could be as high as 50.

Q. Now, Dr. Dost, we talked this morning about absorption rate in the body. Does the dose have an effect on the absorption rate?

A. In the case of TCDD?

Q. Yes.

A. Yes, it does. It's interesting. One would logically expect that the -- that the same fraction would be absorbed with a lot or a little. When I say a lot, I'm assuming that we haven't overloaded the system so far that it simply can't be absorbed. What turns out to be the case is that on both the skin and in the digestive tract that as the -- as the actual dose decreases, the percentage of the dose that is absorbed decreases faster than the dose.

In other words, if there's two doses tenfold a part, the amount that is absorbed from the low dose is a far smaller percentage of the one percent than the percentage of the ten percent that is absorbed, which is very interesting and unusual even and very fortunate.

Q. So that the lower the dose the less the absorption rate?

A. The lower the dose the smaller the fraction of the dose that's absorbed, so the amount absorbed decreases faster than the dose.

Q. Now, I'd like to direct your attention to another

statement of Dr. Silbergeld when she testified that the induction of P-450 enzymes--

MR. CARR: What page, counsel?

MR. HEINEMAN: 148 and 149.

Q. That the induction of P-450 enzymes and the consequent metabolism of dioxin results in a new molecule that is highly reactive and can interact with DNA. Do you recall that testimony, sir?

A. Yes, I recall it.

Q. And do you agree with that?

A. No, I don't. It's a similar statement to one that I believe we heard earlier. It is not correct.

Q. And upon what do you base your opinion that it's not correct?

A. I base my opinion on research that has shown that as the metabolism, as the capacity for metabolism increases toxicity decreases. On research that -- that shows the nature of the TCDD metabolite, on research that shows that the metabolite of TCDD is in fact much less toxic than TCDD, per se, and also the fact that TCDD does not demonstrate any of the characteristics of a substance that goes through the stage of a reactive intermediate, where there is a form of the chemical that is highly reactive and has the potential for interacting with DNA.

Q. Now, well, does TCDD react with DNA?

A. No, it does not.

Q. Now, whose work establishes that, sir?

A. Primarily the work of Allen Poland, and others have studied the problem Thomas Gunther at the National Institutes of Health, the work of, as I mentioned, Poiger and Schlatter in Switzerland identifying the metabolites, and, of course, in Poland's work he used both uninduced and induced animals and was unable to find any difference, any increased DNA binding in animals that did not -- that had excess, if you will, capacity to metabolize, so that in both induced and uninduced animals, there was no response, and, of course, the indirect studies on micro-organisms seeking DNA damage in various ways also do not show an interaction with TCDD.

Q. Now at the same time in her testimony Dr. Silbergeld told the jury that dioxin enhances its own toxicity and creates a vicious biological cycle. Do you recall that?

A. Yes.

Q. And do you agree with that, sir?

A. No, I do not.

Q. For what reason?

A. Well, I really have outlined the reasons already, the fact that TCDD metabolites are less toxic. In addition,

there is again from Dr. Poiger and Dr. Schlatter more recent work, which shows -- in which they have treated animals with TCDD, they have used TCDD as the inducing compound, if you will, and then followed it later with radioactive labeled TCDD and have shown that the rate of TCDD metabolite production is about double. In other words, TCDD is being metabolized and shipped out of the body at a much higher rate in animals that have had a pretreatment with TCDD.

Q. What's the significance of that with respect to enhancement of toxicity?

A. We've already learned that in the same laboratory, as a matter of fact, that the TCDD metabolites, per se, are much less toxic than TCDD itself.

Q. So that if there is more of the metabolites created, then the toxicity is going down rather than up?

A. That's correct, that's correct.

Q. Now, let me hand you, sir, what's been marked as Plantiff's Exhibit 1627 and ask you to examine that and identify it for me, please?

A. This is an EPA document. It is identified as May, 1984 External Review Draft of the Health Assessment Document for Polychlorinated Dibenzoparadioxins, and this is Part 1 apparently of two parts of this document.

Q. Does the document reflect who the reviewers of the

material are?

A. Most documents have a -- here we are. The polychlorinated dibenzoparadioxin peer review panel members -- this group is chaired by Dr. Mukerjee and Dr. Stera. They're both EPA scientists.

Q. Is Dr. Silbergeld on that list?

A. Yes, she is.

Q. All right. Let me direct your attention to page 7-14 of that document. What is the date of that, by the way?

A. This is May, 1984. Did you say 7- 14?

Q. 7- 14.

A. I have that.

Q. First paragraph, last sentence. Would you read that aloud to the jury, please?

A. A comparison of the mortality data at five weeks after dosing indicated that the acute toxicity of 2,3,7,8 TCDD to guinea pigs was at least one hundred times higher than was the acute toxicity of its metabolites.

Q. Now, what does that -- is that statement consistent with or inconsistent with a statement that TCDD enhances its own toxicity, creates a vicious biological cycle, and creates reactive intermediates that react with DNA?

A. I would have to say that it is inconsistent.

Q. Now, sir, Dr. Silbergeld also told us that dioxin

creates a reactive metabolite which initiates cancer as shown by Dr. Eisen. Are you familiar with that testimony, sir?

A. Yes, I -- yes.

Q. All right. Are you familiar with the Eisen study that she's referring to?

A. I'm familiar with a review that Dr. Eisen prepared that, I believe, is the document. She doesn't specify the document, but I believe that it's a review that Dr. Eisen developed in attempting to bring together an explanation of the general question of the-- AH genetic locus.

Q. Do you agree with her statement about what Dr. Eisen is showing in that article?

A. Dr. Eisen didn't specify anything. He discussed TCDD in the article, but he did not identify a mechanism for TCDD.

Q. Let me show you, sir, what's been previously marked as Plantiff's Exhibit 233, and ask you if you can examine that and identify it for me, please?

A. I recall that as a figure that was in the Eisen article that I referred to, the review article.

Q. Now, does this exhibit show that dioxin creates a reactive metabolite which initiates cancer?

A. This exhibit, this diagram does not show that.

Q. All right. Could you tell the jury what it does

show?

A. Well, this is intended as a -- Dr. Eisen is a -- is a very capable biochemist, who has been interested for years in the concept of receptors. I mentioned earlier, I believe, that we depend on receptors for the action of all of our hormones, insulin, for example, in the adrenal cortical hormones as well as for other functions, and one of the characteristics of the AH receptor that starts all of these mechanisms, all of these processes that we been talking about is that it resembles in many ways the receptor for the adrenal cortical hormones, and Dr. Eisen had become interested in this receptor, both for its own sake and as a possible model to help him understand the glucocorticoid receptor, and what he's done here is to try to prepare a generalized scheme that -- into which all of the compounds, all of the polycyclic aromatic compounds might fit in some way.

He is not saying that every one of them follows the whole road map here. What he is trying to do is put together an integrated system and provide a means of relating individual compounds. Now he was, as a matter of fact, my recollection in the article is that his reference to reactive intermediates was one based on his work with 712-dimethylbenzanthrozene, which is a compound that does go

through a reactive intermediate change, and what he is showing is that a route among the variety of prospective routes, the compound comes into the body and interacts with the receptor, and it activates a group of genes, and that causes the synthesis of these enzymes that we've been talking about among them AHH and a variety of others that have generally related functions.

One of the thing that that group of enzymes can do with some compounds benzanthrozene is one, 3-methylcholanthrene is another, is make a reactive intermediate, because this process of making it more soluble has a step where one of intermediates is very reactive, and unless that hydroxyl group can be put on quickly, it could conceivably escape from the point in the cell where it's being made and move to DNA or some other site and cause harm.

It happens that TCDD does not go through that process. It is able -- the organism is able to interact with it and convert it all the way to the hydroxyl without going through this stage. So what he's done is account for the kinds of substances that can be -- that can be interacted that way. The compound not only induces the receptor, but then it interacts with the enzymes with the potential for a reactive intermediates, and we know that those kinds of

intermediates can cause DNA damage and cause all kinds of problems in the cell, at least potentially.

There are question marks here, because noone is really sure where, other than at DNA and a few protein sites, where that reactive intermediate might really be exerting effect. There's evidence of cell membrane effect, there is evidence of interference with the receptor for epidermal growth factor, which is another substance that's synthesized in the cell, in the body to interact at specific sites.

Well, if that happens there are a number of things that might very well occur. Epidermal hyperplasia, that is, where the skin would perhaps tends to thicken; immuno-suppression, we know that there is immuno- suppression with TCDD, for example, and other substances of this sort if the dose is raised sufficiently.

The role in promotion of cancer is not clear. With respect to this there is some evidence that certain kinds of hyperplasia are associated with promotion. A number of other kinds of enzymes that are not part of P-450 pattern that do induce, whenever you induce, whenever you start this process with the AH receptor.

So this is really an excellent description of the overall general process. This group of enzymes, I mentioned the other day that I have done work with ornithine

decarboxylase, but not in this context. There are other things that induce ornithine decarboxylase. So this is just a general scheme. It does not refer specifically to TCDD or for that matter to any other chemical. It's intended to account for the way, to provide a scheme that will allow us to generalize about the way that this group of compounds can have an impact on the cell.

Q. And does that scheme as reflected by Dr. Eisen there relate to the actions of 2,3,7,8-TCDD?

A. To this extent it does. We know that in interaction with the receptor, the cytosolic receptor, we know that it activates these genes, at least we know that indirectly, and the evidence is so clear because of the nature of the enzymes that it induces, and these things all apparently happen. There is a real question about this, but these enzymes are apparently -- well, these are -- these are questionable, but this one probably is induced to some extent.

These certainly are, and, of course, all of the P-450. That's the reason there are question marks here because the evidence is just not sufficiently clear yet to really make a statement about them. So to that extent, yes, and then there are questions here, is there a direct action of some of these chemicals on some of these factors? There is

some evidence, but it's really speculative at the present time. So, yes, some of the things TCDD does relate to, and some of them it does not. It does not -- it doesn't relate to the reactive intermediate idea.

Q. Does it demonstrate a initiation of cancer?

A. No, it does not.

Q. Does this chart demonstrate--

A. This chart does not either, except where certain compounds that are reactive intermediates we have to assume that they have at least the potential for initiation of cancer.

Q. Now, with respect to those parts of the body in the animal that are affected by TCDD, I'd like you to discuss those, if you would, and would you start with the skin please. What does TCDD do to the skin?

A. Well, TCDD in certain species of animals has a effect on the skin. It will cause chloracne, which is a hyperplasia and swelling of cells on the skin. It increases the amount of keratin, the flaky material on the skin, causes inflammation in sebaceous, that is, sweat glands, and ultimately the formation of big blackheads. It does this in the rabbit. It is the same response apparently that is observed in humans with chloracne. Chloracne is caused by other substances than TCDD. There is a genetic strain of

mice that doesn't have any hair, and TCDD will cause a lesion that is something like chloracne, not exactly the same. There is an investigation going on to see whether that would be a appropriate model to use for studying chloracne.

Q. Is there -- well, let me ask this. What animal presents the closest model for human skin with respect to reaction to TCDD?

A. Clearly the rabbit, the rabbit ear.

Q. And has there been a no observed effect level found with respect to chloracneiform lesions and the rabbit ear?

A. Yes, there has.

Q. Okay. Who has done that work, sir?

A. That work has been done by -- probably the first people who really quantitated it properly where Jones and Krizek back in 1962, in which they worked out a dose response in the rabbit, found a clear dose response relationship and a no effect dose that I believe was at the level of about 2 tenths of a microgram, and later on Schwetz and his coworkers at Dow Chemical have also done somewhat similar work and have found a similar dose response, and I believe that the no effect level that they found was on the same order as about, and that is not a dose per kilogram, by the way, that is a dose per site. And in those experiments they treated the animals for an extended period of time. They treated them

five times a week for four weeks applying new material each day.

Q. Now, with respect to -- to humans, has chloracne been observed in humans?

A. Oh, yes, it has.

Q. Has there been any work done on the dosages involved with producing chloracne in humans?

A. I know of only one study in which known doses were applied to human skin.

Q. And which is that, sir?

A. That was a study that was reported by Dr. Roe of Dow Chemical to the EPA hearings on 2,4,5-T, the administrative law hearings that were in progress over about a year and a half until relatively recently. I think that that work was presented, I'm not certain, in 1984, I think, to the hearing, '83 or '84. I'm sorry, I would have to look at it and see the date, I just don't remember right at this instance, but it was a reports of work that had been commissioned by Dow Chemical with the intent of trying to learn where the threshhold for chloracne and other effects might be for TCDD on the skin.

Q. Who was the one that conducted this work?

A. The person who actually did the work was a professor of dermatology at the University of Pennsylvania

named Kligman.

Q. And what was found with respect to a dose response level and chloracne and other effects?

A. This was an experiment that was done -- it was an experiment that was simple in concept, and the execution required some care and timing, because the TCDD was administered in fractional doses. At the low doses they administered half of the dose on one day and then the other half a week later.

At the higher dose, which is what we are primarily concerned with, a dose of 16 micrograms applied in a location. The first dose was 8 micrograms, and then they applied 8 -- they applied that in two halves; in other words, 4 micrograms today 4, micrograms tomorrow, and then starting in a week, two weeks, administered one microgram doses a day for 8 days. It's a simple experiment with a complex dosage schedule.

Q. Leading to a total dose of what?

A. 16 micrograms on that site over that period of time.

Q. Now, you mentioned before the difference in the rabbit experiments, I think, with site dosing as opposed to overall dosing through feed and that sort of thing?

A. Yes.

Q. What is the effect of site dosing?

A. Well, this concentrates -- it's possible to cause chloracne, at least it appears possible in the human to cause chloracne by systemic administration. If the dose is high enough it's possible to cause these kinds of skin lesions by means other than putting it directly on the site. It appears to be possible in the rabbit, although the data is not clear. The difference is that the material is concentrated in one site rather than being distributed through the body. It was placed on the skin in a very small patch, so that it's the effect on the skin at that site is going to be very substantial.

Q. Is there any kind of a solvent used in order to try and get the material through the skin into the animal?

A. In those experiments -- they generally use an alcoholic solvent, methanol, ethanol. Ethanol is alcohol, the kind that you get in drinking alcohol. Methanol is the kind that's usually in rubbing alcohol.

Q. Which did Dr. Kligman use?

A. Dr. Kligman used, he used a mixture of alcohol and chloroform to carry the substance. Because of the relative insolubility of TCDD that was considered by the chemists to be the best solvent, so that it would be certainly dissolved and then distributed uniformly over the spot where it was

being applied.

Q. And at 16 micrograms -- now, you said there was 8 administered at first in 4 and 4?

A. That's correct.

Q. And then there were 8 one microgram doses?

A. In succession, that's correct.

Q. In 8 consecutive days?

A. That's right.

Q. So a total dose was what over what period of time?

A. 16 micrograms over a period of -- total would total about three weeks.

Q. Was that administered at the very same spot on the body?

A. Yes, it was placed right on the same location.

Q. Now, what was the result?

A. There was no evidence of chloracne.

Q. Was there any evidence of any other systemic result?

A. The investigator, Dr. Kligman, had -- I should point out that there were five other groups at lower doses that were run through a somewhat similar dosage schedule. All of those people were given physical examinations, and a number of laboratory tests were run on those animals -- on those individuals, and there was no evidence of any

abnormalities in the clinical chemistry.

I should mention that these were volunteers from a prison population, and the-- the creatinine clearances probably were a tiny bit low, but probably reflect the rather low protein diet. I wouldn't be surprised if they were on a low protein diet, but they were really not outside the range of normal. So there was really no evidence in that of any abnormality that would be detected on the basis of those kinds of tests.

Q. Was there -- was there a subsequent test done that did cause chloracne?

A. This is a -- this is an interesting situation, because Dr. Kligman instead of awaiting instructions from Dr. Roe went ahead with another protocol and used a much, much higher dose of TCDD, and he did cause chloracne in most of the -- I believe he looked at ten subjects, and I believe he caused chloracne in eight of those. In any case it was a high fraction of the individuals.

Q. And was the -- what was the dose that was used?

A. The total dose amounted to 7500 micrograms.

Q. Would that be 7.5 what?

A. Milligrams.

Q. Milligrams. And he produced chloracne in eight out of ten.

A. Something of that order. I'm sorry that I cannot remember the exact numbers, but he produced in chloracne in most of individuals.

Q. And was there any other findings reported other than the chloracne?

A. There was no data presented. There was a statement by him that was included in the document presented at the hearings, which said that there were no significant adverse findings other than the -- other than the chloracne.

Q. Now, based upon the animal data that you have, have you -- have you determined a dose that could be obtained from exposure to soil at the Sturgeon spill site?

A. The only way that I know to do that would be to -- would be to take soil that was under the car when it came to rest, where the material came spilling out, because there was a -- at that point the concentration of the chlorophenols was very high, as you might expect, that is, the concentration in the soil, and if one were to assume that all of the TCDD that was in the tank car remained at the same concentration in the phenol, one could calculate a concentration of TCDD in that soil, and then make assumptions about rubbing the soil on one's skin or something like that.

Q. All right. I wonder if you could use the easel and perform the calculations?

A. Well, all right. My arithmetic has been known to fail on occasions, so perhaps you might keep track of -- the concentration of TCDD in the tank car was represented as 45 parts per billion in the phenols, there was both orthochlorophenol and phenol in that, and the concentration of the phenol in the soil was about 7 percent. I think it was slightly under that, but let's say 7 percent, both the total phenols.

MR. CARR: Counsel are you identifying a sample of soil taken under the tank car as the witness is saying or would you identify the facts that the witness is using 45 parts per billion TCDD, where is he getting that fact and the other findings that he is testified to. Would you do that please. I object to it until it's done, your Honor.

THE COURT: I'll give you an opportunity to do so.

Q. I have asked to you assume 45 parts per billion as the highest level found of 2,3,7,8-TCDD, the highest level reported in a reserve sample tested by Dr. Christopher Rappe. I'd like to you assume, Dr. Dost, that there are a number of other levels reported which are lower than that, but that that's the highest one reported.

A. Okay.

Q. All right. And in addition to that, I'd like you to assume the 7 percent concentration as having been reported

by Environmental Protection Agency on or about January, I believe, 23rd, 1979 at a location directly at the spot where the car came to rest.

MR. CARR: Well now, you mean under the car?

MR. HEINEMAN: Yes.

A. If we have a concentration of 45 parts per billion, that means that there is a 45 -- if it is -- if it's parts per million, it would be milligrams per kilogram, or it would be micrograms per gram. If it's parts per billion, it would be nanograms per gram of OCP, so we've got 45 nanograms of TCDD per gram of TCDD, and then in this -- I beg your pardon -- per gram of the mixed phenols. And then we have -- if it is 7 percent, that means that there is 70 milligrams of OCP per gram of soil. And we could say -- you want to give me a number or shall I assume one, amount of soil?

Q. Ten grams of soil.

A. All right. If we -- let's say then that we've taken ten grams of soil, and we've picked it up and rubbed it on the skin. Now, understand that with this much phenol in it, it is not going to be very comfortable. It's probably going to cause severe skin damage, but we ignore that, so what we have is 70 milligrams of OCP per gram of soil. We've taken ten grams, so that means that we've got 70, 700 milligrams or 0.7 grams of OCP in that, and -- and in one

gram there's 45 nanograms of OCP, and so in ten grams there would be -- there would be .7 times 45, which is -- which is 31.5 nanograms of TCDD. Does that square with -- I hope I haven't lost a decimal place somewhere.

In any case that's the amount of TCDD that would be carried in that ten grams of soil. The absorption would probably be on the order of one percent, about one percent of it would probably get into the -- across the skin, and that means then that -- that we have -- that will give us zero 0.315 nanograms absorbed from that ten grams. So that's the total amount that we might expect to go in.

If -- if we are talking about a 70 kilogram person, 70 kilograms is 154 pounds, so that's not a-- that might be considered a reasonably average rate. Obviously with a larger person, why, it's a bigger number, and a smaller person smaller, but if we were to divide this by 70, it would be -- it's not a complex -- I just done it wrong. The dose would be .0045 nanograms. We are often accustomed to talking about micrograms. If we wanted to refer to this as micrograms, we would bring the decimal back three places and it would be -- it would be that many micrograms per kilogram. What we have done here is to relate the dose. Now what you can see is that even if the one percent is off by two or three fold or if we are talking about a very small

person, we still end up with a very small number here in terms of a dose per kilogram.

Q. All right. Now, how does that compare with the no effect level in rabbits found by Jones and Krizek?

A. That no effect level -- okay, we can consider it in two ways. Ten grams of soil would probably spread over maybe six square inches if you were to really-- I don't know, you probably should have a child who plays in the mud advise us on how much surface that would cover, but let's say about six square inches, and we've spread this amount here, this is this total, we have spread that over an -- over a single area.

Q. More appropriately perhaps we should consider it in terms of dose per kilogram, and the problem with the Jones and Krizek data is that they administered .2 micrograms to the site and if we assume that a rabbit weighs a kilogram and a half, three pounds, that's a fair sized rabbit we could deduce that the dose is something less than 2 tenths of a microgram per kilogram, maybe 1.6 or something like that.

Here, if we were to go through a -- that's assuming that it was all absorbed, and we do not know what the absorption rate is in the skin of the rabbit. We got a good idea what it is in the rat, but in the rabbit we don't know necessarily. So there are a number of ways we can consider

this, but in any case there is a much, much lower dose either if we consider it on the amount that goes on the skin -- this is -- 31.5 nanograms is -- is .0315 micrograms, okay. That's about roughly tenfold lower, perhaps a little less than tenfold lower than this dose we're talking about on the rabbit. At the same time it's spread over a larger area, because in that rabbit they concentrated, they made a very small target.

Q. All right. So which would be lower, the dose from the Sturgeon soil or the dose for the no effect level?

A. Well, the dose from the Sturgeon soil would be vastly lower.

Q. All right. Now, with respect to the Kligman data you just talked about, the 16 micrograms, that were administered, would this dose from the Sturgeon soil that you calculated be lower than that?

A. Well, I guess the thing to do, since we don't the know the absorption rate in the Kligman data is to assume that the absorption here is going to be the same as the absorption there. They applied 16 micrograms to that skin over a period of three weeks, I guess. Here we're talking about a dose over a somewhat larger area, because in the Kligman study they confined it either on the forehead or on the back in this area perhaps so square, and the dose that

we're talking about here compared to in the Kligman study 16 micrograms. Here we're talking about .0315 micrograms, and if we were to divide that, it would tell us how much different they are. The difference is about five hundred fold.

Q. Now the -- in the Kligman experiment you said the solvent was used to enhance the material going through the skin?

A. Well, the solvent has two roles there. One thing you've got -- TCDD is not very soluble even in materials that dissolve it, and so you have to -- you have to get it in some form so that's it's distributed, because if you just put TCDD crystals on the skin, it's not likely that much would happen. It probably would not be absorbed very well even into the superficial layers of the skin. So the solvent will have an effect of helping to carry it through the skin, but it also distributes it, and that's really the role of the solvent as it was used in Kligman.

Q. What if you had TCDD in a 7 percent combination of orthochlorophenol and phenol in soil which you were rubbing on the skin? How would that affect the ability of the TCDD to get through the skin and into the body?

A. Orthochlorophenol is not really the best of solvents, particularly for TCDD, but the soil itself will

have the greatest influence, because there is a large amount of soil, and soil binds TCDD very effectively. TCDD likes to attach to both inorganic and organic solid, inert materials, and it would -- it would diminish its -- it would diminish its potential to get across the skin or to get even into the skin. We're really talking here about a local effect, where the material gets into the skin, and to be sure some is going to go beyond and become systemic, but the main effect is going to be at the site of application in both cases, but that a solvent would enhance the movement, because the skin cells, cells and their membranes tend to be somewhat lipophilic, they have more attraction for fats or fat soluble substances than water, than they do for water, and they would tend to -- to be -- a fat soluble substance in a fat solvent would tend to move across the skin at least a little more easily than otherwise.

Q. All right. Now, sir, the next organ that I'd like to discuss, really a group of organs, I guess, is the reproductive system.

THE COURT: Before you get into that, is this a good point for a short break?

MR.HEINEMAN: Yes.

THE COURT: We'll take a short recess at this time and then resume testimony. The admonishments that I've given

you will earlier will apply during this break also. Court's in recess.

(At this time a short recess was taken.)

(The following proceedings were had in open Court.)

Q. Dr. Dost, before we leave the skin there's another thing that I wanted to ask you about. You mentioned in your previous testimony that today or Friday about a no effect level in Dr. Kociba's studies. Do you recall that, sir?

A. Yes, I do.

Q. Okay. What was the no effect level in Dr. Kociba's studies?

A. That was a extended study in which the material was applied -- oh, in Dr. Kociba's study, I beg your pardon. We were talking about two studies. One was a study carried out over 13 weeks, which was used as a range finding study for one that was conducted over a period of two years for the purpose of determining the carcinogenicity of TCDD and also for seeking other effects, and as I believe I pointed out, they did a great deal of clinical chemistry, hematology, pathology and so forth in those animals, and the no effect dose for -- in that experiment of lifetime exposure -- so when I say a no effect dose, I'm talking about the daily dose that they were given was .001 micrograms per kilogram or one nanogram per kilogram per day over a lifetime.

Q. All right. Now, how would, how would that no effect level relate to the one you've calculated for the ten ounces of Sturgeon soil -- Ten grams, excuse me?

A. Well, I guess the way to tell the relationship would be that we're talking about -- let me -- the no effect level in Dr. Kociba's study was .001 micrograms per kilogram body weight per day. The -- the number that we're talking about here, and we put it into the same terms again, micrograms, is .0000-- when you start getting all these zeros, it's very easy to -- micrograms per kilogram, and it's evident that this is a smaller number by quite a bit, and we can find out how small by just dividing one into the other.

The difference between that single dose that we talked about in this hypothetical situation, where we would run -- we would smear the contaminated soil on the skin is -- and the daily dose that caused no effect in the rat is 222 so the difference is 222 fold.

The no effect level in the rat, the dose, the daily dose that has no effect in the rat is 222 times larger than the dose that would be acquired in this situation, and again if the weight is a little different, absorption rate a little different, it would make some difference, but it's a very small fraction.

Q. Now, has this Kociba level been adopted by anyone

other than the Center for Disease Control, as you mentioned earlier?

A. The Food and Drug Administration considers the no effect dose for TCDD to be .001 micrograms per kilogram, and they use that as a basis for regulatory activity with respect to contamination in food for human consumption.

Q. What would that amount to for a 70 kilogram man?

A. That amounts to 70 nanograms. We're talking about one nanogram per kilogram. A 70 kilogram person would -- that would mean a dose of 70 nanograms.

Q. Now, and the-- and the Jones and Krizek rabbit ear had a no effect level of what, sir?

A. Well, I'm sorry, I made a mistake. I used the lowest effect level. The no effect level rather than being .2 micrograms was .03. I misstated the no effect level.

Q. Now, is that point .03 micrograms?

A. .03 micrograms, yes, 30 nanograms.

Q. Now, would that be pretty close to what you calculated from the ten grams at Sturgeon, isn't it?

A. Yes, as a matter of fact, yes, in terms of a total dose. We -- for those ten grams of Sturgeon soil 31 and a and a half nanograms according to the calculation. This is without accounting for absorption or anything else, the amount that actually reached the skin or that it will assume

that everything was in the soil was in contact with the skin and disregarding the effect of soil on absorption as distinguished from the experiment in which they were putting it on in solvent, but the actual amount in nanograms was this -- approximately the same.

Q. Was just about at the --

A. No effect level ignoring all these other factors.

Q. Okay. Now, if we could move on to the reproductive aspect. Would you tell us what the -- what are considered to be the reproductive effects of TCDD in animals?

A. We would be concerned obviously with anything that interferes with the reproductive process, which means that anything that would cause any adverse effect on the offspring, and that might be identified as small size, fewer numbers. Rats have, for example, very large litters, sometimes 12 or 15 in a litter. If we had an effect that over time caused them to only have ten or nine in a litter, that would be an observable effect. The weight of the pups at the time they're born, any problems with fertility.

The other general kind of effect that we concern ourselves with are birth defects, teratogenesis; in other words, this -- in this context we're talking about the kinds of effects that a chemical can cause on the developing fetus directly that will cause deformities, and this is an impact

that takes place in a relatively narrow time window in development. It has to take place at a time when the fetus is at the point where the limbs and the organs are developing. It doesn't happen late, for example, in the gestation period.

Q. All right. Are there any other effects?

A. The fetus has the same potential for intoxication that the adult has assuming that a chemical can actually reach it. Some chemicals are not able to reach the fetus very easily, some reach it fairly well, and the fetus is subject to the same kinds o toxicity as the adult is except to the extent that certain organs are not adequately developed if the toxicity depends on that organ, so there are some aspects of toxicity that don't appear in the fetus, but in general, we can say, we use the term fetotoxicity, the general kinds of intoxication that affect the fetus.

Q. All right. Now, these subjects have been studied in animals?

A. Yes.

Q. What about the genetic impact, are there any effects in that way?

A. Oh, yes. Obviously the genetic effects that are the genetic strengths and weaknesses that are built into a given species or a given strain appear, and then there are

individual genetic changes that might take place that can result, and when we talk about genetic effects, we're speaking about changes that can arise in one or both parents and then be transmitted forward into some descendant, whether it's the immediate offspring or generations later, so this means genetic effects that take place in the reproductive organs. A genetic change that takes place in the liver only, and that can happen, is not going to have a impact on the offspring. So we have -- we have, as a matter of fact, birth defects that may arise from that kind of cause, and then there are bound to be a variety of other effects that really relate to other kinds of, if you will, weaknesses that could arise genetically.

Q. Well, can't -- can these reproductive effects be studied both in the male and in the female?

A. Yes, they can be studied in both sexes separately or together.

Q. All right. Now, I'd like to review with you what the animal studies demonstrate with respect to reproductive effects and start with the male test results first. What do they show about the effect of TCDD on male dominated effects?

A. The way that -- the way that these kinds of studies are conducted is to treat a male either with a single dose or over a extended period of time and then mate them with

females periodically for perhaps 8 weeks is a good number. Most species have a period of 8 or 9 weeks through which the sperm develops and if -- and, of course, at any given time there are sperm at any stage in a that development scheme, so that if animals, if males are treated at a specific time, and there is a effect, it is going to appear in the offspring that arise from sperm that were at that particular stage of development. So it may be five weeks, 8 weeks, or none at all. So the animals are treated at doses up to the tolerable dose. In other words, it's desirable to see some toxicity in the test animals at the top dose, so that we know that the maximum dose that is practical to use has been achieved and then several lower doses, and the process is relatively simple. The animals are treated they are mated, and then the offspring are examined. In the case of a dominant lethal test, which seeks certain kinds of genetic injury in the -- that would be manifested in the sperm, the -- the offspring simply don't survive. The conception takes place, but the genetic damage is so severe that the offspring don't survive, and the evidence can be seen in implantation sites in the uterus of the female. So that when the uterus, when the female is surgically examined, the sites where development started and then stopped can be observed. So this is one means. A probably more sensitive means is to use a typical

three generation reproduction test, in which usually in the first test the -- both males and females are maintenance on a diet at several doses, at several daily intake rates over an extended period of time prior to mating. They are mated, they continue on the diet so that the off spring and the whole reproductive tract are subjected to the chemical throughout the gestation period and throughout the nursing period, and at the end -- and when the animals are weaned, the original parents are usually taken off the experiment, although not always, but generally they would be taken off the experiment and examined carefully. The offspring are going to be maintained on exactly the same diet throughout their reproductive lifetime, and then their offspring will go through the same cycle so that we have three-fold generation of animals that have been, they and their parents or they and their offspring, if you will, have been subjected to the same diet throughout their entire reproductive cycles.

Now there are variations. Sometimes they'll do two litters from each of these animals and sometimes only one. If nothing appears, that is really sufficient information. If there is a finding that suggests that something has occurred in either the male or the female, you may go back and do this experiment again by administering the test diet only to the males throughout or only to the females. So --

but usually it's done with both species. If nothing appears, then there is a good certainty that neither the males nor the females were affected.

Q. All right. With respect to specifically the effect of TCDD on male animals, has there been any effect seen in the tests?

A. Not from -- not as a result of -- that can be identified with a male animal, no, sir.

Q. What about mutogenic effect on sperm and that sort of thing?

A. The dominant lethal studies do not show any effect at doses that the animals can survive.

Q. Are there any particular articles that you can -- that you can cite to us?

A. Well, probably the best is a study by Lamb and colleagues at the National Institute of Environmental Health Sciences, in which they specifically were seeking evidence of effects of TCDD, 2,4-D, and 2,4,5-T. Their objective was to simulate Agent Orange at various concentrations, so all of these animals had not only TCDD, but the herbicides as well.

Q. All right.

A. The other study that is -- well, that's the one, the most definitive one that deals with males specifically. There is another done by Kera and Ruddik that also was

negative.

Q. And about when was the Kera and Ruddik work done?

A. I believe that was done in the early '70s.

Q. Okay. Now have there been -- what are the results of the three generation reproduction studies on dioxin?

A. They have been -- they have been negative in essence. The consensus of the people who have evaluated those studies as well as the authors is that at .001 micrograms per kilogram per day, which incidentally is the same dose rate that was a no effect level in the Kociba study, that at that dose rate there was no evidence of a compound related effect on the -- on the animals.

Q. Is that for either males or females?

A. Well, that study --- in that study both males and females were treated. If either had been influenced, an effect would have appeared. Now, had that happened and nothing done to follow it up, with just that information alone one would not know whether it was males or females. Since nothing happened with both, it is accepted to -- to -- that there was no impact on either females or males.

Q. At that dose?

A. At that dose, yes, sir.

Q. Okay. Now, who's the author that's written primarily on the three generation studies?

A. Well, this was a study done by a scientist of Dow Chemical. The senior author was a man named Murray.

Q. Now, I'm trying to differentiate between the male and the female studies. Do I understand that the male dominated effects have not appeared at any dose?

A. At any dose that's been -- yes, that's essentially correct.

Q. Now, what about female dominated affects?

A. Well, TCDD is clearly a teratogen; in other words, if it is given -- at least in certain species. It's difficult to induce teratogenesis in the rat. In the mouse it's possible to cause cleft palate and some kidney deformities by treatment with TCDD.

Q. Now, in the Murray study were teratogenic effects found there?

A. The study was not designed to specifically look for teratogenic effects; however, they would have appeared, and it is my recollection that teratogenic effects were not seen in the Murray study at any dose that was used.

Q. All right. Well, are teratogenic effects dose related?

A. Oh, yes, definitely.

Q. What dose do you have to administer to the animal in order to find the teratogenic effects?

A. For some reason the no effect dose for teratogenesis, per se, has slipped my mind. It's up into the micrograms per kilogram, however.

Q. You mean whole numbers?

A. Oh, yes. We're not talking about these very, very small fractions of a microgram.

Q. Now, in those studies that are designed to find teratogenic effects, how many doses are normally given?

A. The way the experiment is done is to administer the chemical daily over what we know as the developmentally sensitive period Now, in the rat that's a period that starts at about day six of gestation and ends at generally on the fifteenth or sixteenth day.

The rat is born at an age of about 21 days. What you must remember is that the rat is born at a stage of development that would be roughly equivalent to a human fetus at about two and a half months of term; in other words, less than a third of gestation period. The rat is cast out upon the world in a very, very primitive way and requires -- it requires enormous care by the mother, you see, so this dose that is administered comes relatively late prior to the birth of the animal, but the birth takes place very early in development.

The period of sensitivity in the human is -- ranges

between about fifteen or eighteen days after conception to perhaps 60 days and for a few structures a little longer than that, so this window, so to speak, of time in which an organism is sensitive to developmental effects on the fetus is relatively narrow. However, the impact in terms of general toxicity, that goes throughout the entire period of gestation.

Q. Now, as I understand it, in the Murray study, which is the three generation study, there is a no effect level found in both males and females of .001 micrograms per kilogram per day?

A. That would be a correct inference yes,.

Q. Now, in the teratogenicity studies in which multiple doses have been given there is a no effect level that's around-- that's in the microgram per day level?

A. Yes.

Q. Would that be higher than the -- what the Murray study demonstrated?

A. Oh, yes, yes, but it's a much less sensitive-- it's a much less sensitive kind of parameter, much less sensitive effect. It takes doses in -- for many chemicals it takes doses up into the range where the mother is seriously injured to cause teratogenesis.

Q. How does that dose relate to what you have

calculated for the Sturgeon dose in rubbing the soil on the arm?

A. The .001?

Q. Yes, sir.

A. Well, if we -- well, all right. We did that with respect to the other study, the Kociba study that we were considering. The no effect dose on a daily intake basis is the same, as it turns out, and so we still have this same relationship here with a difference of something on the order of 200 fold lower than the no effect dose as seen in these studies.

Q. So the Sturgeon dose would be lower?

A. Yes, you know, with the dose with these assumptions that we've made about smearing it on the hand and so forth.

Q. Now, let's assume that the teratogenic no effect level would be as low as one microgram per kilogram.

A. Well, one microgram is a thousand times larger than a thousandth of a microgram. One could then multiply this by a thousand, or if the no effect dose is down in the fractions of micrograms, let's say, half a microgram per kilogram, then it would be a five hundred fold difference.

Q. So you multiply that figure 222 by either a thousand or five hundred?

A. Yeah, depending on the -- and I have to apologize,

because the no effect doses for teratogenesis have simply fallen out of my mind momentarily.

Q. But it would be, if it were one microgram, it would be a thousand times more?

A. That's correct.

Q. And that Sturgeon would be that much lower?

A. There would be 222 thousand fold difference.

Q. Now, Dr. Dost, if Dr. Silbergeld had testified here in court that a strong assumption exists that no threshhold exists for male reproductive effect, would you agree or disagree with that statement?

A. Well, I would disagree with it.

Q. All right. Based upon what, sir?

A. Because we can't find evidence of any kind that male reproduction is affected by TCDD.

Q. Has -- has Dr. Silbergeld written on this subject herself, sir?

A. There was an article, a review really, that was part of a symposium in late 1983, I believe, at Rockefeller University, that -- in which she discussed the reproductive -- in general terms the reproductive potential or the reproductive toxicity potential for TCDD.

Q. What did she suggest for male dominated toxicity?

A. Her suggestion was that there really wasn't much

evidence, if any, that male mediated toxicity by TCDD was at issue, and I can't remember her statement exactly, but there is very, very little data to support any evidence of effect.

Q. On the male?

A. On the male, on the male.

MR. CARR: Counsel, where was it that you quoted Dr. Silbergeld, what page.

MR. HEINEMAN: I think it was -- I have it right here. It's 12th of April, Page 127. On 13th of April, Page 34.

Q. Doctor, I'd like you to move on now to liver effects, if you would, and discuss with the jury the function of the liver in the animal and what it has to do with detoxification?

A. The liver is an-- is a extremely interesting organ, because it has so many functions. It's -- it's -- it has a major function in processing energy substrates for -- so that we can get energy out of our -- the food we eat. The cells -- in each individual cell, of course, does a good part of this work, but when there is an excess of one kind of, say, an excess of carbohydrate or an excess of fat, the liver often has responsibility for making conversions that make it possible to use these various substrates.

The liver is a -- because it has such an enormous

capacity serves in starvation as a protein reserve so that if we are starving, why, when we begin to need protein, we would tend to mobilize it from the liver without losing any function.

It is extremely important in the metabolism of foreign substances as well as substances that are secreted in the body. The steroid hormones, for example, have to be disposed of somehow, because we can't leave them in the organism to just keep working all the time. They have to be disposed of. Foreign compounds are metabolized primarily in the liver and either converted to soluble forms, as I've mentioned many times, or else moved into the bile in a slightly different form and excreted into the digestive tract.

So I guess in very general terms that would summarize the functions of the liver. It manufactures bile, which is necessary as a digestive aid, because it's a detergent, bile is a detergent, it breaks up the fat that we eat into very tiny globules so that the enzymes in the digestive tract can get at it and break it up enough that it can be absorbed.

Q. You previously described for us the metabolic detoxification effect. Does that occur in the liver?

A. That occurs primarily in the liver. Many other

tissues have some capacity, but the liver performs most of that particular kind of work. Even the skin can do it to an extent.

Q. Now, how is it that this organ -- what percentage of body weight of the entire animal is the liver?

A. The liver in an -- in an adult human probably weighs about a kilogram, about 1 and 3 quarters kilograms, so that would be in pounds about what, four pounds.

Q. Now, the blood supply --

A. Roughly four pounds.

Q. All right. As the blood supply is circulated by the heart, does it -- it pumps the blood through the liver?

A. It pumps blood through -- the liver -- the blood enters the liver actually in two ways. It enters in the hepatic artery, which is the artery that supplies the blood from the general circulation; in other words; that you might say is the main line from the heart. There is also a vessel called the portal vein that comes from the digestive tract and brings material that's absorbed from the digestive tract to the liver, so blood is coming to the liver from two different directions.

Q. All right. Now as rapidly as blood circulates through the body how does the liver do all of this function in what is apparently a pretty short period of time as the

blood is going through?

A. Well, actually the circulation time of the blood is not necessarily related. It only takes -- if we were to inject a dye into a vein right here, it would take about a minute before we could find it coming back. It will have gone all the way through the circulation and at least some of it would get back to this point. So blood moves very quickly through the body. What we are concerned with as it goes through the liver, you can think of the liver perhaps as a big filter. There is a lot of circulation going into the liver and each pass -- and as a matter of fact, physiologists use the term first pass, second pass. The idea of the first pass is that how much of material -- and if you inject it into a vessel coming into the liver, how much of it is still there on the other side.

Obviously the liver does not simply clean everything out of blood every time it goes through. It continually takes out more and more and more and more and processes it. Now, if it can't process it very fast, it's not going to take it out of the circulation very fast. If it is working on a foreign chemical, and you recall the diagram I showed with equilibrium related to TCDD, the liver is working relatively slowly. The material that the liver is going to work on is not just simply stacked up outside the

liver waiting its turn. It's only going to come in to the extent that it can be handled. It's possible in the kidney, on the other hand, if there is a lot of soluble material that's available to go through the kidney, the concentration in the kidney may become very high. That's different.

So what we're talking about here is functions that relate to the ability of the liver to do its work and to take material out of the blood.

Q. Now, the liver works on or filters the blood that the body is sending through it, correct?

A. In a sense, yes.

Q. Now, does it get its own blood supply with the cells that are in the liver?

A. Exactly, in the same way. In other words, the blood that's coming into the liver and ultimately that's bathing a liver cell both brings the necessary, the staples, if you will, that the liver requires to live on as well as the work that the liver is supposed to be doing. It's bringing glucose to either be burned or stored, because the liver is able to store a very large amounts of glucose in a form called glycogen, which is a little like starch, so that we have eaten, we've got a ready reserve of energy that we can draw on while we aren't eating anything. But the blood supply as it brings glucose in or brings amino acids for

manufacture of products is also bringing whatever materials need to be detoxified or even raw materials for synthesis of other substances.

Q. Now, when -- if there is dioxin in the blood supply and it is going through the liver, can it have adverse effects on the liver itself?

A. Well, the liver clearly is affected by intoxication with TCDD. If the dose is high enough, we see a variety of liver pathology, because the concentration in the liver has has risen sufficiently -- I mentioned a moment ago that we don't have a lot of piling up of molecules, but I'm speaking of a situation in which there isn't a great mass of material in the -- in the organism. When we have a large amount, when the dose is high, there's going to be a lot of TCDD reach the liver, and it's going to have a variety of deliterious effects if there is sufficient amounts.

Q. If there is sufficient amounts?

A. If the dose is high enough.

Q. All right. Now, what are the effects that arise when the dose is high enough?

A. We can see effects perhaps on the cell wall. One of the things that would happen in a damaged or injured or intoxicated liver cell would be that the cell wall would become more permeable. Now, cells have membranes surrounding

them, and it's possible when the cell has been injured that that cell may, that wall may become leaky and some of the --

Q. Is that what permeable means?

A. That's right. That's right. Permeable, if you put, if you poured this water through a handkerchief, it would undoubtedly go through, it's permeable. If we poured it onto a piece of paper, probably it wouldn't go through until the paper got thoroughly soaked and finally became weak and it started to come through, but it would come through slowly. That would be high permeability, limited permeability.

Well, cell calls are the same way, because they have to bring materials in. The only way that materials can come into a cell is by transport across the membrane, you see. Well, there are a lot of components in the cell, enzymes, which are rather large molecules, for example. Such enzymes as the glutamic oxalacetic transaminase, which has a function in the cell -- I'm sorry about that name, but the importance thing is that it is an enzyme that is in solution inside the cell, and if the cell wall becomes sufficiently permeable, it will start to leak, and we can find it in the bloodstream in a clinical test, and that's one of the clinical tests for liver function.

Pathologically, we can see if that membrane effect

is sufficient. Sometimes cells, two cells will join together, and we'll see a large liver cell with two nuclei or the whole cells will swell, because they will accumulate a lot of fats.

One of the characteristics of TCDD intoxication in the liver is the accumulation of fat in liver cells, and the architecture, the various structures within a cell will tend to become distorted and perhaps even swollen in their own right. So there's a whole gamut, the end of which is actual death of the cell, so that we see in a severly intoxicated animal pockets of dead cells, maybe only a couple at a time or perhaps several hundred, where there's just simply been cell death as a result of the intoxication. This is a fairly typical pattern of many liver intoxicants, as a matter of fact.

Q. That's what I wanted to ask you about. Is TCDD the only thing that has these effects on the liver?

A. No, there are other substances. It's -- many of the effects on the liver are relatively nonspecific. Now, the mechanism by which liver damage occurs as a result of high doses of TCDD is not entirely clear. We do know more about some other chemicals, but this general pattern occurred with a lot of different substances.

Q. Such as what?

A. Well, some of the solvents, solvents, common solvents, even things like kerosene. Very rarely does someone get a dose of kerosene, but you can induce this kind of effect with those kinds of solvents.

An inorganic chemical that I've worked with a good bit called hydrozene will cause very interesting pattern of liver damage, many characteristics of which are rather like what is described for TCDD.

Q. How about alcohol?

A. Well, alcohol certainly will do it. Again it's a dose related phenomenon, and usually that kind of damage does not occur after one drink or even after just one drunken binge, but it will occur in time if excess consumption of alcohol is maintained.

Q. Now, are there -- are there gross effects that the animal studies have found for liver?

A. Well, the liver tends to swell, the liver tends to swell, and that is -- that is not -- there are degrees involved here, because the liver in its -- in its daily work tends to swell. In a well-fed individual ten percent of liver weight, for example, is glycogen, this complex sugar that I mentioned that is stored in liver cells. Ten percent is pretty good sized increase and the liver actually it can be seen to become larger. The liver when it's responding to

foreign chemicals -- a good -- a good solid induction causes enough protein synthesis, increase in endoplasmic reticulum, that the liver will swell, and so this is seen. Now, there is a point beyond which it is no longer a normal phenomenon.

Q. What do the studies show with respect to -- with respect to such liver effects and TCDD?

A. In what sense, the nature of the damage?

Q. Yes.

A. Well, I think I have described a good part of it. That is -- we see the cellular disorganizataion at higher doses. We see -- probably the first thing that would be seen would be the leakage of enzymes through the cell wall. These are -- unless the cell -- unless the cell dies, these kinds of changes eventually -- eventually recover.

Q. Now, in terms of the effects that can be found, you talked about certain enzymes. Well, how about lipids?

A. Well, I mentioned the accumulation of fat in the -- in the cells of the liver, and these are -- these are -- apparently they are triglycerides, which is fat in the same form that -- that we store it in fatty tissue, in adipose tissue, and it -- the liver normally would break that fat up and ship it out into the circulation for either use as a fuel by muscle and other tissues or it would be -- it would be, if it was in excess sufficiently, it would go to adipose tissue

and be stored. So we have an abnormal situation in which lipid metabolism, fat metabolism in the liver cell is deranged in some fashion.

Q. Is there a -- does the dose response --

A. Oh, yes.

Q. -- Principle apply to this as well?

A. It certainly does.

Q. And is there-- What dosage is required for -- you mentioned necrosis or dying of the liver cells. What -- versus the finding of lipids or versus enzyme changes and that sort of thing?

A. One of the difficulties is that the -- much of the pathology has been done on -- with single doses that are known to cause an effect. However, we have a lot of evidence in chronic studies which really probably are more -- are more pertinent.

The Kociba study that we've talked about already shows liver pathology in the higher dose animal, relatively little at a dose of a hundredth of a microgram per kilogram, and nothing that can be described as pathological at a thousandth, at one nanogram per kilogram. So there is a --

Q. Has a no effect level, N-O-E-L, been described?

A. In the chronic sense, yes. I can't bring to mind specific dose response data. It seems to me that Jones and

Grigg have looked at this in acute treatment. I do not remember the numbers offhand.

Q. The -- what about the enzyme induction that we talked about before. Does that have any relationship to liver damage?

A. Not really. That occurs at doses below those that are going to cause liver pathology. That's a -- that's not a pathological response. That's a normal response to low concentrations of a substance with the objective of getting rid of it.

Q. So again with respect to -- I guess if we're back to Kociba study as being one of the primary studies on the liver effects, is that right?

A. Certainly in terms of the pathology, and also they did a great deal of clinical chemistry in those studies, and the effects were -- existed at the highest dose of a tenth of a microgram per kilogram per day, relatively little, if anything, at the -- at the intermediate dose, and nothing at the lowest dose.

Q. Well, then if we were to compare this with the Sturgeon calculation that you made a little while ago that's that same figure?

A. We're right back where we began here keeping in mind that we are talking about a chronic exposure to this

dose as differentiated from this single adventure with the contaminated soil.

Q. What has been found with respect to whether or not liver effects are reversible?

A. There have been -- there have been studies of the pathology of the liver. Bruce Fowler has done such a study in which he's used electron microscopy, which is, of course, much more sensitive then using a light microscope, and he has defined a time course through which in rats the liver after -- after showing such signs as crowding of the -- of the mitochondria, accumulations of fat. The other things that we recognize as being part of the -- part of the pattern of toxicity of TCDD in the liver have reversed over a period of time.

Q. Well, how much time does it take?

A. It would take -- they are well under way, after a single dose they are well on the way toward normalcy by 16 or 20 weeks, and after about 32 weeks they seem to be reasonably well recovered.

Q. Now, are there other effects that are -- on the liver that are also reversible?

A. Well, the enzyme induction, of course, reverses. It is a long -- it persists for an extended period of time, but it does, in fact, it does in fact return to normal. I

suspect that the reason that the induced enzymes stay up is because the TCDD remains in the animal for a longer period of time, and that induction is more or less maintained. If it went in and left, those enzymes would drop more rapidly as they do following other kinds of chemicals.

Q. Now, I'd like to move next, Dr. Dost, to the discussion of heme synthesis, and I wonder if you would explain and describe the heme and the heme synthesis process to the jury.

A. Well, heme is a -- I guess by itself we can consider it a molecule, but its real function is to serve as part of a large number of other kind of molecules. Heme is the component of hemoglobin, which is in our blood that carries oxygen. It's an iron bearing compound that is incorporated in hemoglobin. It's part of a substance called myoglobin that is very, very similar to hemoglobin that's found in muscle.

There are a number of other heme proteins. There are a group of cytochromes so called that are used in the cell as part of the process of transforming energy sources into energy. We call them electron transport proteins. There are a number of enzymes, an enzyme called catalase, which has the function of getting rid of hydrogen peroxide, which is made in large quantities in all cells. A enzyme

called peroxidase, which has a similar function. The P-450 enzymes that have been mentioned any number of times, the so called microsomal drug metabolizing enzymes are cytochrome enzymes. So there is a wide spectrum of proteins in the body that need heme as -- in order to function, and the heme is incorporated in the -- in the molecule. It's a small part of the molecule. The P-450 proteins are -- have a molecular weight of about 50 thousand, and heme has a molecular weight of eight hundred something, so you can see that comprises a relatively small part of the molecule.

The hemoglobin subunit, each molecule of hemoglobin is actually four units, and each of those subunits is 16 thousand and each subunit has a molecule of heme in it that has a molecular weight of about 830, something like that.

Q. Now, how is heme maintained in the body?

A. Well --

Q. Where is it?

A. Well, it's in every cell. Every cell makes its own heme. Heme is not something that's made in some central location and shipped around the body. It's -- the bone marrow would obviously make a great deal of heme, because 90 percent of the heme proteins or 90 percent of the heme is in hemoglobin, and that's made in the adult in the bone marrow.

Q. When you say 90 percent of the heme, you're saying

90 percent that may be found in the body at any one time?

A. That's correct.

Q. Would be in hemoglobin?

A. That's right.

Q. But those levels will rise and fall?

A. Well,they would rise and fall if you're in athletic training, and you're doing things that require high oxygen turnover. As you become better conditioned, you would have -- you'd have more red blood cells. The concentration of hemoglobin within the blood cells might rise a little bit, but that's pretty difficult to accomplish. You would have more red blood cells. If you moved up into the Andes where you are in very rare atmosphere, you -- over a period of time your red blood count would rise in order to improve your ability to bring oxygen from the lungs to the tissues where it's needed.

The myoglobin in the muscle comprises about ten percent of the total. Now, what I've done, I've given you 90 percent and ten percent and that makes a hundred percent, and we haven't accounted for all of the cytochrome enzymes. Those are rough figures. The cytochrome enzymes and other heme containing enzymes only account for less than 1 percent of the total heme in the body, and in the places where those enzymes are made heme is manufactured in that cell for those

proteins, whether we're talking about the cytochromes or catalase or peroxidase or any of the heme proteins, the heme containing substances.

Q. Now, do you recall the testimony of Dr. Silbergeld when she said that the --

MR. CARR: Counsel, what page please.

MR. HEINEMAN: Well, we've got April 12th, we've got Pages 76, 112, and 109, 113.

Q. Where she said that dioxin affects the red blood cells and strains the body's requirements for heme and by induction of enzymes drains heme from the heme pool. Do you recall testimony along that line, sir?

A. Yes.

Q. All right. Would you agree with what she has said?

A. Not at all.

Q. And why not, sir?

A. First, every cell makes its own heme.

MR. CARR: Your Honor, first of all, it was doctor -- she's quoting Doctor Poland, and at that time on page 76 at least so apparently either the witness -- well, I'd like to make that clear that it was Doctor Poland that was being quoted there, counsel.

Q. All right. Would you agree with that statement, sir?

A. That statement is incorrect.

Q. All right. And what is -- why is it incorrect?

A. Well, each cell makes its own heme and as a given group of cells makes heme for its own needs, there is no impact on heme synthesis in other cells. In other words, a requirement for heme at one point does not generate a deficiency in another point.

Q. Has Dr. Poland ever done any work on heme dynamics in the cell, has he ever published a single paper on that subject?

A. The only thing that Dr. Poland has done is to investigate the induction in that -- that might be considered heme dynamics. He's investigated the induction of P-450 related enzymes. He does not do work on heme dynamics.

Q. Now, what is the evidence with respect to whether dioxin affects red blood cells?

A. Well, the evidence shows that -- that dioxin as a matter of fact does not until animals are so intoxicated that they are in a state of collapse and cells are failing all over the body. At that point bone marrow stops making red blood cells, and the animals become exceedingly anemic. These are animals that are essentially in a state of collapse, they are very far along in the process of intoxication.

Q. What kind of levels of exposure cause these kinds of problems?

A. Well, this has been seen in the monkey, and the monkey is fairly sensitive to TCDD. Doses on the order of 2 or 3 micrograms per kilogram would -- would probably carry an animal to that point.

Q. Now, does the induction process require a lot of heme?

A. Not relatively, no. If we look at just the P-450 enzymes, and we have talked about a 25 or maybe 50 fold increase, then obviously there has to be that much of an increase. However, this is an exceedingly small fraction of the total heme required in the body, and it's a relatively small fraction of the -- of the heme demands of the cell in general even with that increase.

What is of most interest is that the animal that's intoxicated with TCDD is still able to induce the enzyme, synthesis goes forward very nicely. Animals that are iron deficient, and iron is a component of heme, can still manufacture the heme protein enzymes.

Q. Well, what about the existence of a heme pool. Is there such a thing?

A. No, there is not. Each cell makes it own. I mentioned that earlier. Each cell makes its heme as it needs

it, and in the cell there is not a heme pool. Certainly this complex process by which heme is synthesized will at one point have some heme that has not yet been incorporated into a protein, but it is driven by demand except in an intoxicated animal.

THE COURT: Mr. Heineman, is this a good point for a short break?

MR. HEINEMAN: Sure, Judge, that would be fine.

THE COURT: Take about a ten minute recess and then resume testimony. Court's in recess.

(At this time a short recess was taken.)

(The following proceedings were had in open Court.)

MR. HEINEMAN: Just a moment, Your Honor. I'm trying to get three exhibits. Your Honor, at this point, if I may, I neglected to offer Defendant's Exhibit 1278 into evidence, which is the Poland and Glover member -- or graph described by Dr. Dost, and I have also had these handwritten pages, Defendant's Exhibit 1279, which was his computation of the Sturgeon exposure, the dose, and Defendant's Exhibit 1280, which is his comparison of the Kociba no effect level and the Sturgeon dose, and I'd like to offer those at this time.

MR. CARR: We have no objections to them, Your

Honor.

THE COURT: All admitted without objection. Thank you.

MR. HEINEMAN: If I may pass 1270 and 1278 to the jury.

Q. Now, Dr. Dost, we were talking about the heme. What is the relationship between heme and chemical porphyria?

A. If -- in the process of making heme there is -- to start with this is a multi-step process. If for some reason there is either a pathological or a genetic influence that accelerates the early steps in this process, it is in a way like any assembly line, if the material doesn't come off the end of assembly line, faster than the parts go into the beginning of the assembly line, there will in all probability be a problem.

In the process of manufacturing heme if the enzymes at the beginning of the cycle are accelerated, there is a potential for a buildup of some of the intermediate products before the final product. If, in addition to that, there is an interference with the terminal process, then obviously the accumulation of material in between is going to be all the worse, and porphyria is an accumulation of the precursors of heme before iron is added to the molecule.

Q. Now, you've talked about the fact that heme is

produced in the cells where it's needed?

A. That's correct.

Q. Where do porphyrins accumulate?

A. They accumulate in those same cells. However, they can escape, because in an individual with porphyria porphyrins are the way we identify those molecules before they become heme, and if they accumulate in the cell, some of them will leak out. They're after all not a large -- not a large compound. They will leak out, and they will be seen in the urine. But they also accumulate in the cells where they are made.

Q. All right. Now, I'd like to ask you if you recall this testimony from Dr. Silbergeld. April 12th, beginning at Page 112. She said, first off since the porphyrin system produces heme, that molecule which carries oxygen in the body, a reduction in the amount of heme through the draining off into that enzyme system that's induced, a reduction in that amount of heme deprives cells of their oxygen carrier, if you will. Nerve cells have the highest need for energy, for oxygen of all cells in the body because they are always active. Therefore, they are among the first to die or be affected when there's a lack of oxygen. She goes on to say another way in which altering porphyrin chemistry affects the nervous system is this insulation, this fatty wrapping around

the nerves, which is made out of lipids. It's very dependent on active porphyrin system in those same cells, and its porphyrin system is deranged as has been shown by workers at Rockefeller University. Now, do you agree with those statements, sir?

A. Well, there is a problem -- there is a number of statements, and it's a very confused, a very confused description. To begin with, we don't have -- I already discussed the problem of a drain on heme. Now, when porphyrins accumulate, they -- they do represent some quantity of the intermediate product, but they are not sufficient to constitute a drain on heme, and as a matter of fact, it can be demonstrated that animals that are porphyric, that is, have been intoxicated sufficiently with TCDD, that they do show porphyrins in the liver as deposited in the liver and in the urine continue to synthesize, they respond to induction of the P-450 series. In other words, there doesn't appear to be any evidence in those cells that there is a -- that there is a drain, any interference with the ability of the cell to make heme proteins.

What is happening is that there is a diversion, and some of the precursors are not being processed into the final product, but there is still enough capacity to make the heme proteins that are necessary. If one looks at red blood cells,

there is no impact of TCDD on red blood cells until, as I pointed out earlier, the animal is in a very, very serious state of intoxication.

Q. Whose work are you relying on there, sir?

A. The work particularly of Joyce Goldstein and of the group headed by Cantoni in Italy. They have devoted the major part of their career to this question of chemical porphyria.

Q. Where is Goldstein located?

A. Goldstein is located at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina.

Q. And Dr. Cantoni, Cantoni is from Italy?

A. Is, I believe, from Milan in Italy.

Q. I'm sorry, I didn't mean it interrupt. Go ahead.

A. I may ask to you read some of that testimony again, because it's ver complex, and I have difficulty keeping it all in my mind.

The question of oxygen supply to the central nervous system. It is true that the brain has a higher consumption of oxygen than almost any other tissue. The brain consumes an enormous amount of oxygen, but there is no impact on the brain's oxygen supply in TCDD intoxication. There is -- for two reasons. One is we can see that there is

no impact on red blood cell counts or hemoglobin levels, no evidence of impaired oxygen transport under TCDD intoxication until it has reached the case where the cells are no longer being made. The -- the central nervous system and the blood cells both have a limited capacity to form porphyrins, because no evidence is found for porphyrin derangement in bone marrow as a function of TCDD, and that's not surprising, because chemicals that cause porphyria almost always are limited in their effect to the liver.'.

Now, there are a variety of genetic porphyrias that do that do have an impact in other organs. Chemical porphyria only attacks the liver, and as a matter of fact, one of the enzymes that is inhibited in the porphyria caused by TCDD is an enzyme called uroporphyrin decarboxylase, and it comes at the end of this cycle of-- it's the finishing enzyme, so to speak, because it trims a lot of the useless side chains off the porphyrin molecule before it becomes heme. If it's inhibited, obviously production is going to pile up. There will be porphyrin intermediates formed, and there will be interference, some interference with heme synthesis. That enzyme exists in the precursor cells for red blood cells, in the bone marrow, and when red blood cells are formed in the bone marrow, they're derived from cells that are able to form a variety of other cells. As they differentiate, they

eventually lose much of the machinery in the cell, because all the red cell really is a just a bag of hemoglobin, and it lasts in the circulation for a hundred or 120 days, and then it breaks up and then the material is salvaged or discarded.

But the enzyme, uroporphyrin decarboxylase, happens to be carried along. It's left in the cell. When the nucleus comes out, the mitochondria, which is an energy producing mechanism in the cell, when that comes out, some of the enzymes are left, and uroporphyrin decarboxylase is left,and it is not impaired in red blood cells by TCDD. It's only impaired in the liver.

I would appreciate it if you would read the part again about the relationship between effects of porphyria on the central nervous system. I became a little confused there.

Q. All right. What I read was that the porphyrin system produces heme. A reduction in the amount of heme through the draining off into that enzyme system that's induced, a reduction in that amount of heme deprives cells of their oxygen carrier, if you will. Nerve cells have the highest need for energy, for oxygen of all cells in the body, because they're always active. Therefore, they're among the first to die or be affected when there's a lack of oxygen.

A. With respect to that, there have been specific

experiments done, particularly by George Lucier at again the National Institute of Environmental Health Sciences. That group, by the way, does a great deal of research of TCDD, because it is a major national concern, and they can't find any evidence of an effect on energy metabolism.

In other words, there isn't any -- there isn't -- there's no impact on the electron transport system, which depends so intimately on the cytochrome system, the cytochrome enzymes that are responsible for helping to transport electrons to oxygen so that we can make -- complete the energy retrieving cycle in the cell.

And there's really no evidence that demonstrates any impact on the ability of the organism to transport or utilize oxygen either throughout the whole body or within a given cell.

Q. With respect to TCDD intoxication of animals, has there been any evidence of brain pathology?

A. No.

Q. Has there been any effect shown on porphyrin synthesis in the brain?

A. Well, the brain -- no.

Q. Has there been any effect on hemoglobin synthesis in animals?

A. No, only, as I said, until at the time the cells in

the bone marrow are ruined, and they simply are unable to -- and that's probably a nonspecific effect of some kind. It certainly isn't anything that's attributable to a direct effect on heme synthesis in the -- or hemoglobin synthesis, either heme itself or the ultimate heme protein in the bone marrow, where blood is made.

Q. Now, what particular studies do you rely upon to reach those conclusion?

A. A variety of pathology studies particularly, because -- and evidence also by Cantoni again, where porphyrin metabolism or porphyrin accumulation in the central nervous system as well as the activity of the uroporphyrin decarboxylase, this terminal enzyme in this chain, were measured, and there is not an accumulation of porphyrins in the cells of central nervous system.

It may be useful to point out that there is in individuals who have genetically derived porphyria, neurotoxic symptoms, neurological symptoms that are apparently a function of the development of porphyria. This is not seen in the chemical porphyrias. It also only arises after a long history of porphyric episodes that are genetically based.

In the case of TCDD there is no evidence at all for a -- a porphyric impact in the central nervous system as a

result of TCDD intoxication. Now, as far as the pathology of the central nervous system is concerned, the brain is always a primary organ that is examined in any pathology study, in any toxicology evaluation. Again Kociba in animals treated for an entire lifetime, both in the studies that they did for two years and the studies that they did preliminary to that for 13 weeks at which they actually studied pyradosis. James Allen's work in monkeys, for example, showed no brain pathology. The only thing that appears in the nervous system of heavy intoxicated animals is capillary failure, because at high doses the capillaries become more permeable, and they start losing blood, and in the central nervous system this will occur around nerve cells. It's a circulatory lesion, not a nervous lesion.

Q. Now, if I may I'd like to read you another statement that Dr. Silbergeld made. Well, I guess I already did. It had to do with the myolin?

A. Oh, I missed that.

Q. All right, let me read it. Another way in which altering porphyrin chemistry affects the nervous system is this insulation, this fatty wrapping around the nerves, which is made out of lipids. It's very dependent on an active porphyrin system in those same cells, and its porphyrin system is deranged, as has been shown by workers at

Rockefeller University and elsewhere. Some of the first cells to die are these wrapping cells, what's known as myolin around the nerves. Do you recall that testimony?

A. I do recall it now, yes.

Q. Do you agree with it?

A. No, I'm afraid not.

Q. Why not, sir?

A. Well, to begin with the pathology studies do not show any evidence of demyelination. Demyelination is very easy to see under a microscope. I've observed it in my own work on hexachlorophene. Hexachlorophene happens to be a very effective demyelinator. That is in part the reason why it exerts pathology, why it has caused neurological damage in-- in humans, and why it is no longer used as a skin disinfectant. So there is no evidence to begin with for any myolin lesions, any lesions of the myolin sheath. Now, the myolin sheath is a structure that surrounds the nerve fiber, and it is relatively inert metabolically, it doesn't do much, but it's there as insulation, and it's sustained by a cell that is located -- cells are located periodically along the nerve fiber that -- and this is not a nerve cell, but it's a separate cell called a schwann cell, and it is responsible for maintaining the myolin sheath, which is really a wrapper. It would be very much like taking a pencil and

making several wrappings of a membrane around it, and in between each layer of the membrane is this material, myolin, which is more or less homogenous, that is, it doesn't have much structure.

I have been trying to find evidence of any role for porphyrin synthesis or heme synthesis in maintaining myolin, and other than the necessary heme proteins that are needed for the maintenance of energy metabolism in any cell, including the schwann cell, even a fat cell has these kinds of functions. I cannot find anything that is specific to effects on maintaining myolin, but the bottom line, if you will, is that there is no evidence that TCDD causes demyelination around nerve fibers in any experimental animal.

Q. Whose work do you rely on?

A. Again, I rely on the pathological studies done by Allen on monkeys, on Kociba, and particularly -- there are a number of others, the brain is routinely examined in any pathological screening, but those are perhaps the most important.

Q. Has there been any work done in the field by a man named Calder?

A. Yes, as a matter of fact. Calder is a researcher in New York who in contemplating the problem of TCDD and what appear to be some evidence of peripheral neuropathy or at

least suggestions that TCDD can cause a peripheral nerve derangement that could take many manifestations, but particularly tingling and pain in the peripheral nerves, treated rats specifically for the purpose of doing pathology on the peripheral nerves, and by peripheral nerves, I mean the nervous system outside the brain and spinal cord. The nerves that serve my fingers are part of the peripheral nerve network.

Calder and his associates were not able to find evidence of the -- that they could attribute to-- that would be associated with peripheral neuropathy, particularly no demyelination, no other lesions that they could associate with such an effect in the rat.

Q. Now, with respect to porphyria and the causes of it, you mentioned chemicals such as hexachlorophene?

A. Not hexachlorophene. We know that TCDD will cause porphyria. Hexachlorobenzene will cause porphyria.

Q. All right. How about any metals or any other materials?

A. Lead will cause porphyria.

Q. You mentioned the genetic cause?

A. And the genetic problem, which is really defect in the -- somewhere along the enzymatic trail of porphyrin synthesis.

Q. Has there been any investigation to see whether -- well, let me start over again. What is a subclinical porphyrin result?

A. A subclinical result, I would say, is one that can be measured if one examines the urine, and usually urine is the body fluid in which we would attempt to detect porphyrins. There are going to be porphyrins appearing in the urine all of the time anyway, and one would, I presume, look for a change in the pattern or a change in the amount. I would say that a subclinical change in porphyrins would fall within the realm of those that are occurring just as a result of our daily life without any particular evidence of toxicity, illness, or other effect. If it were clinical, clearly it would be associated with some kind of symptomotology that made us go and look for it.

Q. What is porphyria cutanea tarda?

A. Porphyria cutanea tarda is a form of porphyria in which porphyrins accumulate in the skin, and this creates a problem, because it causes a stiffening of the skin, it causes, but the main reason that it is -- that it causes an effect is because porphyrins are -- have the ability to trap light. In other words, they are -- and in the skin they tend to cause an excess of ultraviolet damage in the skin, and that combined with the excessive amount of those materials

creates some pathology in the skin, results in discoloration, extreme sensitivity, skin injury in the sunlight and so on.

Q. Is porphyria cutanea tarda found in humans?

A. It is found in humans, yes.

Q. Is it found in animals?

A. I don't believe that there is an adequate model in animals for porphyria cutanea tarda.

Q. What -- what are the doses which have been used to give rise to abnormalities in the porphyrins in animals?

A. Well, Goldstein was able to produce porphyria at doses of a tenth of a microgram per week per kilogram. Cantoni, I believe, was -- I'm afraid that I would want to look at the papers to confirm that, but it seems to me that Cantoni conducted an experiment over a 45 week period with somewhat similar doses that were at the -- were porphyria -- forgive me -- porphyrin accumulation in the liver was beginning to show up at marginal levels.

Q. Has there been a no effect level discovered in animals for abnormalities in porphyrins?

A. In all of the studies that show a sufficient dose range, yes, a no effect level has become apparent. Clearly there is a dose response, and if there is a wide enough range of doses, a no effect level becomes evident.

Q. The -- what is the difference between urinary

porphyria and hepatic porphyria?

A. Urinary porphyria is simply the discharge of porphyrins that have escaped the liver cell. We're talking here about a chemically induced porphyria, which is restricted to disorder in the liver. Porphyrins that escape the liver cell and then find their way into the circulation, they are relatively small, are excreted in the urine are the urinary porphyrins, and the accumulation that remains in the cell is, I guess what we would describe as hepatic porphyria, but they both arise from a liver disorder, a hepatic disorder.

Q. I'd like to deal with the cardiovascular system. Dr. Dost. Would you describe what constitutes the cardiovascular system?

A. The cardiovascular system is the entire -- well, it's the system that transports, pumps and transports blood throughout the body, it's the plumbing. It includes the heart, and all of the blood vessels. Interestingly enough, in an ideal sense we don't consider blood to be part of the cardiovascular system, we think of it separately. It's carried by the cardiovascular system. Without blood there would be no point in having a cardiovascular system, but the system itself is really the heart and the arteries and the veins coming back and all the capillaries in between.

Q. Now, in terms of dioxin exposure to animals, has there been seen any effect in the cardiovascular system?

A. At higher doses the capillary bed tends to become leaky. I think I mentioned earlier how at high doses there is -- are capillary hemorrhages found around nerve fibers, around nerves. This is -- this is fairly characteristic. The cardiovascular system structurally does not suffer a great deal from -- from TCDD intoxication other than this increased permeability of the capillaries at high doses.

Q. When you say high doses, what -- how high are you talking about?

A. Well, those kinds of doses are up into the -- in the really long chronic experiments on the order of a tenth of a microgram per kilogram per day. Shorter term experiments, single doses up into the micrograms per kilogram per day.

Q. So it would be from a hundred to a thousand times higher than the no effect level?

A. Yes, yes.

Q. Now, has there been observed any degeneration of heart muscle as a result of dioxin exposure?

A. Well, okay. That has been seen in -- at again to some extent in sustained -- in sustained intoxication at higher levels.

Q. All right. In what kind of animals?

A. Well, in -- I believe in the rat.

Q. Now, we talked before about elevated serum lipids in connection with dioxin exposure?

A. Yes.

Q. Is that really a cardiovascular effect?

A. Well, it has its impact ultimately or at least -- let's put it this way. Elevated serum lipids and serum cholesterol represent a risk factor for cardiovascular disease. All right. In terms of the effect of TCDD, it's probably an impact on the liver, although it could very well be -- it could be an effect to some extent on metabolism out in the periphery where lipids are being used for energy. There is no evidence of that, but the derangement in the liver that results in increased serum lipids if it were sustained for a period of time, could imaginably be associated with an increased risk factor for some kind of cardiovascular disease, because we know that particularly elevated serum cholesterol is associated with increased arteriolosclerosis, which is a factor in heart and artery disease.

Now it has to be kept in mind that that relationship applies primarily to sustained situations; in other words, in which the individual has a naturally

occurring -- well, naturally it's going to be a function of diet, lifestyle, or even genetics, so that the elevated blood lipids are maintained over an extended period of time, and that would increase the risk of cardiovascular disease. It's questionable whether a short period of elevation would have that much significance. We have to consider it at the same time in that light.

Q. When you're talking about a short period, what period of time are you talking about?

A. Well, if it were to occur as a result of an intoxication, a few weeks, a few months.

THE COURT: Mr. Heineman, is this a good point to break for the day?

MR. HEINEMAN: Yes, sir, that would be fine.

THE COURT: We'll break for the day at this time. We'll resume again tomorrow morning at 9:30. I would remind you that you're not to read, listen to, or watch anything about this case in particular or the subject matter in general in any of the media. Thank you for your attention and cooperation. Court's adjourned.

STATE OF ILLINOIS)
) SS.
COUNTY OF ST. CLAIR)

I, MARSHA SCHNIPPER, certify the foregoing to be a true and accurate transcript of the testimony and proceedings in the above-entitled cause.

Dated this ____ day of November, 1985.

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STATE OF ILLINOIS)
) SS.
COUNTY OF ST. CLAIR)

I, Richard P. Goldenhersh, one of the Judges in and for the Twentieth Judicial Circuit, do hereby certify that the foregoing transcript is a true and correct transcript of the proceedings had in said cause.

Dated this ____ day of November, 1985.

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RICHARD P. GOLDENHERSH, JUDGE