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

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

Before the HON. RICHARD P. GOLDENHERSH, Judge

TESTIMONY OF DR. FRANK DOST

November 5, 1985

APPEARANCES:

MR. REX CARR, Attorney at Law, and
MR. JEROME SEIGFREID, Attorney at Law
Appeared on Behalf of the Plaintiffs

MR. KENNETH HEINEMAN, Attorney at Law, and
MR. JOSEPH NASSIF, Attorney at Law
Appeared on Behalf of the Defendant

PATRICIA GANDY, CSR, RPR
Official Court Reporter

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BE IT REMEMBERED AND CERTIFIED that heretofore, on to-wit: Tuesday, November 5, 1985, being one of the regular judicial days of this Court, the matter as hereinbefore set forth came on for hearing before the HON. RICHARD P. GOLDENHERSH, Circuit Judge in and for the Twentieth Judicial Circuit, State of Illinois, St. Clair County Building, Belleville, St. Clair County, Illinois, and the following was had of record, to-wit:

* * * * * * *

THE COURT: Good morning.

CONTINUED DIRECT EXAMINATION

BY MR. HEINEMAN:

Q Dr. Dost, I'd like to take you briefly back for a minute to the discussions we had yesterday on porphyria and the no observable effect level, the NOEL. Have you caused a chart to be prepared which would assist you in explaining this situation?

A Yes.

(At this time Defendant's Exhibit 1281 was marked for identification.)

Q All right, sir, I'll show you what's been marked as Defendant's Exhibit 1281 and ask you to examine that and identify it for me, please.

A This is a chart prepared from tabular data that was

published by Smith, et al., in the journal Biochemical Pharmacology, Line 30 in 1981, and this is a description of the effect of an acute or of acute doses, single doses of TCDD as distinguished from the experiments that you have mentioned, I believe, yesterday in which lower doses were given on a weekly basis over an extended period of time.

Q Now, would you be so kind as to step down here and explain that to the jury please?

A This is another dose response curve. It looks a little different from the ones that we talked about the other day. You recall that in the ones that we've seen earlier there is kind of an S shaped arrangement. This is really the same, the only difference that the, the change is very, very rapid. The slope here is extremely steep, and what it shows is that at a dose, and this differentiates between males and females because there is some difference in the response of males and females to most of the effects of TCDD. It isn't much, but it's measurable. And again this is a logarithmic scale and this would be a dose, I believe, of about 6 micrograms per kilogram. And you can see that the diamond and the circle are superimposed here, they both have got the same doses, this I think would be 12 micrograms per kilogram and this would be 16. And what it shows here is that these rather substantial single doses, now this is just a single

dose, not a chronic exposure but a single dose, that it requires a very substantial single dose before this kind of response emerges. The measurement that is being made is the appearance of porphyrin actually depositing in the liver. You recall yesterday I mentioned that they can appear in the urine and/or in the liver, and here we are talking about hepatic porphyrin, that is these intermediate product in the synthesis of heme accumulating in liver cells and in the response the animals manage a degree of impairment in heme synthesis very, very well until they get up to some substantial dosage, and then the accumulations in the tissues becomes very, very rapid because the -- presumably because the impact at the enzyme level becomes so substantial that the material is not being disposed of very readily.

Q Now, Dr. Dost, yesterday you spoke about the chronic level or the no effect level for chronic dosage being at what level?

A Well, it is at a level down on the order of a tenth of a microgram per kilogram per week or less. Statistically if you run, if you run the dose response curve all the way down, the no effect level approaches a hundredth of a microgram per kilogram per week. Statistically the no effect level is on the order of a tenth of a microgram, and by that I mean that it comes up a little but it is not enough that a statis-

we start dealing with a disease state, again, increased blood lipids all by themselves could not provide a diagnosis. A diabetic will often have elevated blood lipids, but that wouldn't diagnosis diabetes. It depends on other kinds of effects. It is associated with diabetes. Smokers may have elevated blood lipids, but they may not, too. But it seems to be -- there seems to be some association. People with hypertension often have elevated blood lipids, but here we're dealing with a chicken and egg situation in which the condition that led to elevated blood lipids may have caused over a long, long period of time a change in the arteries that we would call arteriosclerosis that resulted in the hypertension, so what I am saying here is that a long term, a long sustained elevation in blood lipids and blood cholesterol could very well lead to and in fact the statement is sometimes made that prolonged elevation of blood lipids causes cardiovascular disease. Other people are more conservative and say that it is associated with it. Whether that's a weasel word, I don't know. In any case, there is at least an association with cardiovascular disease, and that's why we're talking about it perhaps in the context of cardiovascular disease. But there are a variety of other conditions that will cause either a prolonged or a relatively foreshortened period in which blood lipids are elevated.

Q And are the results in the animal data dose dependent?

A Yes, they are dose dependent. The difficulty is that in the case of lipid abnormalities with respect to TCDD at least, the doses have not been carried downward all the way to clear no effect levels. The dose response is very clear, but we don't identify a specific no effect level below which no abnormalities in lipid metabolism appear. Many of the studies use only a single dose because they are trying to understand the mechanism by which this event occurs. But it would appear that the no effect level is substantial upon the order of a microgram per kilogram.

Q Now, would you say it would appear that it is substantial?

A By that I am just making a judgment on what the dose response information is. You recall that the other day I told you that if you don't have enough points on a dose response curve to carry all the way to a no effect level, it is possible to project within limits from what data there is where the dose response, where the no effect level might be expected. However, one cannot simply say here is where the dose, here is where the no effect level is. I would judge on the basis of the existing data that it is on the order of a microgram per kilogram.

Q Now, based upon a judgment of a microgram per kilogram and the dose that you calculated with respect to the Sturgeon

exposure of the 10 grams of soil, would that Sturgeon dose be sufficient to generate elevated blood lipids?

A Well, we calculated that a dose of .001 micrograms per kilogram per day would represent a 222nd, if you will -- I beg your pardon, that is 222 times higher than that exposure that we postulated, and since we are talking about 1 microgram, then that number would be expanded by a thousand.

Q So the Sturgeon dose which you calculated would be 222,000 times 2 low?

A It would be that much less than the 1 microgram per kilogram that I am postulating.

Q Now, I'd like to talk to you this morning about neurological effects of TCDD. Now, briefly describe the neurological system that we are talking about.

A Well, the neurological system is all of the -- it is the central nervous system, the brain and the spinal cord, and all of the peripheral nerves. So the neurological system is our system for transmitting rapid messages from one part of the body and for integrating them and acting upon them in the central nervous system. It is all of the nerves of the body.

Q Now, had there been neurological effects observed in humans exposed to dioxin?

A To my recollection, no. I am not entirely familiar

with all of the epidemiology, but I do not recall any in humans.

Q All right, are you familiar with the examination by Dr. Suskind of workers at the Nitro Plant who were exposed to in the cleanup of an accident there in 1949?

A I remember some of that information, yes. I remember one individual that from which a nerve biopsy was taken that was found to have suffered some demyelinization.

Q Now, do you recall whether or not there were reported neurological effects in those individuals who were examined by Dr. Suskind?

A I'm sorry, I don't remember.

Q All right, let's deal with the animal data. What did the studies show with respect to animal data and the effect of TCDD in causing neurological effects?

A It shows that TCDD is essentially inactive as far as either the central or the peripheral nervous system. There are reports of animals at high doses showing listlessness and effects like that, but that can hardly be described as an effect on the nervous system. These are very, very sick animals and any sick animal is going to be listless, and so I don't think that data like that or statements like that can be interpreted as reflecting neurological change in the experimental animals. What we have to rely on are the --

primarily the pathological examination of animals in which the central and the peripheral nervous system are examined microscopically in detail to determine whether there has been any change in the architecture of the nerve cells.

Q Now, when you say that someone reports that the animals look listless, at what doses have those reports been made?

A Well, those have been at high doses that are producing, they are on the verge of a lethal effect. These are animals that are clearly intoxicated by the TCDD, they have been badly injured.

Q All right. And so the list -- to what do you attribute the listlessness?

A A very sick animal, and I would not try to assign any specific -- any sick animal is going to be listless. These animals don't convulse, they don't show signs that would suggest that their central nervous system has been stimulated as far as depression is concerned. There is no way of assigning that kind of effect. However, those animals have suffered a great deal of other kinds of damage, and a sick animal is listless.

Q All right. Now, what do the studies demonstrate with respect to neurological effects on animals due to TCDD?

A Really, nothing. The only effect that, for example,

that Kociba reported in his lifetime studies on rats was, and this is at a dose range that was actually increasing the mortality of the animals, there was a good deal of pathology generally in those animals. There was, and I believe I mentioned something about this yesterday, some capillary breakdown in the central nervous system so that there was bleeding around nerve cells, around nerves, but that's not a function of effect on the nervous tissue. That's a function of an effect on the circulatory system.

Q All right, now, Kociba, he carried on two different sets of studies?

A This is the two year studies. In the thirteen week studies in which they ran still higher doses of microgram per kilogram per day, which again caused a good deal of other kinds of pathology, there was no mention, they specifically describe the parts of the brain that they examined pathologically and they reported no information about any effects on the brain. The custom is in a pathological study to identify every tissue that is examined, and then to report only on the tissues in which some kind of abnormality has been found, and for this reason there is often a statement in the methods of the paper describing the methods of examination and the tissues examination and no further discussion of certain organs, and that means that there has been nothing identified as being abnormal.

Q Have there been any studies done? You mentioned yesterday a Dr. Calder having done some studies?

A Yes, well, he was concerned about effects on the peripheral innervation, that is out in the legs and arms and so forth, of course he is studying rats, so they have four legs, and I looked specifically for evidence, pathological evidence of injury to peripheral nerves after substantial doses of TCDD and found none.

Q Now, with respect to myelinization, we talked briefly yesterday about myelin, what had the studies demonstrated with respect to the effect on the myelin surrounding the nerves after exposure to TCDD?

A Demyelinization is one of the easier morphological -- by that I mean anatomical changes to detect in nerves, in nervous tissue, and there have been no reports whatsoever of changes in the myelin sheath of nerves in animals intoxicated with TCDD.

Q This is an effect that can be observed upon examination?

A As it happened I am not a pathologist, you must understand, but I've done research on hexachlorophene, which is a demyelin agent, and while I had a pathologist direct and analyze pathological effects on those animals, I was able to identify without any instructions at all just with the pathology

that I learned as a veterinary student thirty years ago or more, I was able to identify the areas of demyelinization. It is a very easy thing to see.

Q Now, Dr. Silbergeld has testified to this jury that there's been work to demonstrate that 2,3,7,8 TCDD causes peripheral neuropathy and she cites the work of animal studies done by, among -- she talks about the Strik Group in the Netherlands, Elovaara's Group in Finland, and workers at NEIHS, are you familiar with Dr. Elovaara's work?

A Elovaara was, I believe, a postdoctoral -- in any case, I was a worker in the laboratory of Harry Vanio in Finland who has done a good deal of work on TCDD and phenoxy herbicides. It had nothing to do with myelin. They were working with a strain of rat that has a genetic defect that -- let me see if I can explain this. When hemoglobin or heme proteins are used up, when they break down, the heme has to be, has to be also destroyed. And one of the products of the heme breakdown is a pigment called bilirubin, and it is excreted in the bile, by and large. That has to be done actually in the cells just as heme is made in each cell that uses it, that breakdown has to take place in the cell when finally that enzyme becomes ineffective and has to be disposed of. But you don't repair proteins, you don't repair enzymes, discard them and start over again. The strain of rat that Elovaara was using, and

he did this deliberately, was a strain that does not dispose of bilirubin very well. I think I mentioned earlier that in mice, why, there are strains and strains and strains of rats. There are probably a hundred different strains of mouse used in research, and they are almost designed for certain kinds of investigation, and the Gun rat, which is what Elovaara was using, is a rat with a genetic defect that doesn't permit it to dispose of bilirubin properly, and what they found was in those rats there was in effect, in rats treated with TCDD with that genetic defect, they were able to find a somewhat amplified toxicity, some biochemical changes in the nervous system of the rats with that genetic defect. The doses again were fairly substantial, we are not talking about fractions of microgram as we have been.

Q Did those studies demonstrate peripheral neuropathy?

A No, they had nothing to do with the peripheral nervous system. They were working with the brain.

Q Now, I'd like to deal next with the immune system. I wonder if you would describe the immune system in animals and humans.

A Well, the immune system is in animals and humans is in essence the same. There are differences among species but the function and the general concept are the same. The immune system first is the protection system that the body has for

dealing with infection, that is external organisms that can cause damage to the organism. It is also intended to deal with foreign substances, foreign proteins. One of the reasons that oftentimes an insect bite can cause such a ferocious response is because of foreign proteins that the insect injects into the skin and the body immediately sends cells in to try to deal with that foreign material. The mechanism that rejects organ grafts, and I think everyone is aware of the present medical advances in grafting organs, kidneys, heart, etc. And one of the problems that they have is preventing rejection. That's an immune response to the foreign protein of the new part. The immune system apparently also responds to cells which are becoming cancer cells because they have become new and strange. So what we have here is a protective system that is primarily circulated in the blood that responds to external assault, primarily infectious attack. It has two general divisions, it is an exceedingly complex system, and I would never claim to be immunologist, but it is a very complex system that can be divided into two parts. One part is known as the cell mediated immune response, which means that for the most part it consists of cells which attack foreign organisms, which attack foreign cells. If we have cancer cells starting to develop, it may attack those cells and it in general responds to, if you will, things that are particulate in nature by

oftentimes foreign inert material, some components of the cell mediated immune response may actually engulf them, may actually surround them and remove them. The other part of the immune system is called the humoral immune system. Humoral arises from the same root as hormone, and what it means is that this is related to materials that are actually in solution in the blood, primarily antibody, primarily antibodies, and antibodies are proteins that have -- that arise from experience. In the immune system there are some proteins that are very nonspecific, the gamma globulin is a nonspecific immune response that will tend to interact with foreign organisms, and it's there all the time. If you have been vaccinated against some disease or if you have had exposure to some disease, you will develop antibodies and you also develop a memory in the immune system, so that if you are exposed at another time, the body immediately makes more antibodies to respond to the challenge. In other words, if you have had smallpox or chickenpox, almost everyone my age at least had chickenpox at sometime or another. You don't get it a second time, and the reason you don't get it a second time, it happens to be a viral disease, but the reason you don't get chickenpox a second time is because the body has learned about chickenpox and it is sitting there poised to respond to the next infection. So the humoral immune system has a memory that responds to the second assault, let's say,

there is probably a genetic change in these cells as a result of some interaction with the epithelium of the thymus and that is not clearly understood, the mechanism. However, these cells are given the capability of responding to the kind of challenge that the cell mediated system has to meet. That is, they will go and attack any organisms that are foreign, that don't belong. It will attack cells that don't belong, and these lymphocytes leave the thymus with this change in their genetic instructions. They go to the thymus and they form clones. Now clones are just collections of cells. A cell has divided, made another cell just like it, they divide and make cells just like them, and so we have a colony of cells in each lymph node and they are continually shed into the circulation. There are also cells in reserve, and when some kind of infection that requires their services arises, then they move into the -- sometimes right out of the blood vessels into the tissues to respond to the challenge. These are the kinds of -- I would imagine that when you have an infection on a skin wound and it gets pusey, that primarily is lympocytes of this sort, as well as certain kinds of cells called macrophages that actually engulf foreign material and they move into the area to clean up the mess.

Q Now, what is the relationship between the thymus gland and the age of a person, whether he be in infancy or

adulthood?

A The thymus has its greatest importance in early life, very early. And in the human it begins to function, and as a matter of fact has accomplished a major part of its activity probably before birth. It begins to function several months before birth and is going through this process, because the fetus has its own circulation, it is processing lymphocytes for a long time prior to actual birth. So that the human infant is born with a fairly effective immune system, it isn't perfect, but it is very nearly in place. And the thymus probably has, will have disappeared in a child of perhaps ten years. In the rat, and most other species in which we do experimental work, the animals are born very very early, I think I mentioned yesterday that the rat pup is born at about the same stage of development as a two and a half month human fetus, so the rat has got a long way to go in terms of its development before it actually is competent. And this function of the thymus in the rat is not really fully developed, and the thymus as a consequence is really very sensitive in a very young rat.

Q Now, are T cells those cells that are coded or programmed in some way in the thymus gland?

A Yes.

Q Humans are born with circulation T cells?

A Apparently, yes.

Q How about rats?

A No, they don't seem to be, although at the time of birth it is a little difficult to examine them. But they appear not to have started the process of T cells until after birth.

Q Now, what does that mean then in terms of whether a chemical would have an immune effect on a rat as opposed to a human? Does this difference in development between the rat and the human have any effect on your ability to extrapolate data from one to the other?

A In the rat during the earlier stages of development up until weaning and even for a period afterwards, the thymus is extremely important, and if you interfere with thymic functions, then almost certain, well, you're going to probably interfere with development of T cells. The human infant is probably past the stage of maximum dependence, keep in mind that the thymus still functions in a young child. But the initial function of delivering coded, to use your term, instructed lymphocytes has been done, and those lymphocytes once out in the periphery maintain their own population. There are still some being processed by the thymus, but a lot of the effect, a lot of the T cell activity arises from the reproduction of T cells out in the lymph nodes. So a good part of the work has been done, much of the work has been done as far as the thymus is concerned in an infant.

Q Well, does dioxin have an effect on the immune system in experimental animals?

A Oh, yes.

Q All right, and what sort of effect does it have?

A Well, it has a general depressing effect on the immune system. It has a more -- it has a greater effect on cell mediated immunity than it does on humoral immunity. However, there are some effects on humoral immunity. A lot of it depends on what kind of tests are done, and there are a very large number of different kinds of tests available to examine the function of the immune system, and many of them do show effects of TCDD and there are some of course that do not. But I think that it is, as a general statement, yes, TCDD is able at sufficient doses to have an effect on the immune system.

Q Because of the differences between the rat and the human being in this particular parameter, the immune system, how reliable is that data on rats in terms of predicting what's going to occur in a human being?

A Well, there are two kinds of information. One is, of course, what I just mentioned, that the cell mediated system is fairly functional in a newborn human. The other is that the impact of maternal treatment, that is treatment of the mother prior to birth in experimental animals does not seem to be as substantial as dosing afterward when the animals are nursing

because it is well-known that TCDD is able to find its way into milk, that's not at all a secret. And there seems to be a greater effect on the rats after birth during the period when the thymus is actually starting to do its work. So I would suggest that the human infant is not as vulnerable to infection to failure of immune function, it is protected by the maternal immune system of course prior to birth, just as the rat fetus is, after birth it is in considerably -- it is much more competent in terms of its immune function. You have to keep in mind that the effect on the immune system in the -- on the cell mediated immunity apparently is not an effect on T lymphocytes per se. They don't seem to be, they don't seem to be injured, even though the thymus is very severely affected, it is one of the most sensitive organs to TCDD.

Q So the effect is on the -- is on the thymus gland and not on the lymphocytes themselves?

A It would appear so.

Q What happens to the -- what do the studies indicate happens to the thymus in animals?

A Well, if you follow almost any experimental animal, and young animals are usually used because the thymus is functional in young animals, and therefore more vulnerable, you find that after TCDD treatment, assuming that the dose is sufficient, the thymus shrinks, it becomes smaller and smaller.

One of the reasons it shrinks is because all of the lymphocytes that are contained in it leave. Now, there is a continual, probably it isn't that the lymphocytes leave, it is more than likely that no new lymphocytes are coming in for processing and the ones that were going to leave do. It appears that the effect is on the, well, the thymus is a gland that is comprised primarily of epithelial cells. In other words they are cells interestingly enough that are related back in early development to the cells that form the skin. Those are the cells apparently that are affected by TCDD. There is recent work that is described in Dr. Greenlee's Group has been working on this for quite some while. So the effect on the thymus is really to stop or interfere with the function of the cells that line the thymus, and the lymphocytes that it processes are simply not brought out anymore and the thymus shrinks way down.

Q What are the clinical manifestations of an impairment of the immune system in animals?

A Well, there is a, you know, we are exposed constantly to every kind of infection that you can imagine, just about. And we occasionally catch cold, we occasionally get gastro-enteritis, or we may even get an infection in some tissue. The manifestations of a failed immune system would be infectious disease. Now, that shouldn't be interpreted to mean that every infectious illness is a function of a failed immune system

because we do get infections. I have been fighting off a cold for three weeks and I haven't fully succeeded yet, but I'm fighting it off. And that cold got a foothold, now maybe if my immune system had been perfect I wouldn't have caught cold. But if it requires perfection, then we are all defective because I don't know of anyone who never gets colds. But once I got the cold, then all of my immune functions are coming to bear in trying to get rid of it. So if we are looking at the experimental context, we would do experiments in which we administer an infectious organism to an animal and observe its ability to respond, and lots of experiments like that are done. We continually monitor animal colonies to see whether there is an increased incidence of this. If we are seeing an increased incidence, far more animals with the sniffles or other kinds of disease, we know that we've either got a massive infection in the colony or that we imaginably might have some kind of an immune dysfunction.

Q Well, when the animals are treated with TCDD, does the immune system just fail completely?

A No, it appears that it doesn't fail, because at doses -- there are effects, and again the dose response relationship applies in immune function just as it applies everywhere else. It appears that there is an upper limit. It is known very clearly that animals that die of TCDD intoxication do not die of infection.

Animals that are maintained, it seems to me that I made some mention of this earlier, animals that are maintained without any microorganisms in their environment at all are just as sensitive to TCDD as animals maintained in a normal environment. Animals that are maintained over a lifetime, we continually seem to mention Dr. Kociba's work, but lots of other long term studies as well do not show any increase in infectious disease, and even though they don't speak specifically of infectious disease, infectious disease causes pathology just like chemicals do, and effects in the lungs and so forth would be identified in the pathology reports on these kinds of studies. So there is a clear upper limit to the degree of impairment that TCDD might cause. And we can see this in experiments in which animals are infected and their mortality is measured following TCDD intoxication.

Q Well, is there a no observed effect level for immune response?

A In every experiment where there has been an adequate range of doses, no observed effect level becomes quite apparent. Whether there are examinations of specific components of the immune system or whether they are looking at the ability of animals to withstand infection.

Q In connection with what you are discussing there, Dr. Dost, have you caused to be prepared a chart dealing with that

subject?

A Yes, I have.

Q Would the use of that chart assist you in explaining it to the jury?

A Yes, it would.

(Defendant's Exhibit 1282 was marked for identification.)

Q All right, sir, let me show you what's been marked as Defendant's Exhibit 1282 and ask you to examine that and identify it for me, please.

A This is a graph that is prepared from data, tabular data that was reported by a researcher named Thigpen, that's an unfortunate name, in the journal Infection and Immunity, Volume 12, 1975 in which he treated mice with TCDD for four weeks and then infected them with an organism, that is, with a salmonella, the species is burned and this is an organism that when administered to mice at sufficient doses is in fact lethal, and this is one of several experiments that he did, but it does represent the general findings in the studies.

Q Now, S-burn?

A Salmonella, salmonella burn, salmonella is a very common genus of microorganisms. This particular one is one that is used in the laboratory and it does appear out in the general environment also. This is again a dose response curve, and again it looks a little funny because the dose response is

so steep. As usual, the dosage is expressed logarithmically, that is .1, 1, and 10 micrograms of TCDD given weekly. And these are in young mice. And what we see here is that there is an upper limit to the -- this is the mortality, this is the percentage of animals that died given that treatment. This is the control level, the number of animals, the percentage of animals that were given no TCDD which succumbs to the infection. What we have here is at this highest dose which is clearly an upper limit because you can see at the next highest dose we are up here on somewhat less than 70 percent, and then we are up here to 75 percent, something like that, and that's as high as the mortality would go. And when we're up into doses like this at, let's say 50 or 60 micrograms, some of the animals will simply die of the TCDD at the lowest dose that they use here, which is at about, what's that going to be, something on the order of probably .4 micrograms per kilogram per week in four weekly doses, there was no effect. In other words, that dose of TCDD did not increase the susceptibility of these animals to infection. And that is a common finding wherever a dose response has been worked through.

Q What is a direct challenge test?

A Well, a direct challenge test, if you are familiar with a tuberculin test, what's done in tuberculin tests is to inject into the skin, I think they do it on the forearm if I

remember correctly, into the skin, not under the skin, the skin is thick, you realize, and so they inject right into the dermal layer of the skin, either -- well, usually it is a killed preparation of tuberculin organisms, and then what we're looking for is to see how the body responds to it. Now if you have never had tuberculosis the response will be minimal because the body has never learned about tuberculosis. If you have had an infection relatively recently and it's been active, why there may be a very vigorous response. And if you had an infection twenty years ago it may be somewhat lesser because there has been a lot of forgetting going on during that period of time. So what we're looking at here are invasion of white cells to attack that presumed invader.

Q Now, what does the line on this chart mean, the horizontal line in the middle of the chart?

A That just identifies the control level of mortality. In other words, animals that were not given TCDD but were given the information, and this is a challenge, too, for that matter, it is an infectious challenge. We are given the microorganisms in 25 percent of the animals that were not treated with TCDD died of the infection. The no effect level is .5, I don't remember what I estimated it at, but it was calculated.

Q As .5 micrograms per kilogram?

A Yes, it intersects the .5.

Q With respect to the dose that you calculated from Sturgeon soil, would that dose be sufficient to effect the immune system in the mice that were --

A As reflected here, no, this no effect level is a half a microgram, and this is a thousandth of a microgram, that's a difference of, what, 500 fold.

Q So it would be 500 times less than the dose necessary to cause an immune --

A I beg your pardon, I'm looking at this other no effect level. I misstated myself. We have, or have I? No, we have with respect to the dose at, that we calculated at Sturgeon, for the sake of simplicity call this .5 or change it up to 5, and that means that we have a 10,000 fold difference.

Q 10,000 fold difference?

A Well, the difference between point -- we have the difference between .5 and .00005, essentially.

Q So the Sturgeon dose calculated by you would be 10,000 times less than that required to begin to show an effect in the mice?

A In this direct challenge experiment, yes.

Q Now, when the animal's immune system is affected by a sufficient dose of TCDD, is this a permanent effect?

A No, it will recover, but it takes -- it takes a substantial amount of time in an experimental animal; it will

take months.

Q To recover?

A To recover.

Q But does recovery occur?

A Eventually these return to normal.

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

MR. HEINEMAN: Yes, sir, fine.

THE COURT: Okay, we will take a short break at this time. I would remind you, and this would go for any other breaks we take during the day, that you are not to discuss this matter among yourselves, with anyone outside the jury panel, or as of yet form any opinions or conclusions about the matters on trial. The Court is in recess.

(At this time Court was in recess.)

MR. HEINEMAN: Your Honor, at this time we would move the admission of Defendant's Exhibit 1282.

(Defendant's Exhibit 1282 was offered into evidence.)

THE COURT: Any objection?

MR. CARR: None, your Honor.

THE COURT: Admitted without objection. Thank you.

(Defendant's Exhibit 1282 was admitted into evidence.)

Q (By Mr. Heineman) Dr. Dost, in your review of the testimony of Dr. Zahalsky on behalf of the plaintiffs, do you

recall his testifying about a study by a man named Boorman?

A Yes.

Q Let me show you what's been marked as Plaintiffs' Exhibit 260, yes, it is 260. Have you seen that before, sir?

A I've seen a photograph of it.

Q All right, is this the photograph that you have seen, sir?

A Yes, it is.

(Defendant's Exhibit 1283 was marked for identification.)

Q What has been marked as Defendant's Exhibit 1283, sir? Is that a photograph of Plaintiffs' Exhibit 260?

A Yes, it is.

Q Now, are you familiar with the Boorman article itself, sir?

A Yes, I am.

Q And did you cause a chart to be made of some of the data from some of the data in there --

A There is a table in the paper, and it was copied directly from the, that is, it isn't a photocopy of the page, but the data has been copied in essence into a table.

Q And based upon your review of the testimony of Dr. Zahalsky, is that the table from which Dr. Zahalsky purported to create this exhibit?

A Yes, it is.

(Defendant's Exhibit 1284 was marked for identification.)

Q Now, let me show you what's been marked as Defendant's Exhibit 1284, sir. Can you examine that and identify it for me, please?

A Yes, it is a copy of a table that was in an article by Boorman that was published in Environmental Health Perspectives, and we do not have a specific reference on here, but it is the same article.

Q It is adapted, it's taken directly from this Boorman article?

A It is taken from the article and it is the data from which Dr. Zahalsky's chart or table was derived.

Q All right. Now, do you agree, sir, with the testimony of Dr. Zahalsky with respect to that chart?

A No, I do not.

Q All right, I wonder if you would step up here and explain to the jury why you don't agree with this chart.

A I'll put this up here where it can be seen. The reason that I don't agree is because some very important data has been left out of the chart and there has been an incorrect representation of the dose, and also in reading the testimony, I saw no description of the experiment and the nature of the experiment is of considerable importance because the animals -- and it was necessary to go to the original article, Boorman's article

is a review, and they reproduced from an earlier paper done by the same group, these data, and in the earlier paper they describe more completely the manner of treatment of the animal. What they did was to administer to pregnant mice TCDD seven days prior, I believe, to the birth of the pups, and then at seven and fourteen and twenty-one days following during the period when they are nursing, so that the animal, the pups were presumably exposed while in the uterus, and then exposed through the milk, and this a common way of exposing very young rodents in the experimental context, that is, to treat the mother and deliver the material in milk. There is a very serious error made in representing the dosage. When we speak of parts per trillion or million or billion, we are talking about concentration. We do not use this representation in discussing dosage, because the only thing that dosage tells us is how much went into the animal for unit of body weight. It doesn't tell us anything about the concentration in the liver or in bone or in the kidney or the brain or any other tissue. The concentrations within organs is going to vary with the organ. So this tells nothing about what is, it doesn't represent what's happening in the animal, neither does the dose in milligrams per kilogram, but it is the only information we have when we administer a chemical to an animal. We don't know where it went, we know enough about a chemical we can say, yes, so much of it is in the liver and the kidney and

so forth. The other error which is extremely critical, even if we accept this idea after parts per trillion as representing the dose, if it were parts per trillion, it would mean that the dose was .005 micrograms per kilogram and .015 micrograms per kilogram, accepting for the moment that this improper reference is the way to use the language. The fact of the matter is that the doses that were administered were a thousandfold higher. This is milligrams per kilogram, a thousandth of a milligram per kilogram is one microgram. This is an erasable -- the dose is one microgram per kilogram per week. So the dosage here has been represented as a thousand times lower than it really was. I don't have a great deal of objection to changing the terminology because Dr. Zahalsky was trying to explain the nature of these cells. He did use a different convention than the authors did for describing the results. He talks about percentage of inhibition, that is, that means how much of the activity of the those cells was decreased. Whereas, the authors speak to how much activity was left. That's not serious, that's just a convention. But I want to point out that he is talking about in this case the loss of competency and the loss of competency obviously would be greater as the dosage increases. The important thing is however that he left off the lowest dose, he left off the lowest dose. Now, this information, this is the number of cells in the bone marrow. It is not just cells making red

blood cells, but all cells in the bone marrow. These are cells that will eventually form red blood cells as he states, these are cells that eventually will form macrophages, similar cells that actually, I mentioned cells that go out and surround, engulf foreign material. The important information in this paper is the dose response relationship. Now, these other chemicals, don't worry about -- we simply wanted to reproduce all of the material so that you can see what the original table looks like. This is the data that we're concerned with, and surely enough there is a dose response, one microgram per kilogram per week, 5, 15. And as you might expect, here is an effect, 15 micrograms is a good size dose for any animal, any rodent, and here only 61 percent of function has been left, and they describe the statistical validity of that. In other words, that is a result in which they have great confidence. This is also a result that they have confidence in, but somewhat less because it isn't that far removed from the control. These numbers are percentage of control, and that's the reason we don't see a separate line for the control information. In this one, they didn't determine the effect for whatever reason. At the lowest dose, but again there is 68 percent of the function left here, 37 percent at this higher dose, which is not surprising. In this case there was a more severe effect even than the others at 15 micrograms per kilogram. Very little effect at 5, and you

can see here that they consider, or the statistics tell them that 90 percent is so near to a hundred percent in this experiment that you can't tell the difference. And here at one microgram per kilogram is actually these animals have more than the normal amount of cells, don't take that as meaning that a low dose of TCDD makes the bone marrow work better. This is the kind of variation that one is going to expect in an experimental context. What I want to show you here, though, is we have a no effect here at one microgram per kilogram. A plot of a dose response curve which suggests that you are going to have a no effect level at this point, but the data isn't there and all we can do is say that is suggested. Here we clearly have a no effect dose with given the statistics, the animals are even better than controls. Again, when I see information like that it doesn't mean they are better, it means that there is enough statistical variability, but given the location of this this point and this point, it is very clear that there is a no effect dose here. So at the dose level that he did not include in his testimony there is clearly a dose, a no effect dose.

Q Now, what is the no effect level there, Dr. Dost, is one microgram per kilogram?

A In this experiment, yes.

Q Now, was this an acute exposure?

A Well, it took place over a period of, I think, four

weeks in which the material was administered prior to birth of the pups, and administered periodically after the birth of the pups, so that there were four doses at this level and I think the total period was over four weeks.

Q So would that be called a chronic exposure?

A No, it would be -- of course when you define those terms, why everybody has his own definition of what is acute, I guess I would call it, it is a short subchronic experiment, but it could even be called a long acute experiment. It is a little difficult to say when you have that kind of a dosing schedule. Their objective was to maximize, at a given level of input they wanted to maximize the exposure to those pups.

Q Now, what's the difference between the no effect level of TCDD demonstrated on that dosing of those rat pups and the concentration or the exposure level or the dose, pardon me, the dose computed from the Sturgeon incident?

A Well, we're back at microgram per kilogram again, and if I remember that would be, the difference would be 222,000.

Q 222,000 what?

A 222,000 fold difference between, that is the dose that we've just been speaking of here at one microgram per kilogram per week, or whatever the period would be, is but one of those doses, one of those four doses is 222,000 times higher than

this exposure that we hypothesized.

Q From Sturgeon?

A From Sturgeon.

Q Now, and that was a no effect level when it was administered -- when that dose, 220,000 times more was administered once a week for four weeks?

A It is my recollection that that was the schedule, it was one time prior to the birth of the pups, and then three times afterward.

Q Now, Dr. Zahalsky testified about proteases. Do you recall that testimony?

A I remember something about it, yes.

Q He suggested that proteases --

MR. CARR: What page, Counsel?

MR. HEINEMAN: 135.

MR. CARR: What day?

MR. HEINEMAN: May 3rd.

Q He suggested that proteases act on proteins and attack tissue. They attack protein in tissue, do you recall that?

A Yes.

Q Do you agree with what Dr. Zahalsky said?

A Only in part.

Q All right. Would you tell us about your disagreement there?

A Well, proteases are in fact enzymes that break down protein. They do not act, when you say tissues, I am assuming that is living tissues.

Q Yes, sir.

A They do not act in living tissues except under extraordinary circumstances. They only act, let me explain. Proteases are present in all cells. Now, I'm using the term very generally, it is my recollection he mentioned a couple of proteins in here. But proteases exists in cells for the purpose of degrading proteins, enzymes, other proteins in the cell when they are no longer working and have to be disposed of. In fact, proteases are responsible for regulating the lifetime of certain proteins in the cell, and this is a very effective system. If a protein should not stay there too long, it will be in the course of time depending on its size be degraded by the proteases that are always in the cell. There are also proteases in certain cells in the circulation and their responsibility, and they are part of the immune response, too, in a sense because their function is to break down. If a cell engulfs another cell, it's got to do something with it, it can't just continue to hold onto that cell. It's got to break it down, it's got to digest it so the cell eventually, that is picked up eventually is digested. There are a certain amount of free protease in the circulation as well because they will tend to leak out of cells that have failed,

they will leak out of the macrophages that are in the tissues and in the blood, just in the normal course of attrition of the cells. So there is always a level of a variety of proteases that break down various kind of protein in the circulation. And if a cell is in, a cell is severely damaged, for example, a lung cell, if a heavy smoker, the cell is alive and it is functional but it is not well. And in that circumstance a cell in that marginal zone between function and nonfunction or life and death could very well be attacked by proteases that circulate. But they don't attack healthy tissue.

Q Now, what is the purpose of them? As I understand it, a protein is a chain of amino acids, didn't you tell us that the other day?

A That's correct.

Q Okay, now when the body -- and does the body normally dispose of protein as an everyday occurrence?

A Oh, certainly, because they either wear out or they are no longer needed, and since we do not repair a protein that isn't working properly, the body degrades it, makes use of those amino acids, starts over again, uses them for whatever it needs amino acids for.

Q Is it something like using building blocks?

A Well, the structure of a protein, yes, is like building blocks. There are a relatively limited number of amino acids,

probably twenty or so, that are incorporated into proteins, and the proteins are very, very long, the molecular weights I think I mentioned the P450 enzymes as having the cytochromes as having molecular weights on the order of 50,000, well, the molecular weight of a typical amino acid may be anywhere from 50 to 150 perhaps, or somewhat greater. They are small components, but the pattern, the sequence in a given protein is very, very specific. And in fact it is even specific across species. Insulin, for example, which is a complex protein from the pig works perfectly well in the human because it is essentially the same structure. There are minor differences, but it is possible to go through and identify every amino acid and establish its sequence in a protein. So we have in all our cells all of these proteins that keep us functioning, and that are really the structural framework of the cell. If there is failure of a molecule, and every molecule is going to fail sooner or later, something has to be done with it. We can't leave the debris lying around like the trash in my back yard. It is necessary to dispose of it, get it out of the way and make use of the parts.

Q And is that what the proteases do?

A And that's what the proteases do, and they are within cells, normally. I suppose in a perfect organism we wouldn't find any in the circulation, but no organism is perfect, there

is always leakage and they appear in the circulation.

Q Now, Dr. Zahalsky on about the same page or the next page, I guess 136, talks about some materials called Alpha 1 anti-trypsin and Alpha 2 macroglobulin, do you remember?

A Yes.

Q Those terms in that testimony?

A Yes.

Q Now, do you agree with what he said about those molecules in that testimony?

A It was a little difficult to understand, but basically no, I don't agree.

Q All right. Point out to the jury if you would what Dr. Zahalsky said about these materials and the area of your disagreement.

A Those two substances are inhibitors of proteases and they exist in the circulation. They are very important components of the plasma protein profile. In fact, they're even greater quantities than, let's say, the immunoglobulins, which we depend on for immune response. Their function is to inhibit the activity of these proteases that we have been talking about that inevitably escape from cells. In other words, they protect us from proteases. And there are a number of genetic diseases in which these protease inhibitors, particularly the Alpha 1 anti-trypsin, the reason it is called anti-trypsin is because

it is very efficient of the enzyme trypsin which is found in cells and that is also an enzyme that is secreted into the digestive tract to digest meat. But there are genetic defects in which there is a deficiency of the anti-trypsin and people with that deficiency seem to have a greater incident of pulmonary emphysema and probably the reason for that is in the normal course of assaults that our lungs take, whether we're smokers or not, there are injured cells and those cells are vulnerable or if trypsin and other enzymes have access to those cells, there's going to be some other kind of break down and that results in emphysema. The main thing about his statement is that he describes anti-trypsin more or less effectively, but then he talks about the Alpha 2 macroglobulin which is just another kind of inhibitor as being some kind of a regulator for anti-trypsin, and it is not. He also makes a peculiar statement that if you increase the amount of Alpha 2 macroglobulin, and I don't know how that is going to come to pass, but if you increase the amount of Alpha 2 macroglobulin you increase the activity of the proteases, and it is just the other way around, it is just the other way around.

Q What do you mean "just the other way around"?

A Well, if you increase the amount of Alpha 2 macroglobulin, its function is to inhibit those proteases, not to increase them. It protects the body against the proteases.

Q Well now, if proteases don't attack healthy tissue, why does the body have inhibitors, protease inhibitors?

A Well, I already mentioned that even in the best of circumstances cells lose, they break up, they're going to lose their -- the proteases, the enzymes that break down protein that are inside the cell, that are there for normally to dispose of unwanted or nonfunctional proteins in cells, and I think I mentioned the other day about when the liver is injured, it spills some of its enzymes into the circulation. The cells are not broken, but they've become leaky. The same thing can happen, a leaky cell presumably can lose its proteases, its trypsin, etc., or a cell that completely breaks up. So there's always going to be some level, even though in an ideal state there wouldn't be any, there's going to be some, and we have evolved that kind of protection against that activity because we always have cells in our body that are perhaps not perfectly healthy, and a cell that is marginally healthy and has been injured in some way would quite imaginably be affected by an enzyme that will break down protein. And so we need to protect against that.

Q Now, Dr. Dost, is there any evidence from animal studies or from anywhere else that you are aware of that TCDD affects proteases or the protease inhibitors Alpha 1 anti-trypsin and Alpha 2 macroglobulin?

A I know of no evidence, I don't think the question has

even been examined.

MR. HEINEMAN: Your Honor, it's noon, I notice, if it's all right with the Court.

THE COURT: Okay, fine. We will break for lunch at this time. We will resume again at 1:15, and the admonishments that I have given you earlier will apply during this break also. The Court is in recess for lunch.

(At this time Court recessed for the noon hour.)

Q (By Mr. Heineman) Dr. Dost, when we broke for lunch we had just finished talking about the Boorman study, and I wanted to ask you a little more about that. Do you recall that Dr. Zahalsky testified that the effect of dioxin on the immune system could be initiated by one molecule of dioxin?

A Yes, I recall that.

Q All right. Now, do you agree with that statement?

A No.

Q Now, what would be the possibility of an exposure to or a dose of one molecule causing an adverse reaction in an organism?

A No possibility at all.

Q Why is that, sir?

A There are a number of reasons, even if the substance was highly reactive it is necessary for a reaction to take place that there be a population of molecules. There would have to be

a lot of molecules present, a reaction that involves a single molecule out of a population, a large population might imaginably take place, but there are time factors. Molecules are very, very lively things. They don't just sit in one place all by themselves. And for a reaction to take place, time is necessary. Chemical reactions are not just instantaneous. It may seem like it when we look at them in a toy chemistry set and pour two clear liquids together and they suddenly turn purple, but there is a great deal of time involved. A molecule has to get into a position where it can react, and then it has to be held there for some finite period in order for whatever it is going to interact with to react, for the two of them to interact together. Otherwise, it may come into contact and be gone again in an instant and more than likely never to return again to that site.

Q Well, a molecule upon entering the body, what happens to it? Where would it go?

A Anywhere. One molecule, we are not accustomed to thinking in terms of one molecule. We are accustomed to thinking in terms of large populations of molecules, and even a dose of a microgram or a thousandth of a microgram is a very, very large number of molecules.

Q Well, how does the fact that the Boorman study establishes a no effect level affect Dr. Zahalsky's theory that one molecule

can initiate this immune function difficulty?

A Well, in terms of this particular study, we see a no effect level as a microgram per kilogram. There are other studies looking at other parameters that show somewhat lower no effect levels. We are still talking about enormous numbers of molecules.

Q Now, Dr. Zahalsky has said, sir, that when this one molecule would initiate this immune function problem, then other dioxin molecules would be required to deal with the additional messenger RNA and receptors, do you recall that?

A I recall something of that sort, yes.

Q Now, would a small number of molecules, a handful of molecules have any possibility of causing the immune function effect that is reflected in the Boorman study?

A Not really. Maybe I should illustrate what I talk about when I say there would have to be an enormous number of molecules, because --

Q All right.

A It happens that we know how many molecules are in a given amount of a chemical. It may seem like an awfully esoteric kind of thing to know, but I guess what I have to do is give a brief lesson in chemistry. But we are talking about TCDD, and I will draw the molecule and show you what I mean. Every atom has a relative mass or relative weight. It isn't very much, but it exists. And we've got a molecule here with chlorine and

oxygen and carbon and hydrogen. Now, you remember yesterday I pointed out that at each of these corners there is a carbon atom and there is also a hydrogen atom. And we rarely show these in a structure unless they have some kind of meaning. It happens that the atomic weight of hydrogen is 1 and the atomic weight of carbon is 12 and of chlorine is 35 and a fraction and oxygen is 16. And if we were to add those all up, we would have the molecular weight, so to speak, well, it is the molecular weight of 2,3,7,8 TCDD. We have four hydrogens, and the atomic weight is 1, so that means we have a total of 4, and we have two oxygens, and the atomic weight is 16. So that means 32. And we have four chlorines, and we will call that 35. It is a little more than 35, there is a decimal in there, but let's keep the decimals out of it. So we've got four chlorines, and then we've got twelve carbons and they are each 12. So this comes out 4 times 35 is 140 and 12 times 12 is 144, and that adds up to 320. Now, that's the molecular weight of TCDD. And a man named Avigodril back in the last century somehow determined that for every chemical compound, if we take its molecular weight in grams, in other words, the gram molecular weight, if we take 320 grams of TCDD, it has in it a very, very large number of molecules, as you might imagine, the number is 6.023 times 10^{23}. What that means is then since 10^{12} is a trillion, this means that there

is 600 billion trillion molecules in this much TCDD. Now, if we were talking about, if we were talking about phenol, phenol has a molecular weight of 94 and we would arrive at this in the same way, 94 grams of phenol has the same number of molecules in it, 180 grams of glucose has this many molecules. This is a constant for all molecules, and it is a very useful number as you can imagine. I guess to make use of that --

Q Dr. Dost, let me ask you a question. Originally when Avigodril came up with that number, there couldn't have been any way for him to count that. How in the world did he arrive at it?

A I'm not exactly sure. He certainly didn't have instruments to count that. He deduced it from the Table of Elements and other information known at that time to chemists. It was a rather astonishing intellectual feat, really.

Q Has it been established now as being accurate?

A It's still valid, and apparently now will remain that way. In other words, his calcualtion was correct. So if we want, we have 360 grams equal 6.023 times 10^{23}.

Q Excuse, was that 360 or 320?

A I'm sorry, 320. Then 320 milligrams which is a thousand less would have times 10^{20}, 300 micrograms is 10^{17}. Well, if we wanted to find out how many molecules are in one microgram, well obviously we would take 1/320th, and I don't

do arithmetic like that in my head. What we really do is divide this by 320. I've got the wrong exponent here, I'm sorry, I would be giving you a million more than you really want. Okay, we come up with a number that is 1.8822, we don't care too much about these late decimals, times 10^{15} molecules in one microgram. Now, we're talking about a microgram per kilogram, so we are talking here about the number of molecules in a kilogram of mouse, there are about forty mice to the kilogram. So if we wanted to know how many molecules were in each of these mice, then we would divide that number by approximately 40, a mouse weighs about 25 grams. So since we are talking in this experiment about a dosage per kilogram, we can leave it at that. Here is the number of molecules per kilogram in this experiment which did not produce an effect. Now, if we wanted, let's say, to consider how many molecules, if our no effect level had been a thousand times lower, then the number would be 1.9, I'm just rounding this off, times 10^{12}, which is 1.9 trillion molecules in a thousandth of a microgram, which is more than I can count in an afternoon.

Q Well, how does that relate to the Boorman study? I'm not sure I understand.

A I'm sorry, the Boorman study found a no effect level at one microgram per kilogram. So the number that we are concerned about is 1.9 times 10^{15} molecules.

Q So at no effect at all in the animal there was nearly 2 trillion molecules of dioxin.

A Per kilogram?

Q Per kilogram.

A Of body weight.

Q All right. Well, what does that number, how does that effect what Dr. Zahalsky testified to as to the causation of this immune effect?

A Well, it says that one molecule or even a few or even a thousand are not likely to have any effect or will not have an effect.

Q All right, now I believe you've testified the other day that the Food and Drug Administration has arrived at a no effect level in a human being, in a 70 kilogram man, is that right?

A They have arrived at a conclusion. This is not measured in human toxicity studies, this is information derived from animal experiments and they have determined or decided that the no effect level in man -- that they can establish a no effect level in man.

Q All right, what is that no effect level?

A It is one nanogram per kilogram. They use a figure of 70 grams just on a general assumption of a 70 kilogram person.

Q So that in a 70 kilogram man, which is what you say, 150 some pounds?

A 154 pounds.

Q There is a 70 nanogram no effect level?

A That's correct.

Q All right, how many molecules would that be?

A Well, we will multiply this figure by 70, and that's for a 70 kilogram individual, it would be 1.31 -- well, let's call it 1.32 times 10^{17} molecules, which would be to put it in manageable form, a trillion is 10^{12}, so we've got 1.32 times 10^{5} or 1.32 thousand trillion.

Q Molecules?

A Molecules.

Q Of TCDD?

A Of TCDD.

Q At a no effect level?

A If we consider this to be a no effect level, yes.

Q Now, in terms of the immune system, sir, when you say that an adverse effect on the immune system will cause additional infections, is that correct?

A Yes.

Q What kind of infections? Are you talking about a cold or a runny nose or what kind of infection is evidenced in an immune suppressed organism?

A Well, an organism that has a significant -- well, to start with there is a lot of excess capacity. We're always

fighting off infections of one kind or another. An individual that has a noticeably compromised immune system is suffering probably continuous assaults, wounds don't heal, probably continuous respiratory ailments. The other problem is that these tend to progress, not only are they allowed to take root or take foothold, but it would seem to me that they would progress. And I think that the, once the barrier, so to speak, had been overcome of our excess capacity for immune protection, it would seem that the individual animal,human or any animal presumably, or for that matter any organism that relies on an immune system would, I think, suffer fairly substantial infectious disease.

Q Let me start, first of all, with your statement about excess capacity. What are you talking about?

A Well, the immune system has far more capability than is necessary to help us get over a simple cold or to get over a case of pneumonia. Now, to be sure, if the infection is massive enough, if it just absolutely overwhelming or if the individual has a protein deficiency and is unable to synthesize protein, and this is a significant problem with heavy protein malnutrition, people cannot manufacture antibodies. It seems to me that if the failure of immune function is really significant it is going to mean pretty nearly a collapse of the immune system. There is a great deal of excess capability available. If we interfere with part of the immune system we

do not necessarily cause animals at least to succumb to infection. We can show a lot of different parameters of immune function, we can look at the ability of the rat, you can inject a challenge into the rat paw and to see whether the paw swells as it ought to because cells are being brought into the paw, or we can look at titers of antibodies.

Q Come again now?

A We can look at the amount of antibodies available in the blood.

Q What is a titer?

A A titer is a measurement of the amount that is available for reaction with an infectious system. So there is lots of ways of quantifying depressed immune function, and we know that TCDD at substantial doses depresses immune function, there isn't any question about that. Not all immune function, there doesn't seem to be any impairment of the response to viruses, for example, to the extent that laboratory experiments have demonstrated, but animals do not die of infection that are heavily attacked by TCDD, and we know that their immune function must be at least partially impaired, you see, so --

Q Now, when you say that there is excess capacity, why then if the immune function has more capacity than is affected by dioxin exposure, why is it that takes so long to fight off a cold? Why is it that an infection can last a while in an

organism?

A If I knew why it took so long to fight off a cold, I would have probably become famous long since. That's a problem that has yet to be solved. The fact remains, though, is that colds, the cold that I have didn't just sweep in and wipe me out. There were a couple of days where I was worried about that, perhaps, and then there was a day or two perhaps that I was worried that I wouldn't die. But nonetheless, that interaction took place, and it has protected me, and I am having a little trouble getting the last of it resolved, but if I didn't have an immune system, I would have dropped very quickly. Why it takes so long is a good question. Certainly these, the antibodies are continuing to be produced. The cells that are fighting off specific organisms, lymphocytes and so forth, are continuing to be produced. Why they can't just step in and mop up instantaneously is something that I don't understand.

Q If the type of disease for a quenched immune system would be something more than just a cold --

A Not necessarily. I guess what I am saying is that if you were to reduce the function of some component of the immune system by half, let's say, by intoxication, that doesn't mean that you have eliminated capacity sufficient to cause that animal to succumb to some kind of an infection. There is reserve capacity. We have impaired the animal, but we haven't

impaired it to the point where his ability to fight off this infection is really affected, and that's really very clear in all of the experimental work, because when animals are challenged with a lethal dose of, well, I showed a dose response relationship earlier. At the highest dose of TCDD, there is still survival. A quarter of the animals survived this impact that in untreated animals killed a quarter of the animals, and we see in animal colonies where TCDD is being administered to animals, there is no change in their response to the host of infectious diseases which exist in most animal colonies.

Q Well, if the immune system were devastated, were quenched, as I think Dr. Zahalsky used the term, what would happen?

A We would have no, we would have no protective response to infectious disease, and this is the kind of problem that happened. People who have leukemia, who has a bone marrow transplant, have to go through a process by which all of the bone marrow is destroyed, usually by radiation or by chemotherapy, and then new bone marrow is transplanted or, yes, new bone marrow is transplanted into those bones, which is a very painful process but interestingly enough, and that new bone marrow will then proliferate and will form these kind of cells that we are talking about here and repopulate with normal white cells. A person who is in that stage where they have had bone marrow

destroyed have had virtually all of their other immune system destroyed. They are very vulnerable to infection and it is necessary to protect them with great care. People who have organ transplants are usually maintained on drugs that depress the immune system very, very sharply. There are many such drugs, and they depress the immune system to the point where that heart is not being rejected or that kidney is not being rejected. If a person is not fortunate enough to find an organ that is compatible, they are in a continuous state of depressed immune function, continuously vulnerable to major infection and have to live a very special kind of life in order to make that possible, a great deal of protection, antibiotics a lot, and so forth. And of course we occasionally read about the children born completely without an immune system, and no one has solved that problem yet, and they live in a bubble and not all that long. They have to be completely protected from any contact with other humans or any kinds of organisms, and their food is sterilized before it is sent into them and so forth. They have no protection.

Q So that if an organism, an animal does not have a catastrophic illness or if a scratch doesn't become blood poisoning or if a minor infection doesn't advance to a very serious infection, does that indicate that the immune system is working?

A Yes.

Q Let me go next, Dr. Dost, to the kidney as an organ, if I may. Dr. Silbergeld testified on April the 12th, 1984, Page 97, that the animal demonstrates that TCDD destroys kidney cells. Do you recall that testimony?

A Yes.

Q Do you agree with that?

A No.

Q Why not?

A Well, the animal data show really just the opposite. TCDD at survivable levels does not have an appreciable effect on the kidney even over long periods of time. If I may refer again to Kociba's work and other chronic studies, long term studies, the kidney is not significantly affected. One of the interesting aspects of the work that Dr. Kociba did was that he found that the normally occurring degenerative kidney disease that occurs in all old animals was significantly diminished at the high dose of TCDD. What that means, I don't know, but it certainly does mean that there was no increased incidence of kidney toxicity in those animals, nor was there any at the lower doses.

Q Now, how exactly, I want to get back to these Kociba's studies in just a minute, but how exactly does the kidney work? You were talking before about organs in which -- that handle

large volume of blood and organs that have a lot of fat so that TCDD can be stored in them. Where does the kidney fall in that spectrum of types of organs in the body?

A Well, the kidney is our primary excretory organ for soluble substances, and it would -- I think I'll draw a picture if I might. This by no means is a precise rendition of a kidney tubule, but the kidney is comprised of a large number of individual units, kidney tubules, and the way they work is there is a filter, any fat. The blood breaks up into small vessels and it comes in and there is just a big clump of vessels here surrounding, that is a kind of a cup, and it is really a filter, and as the blood goes through there, probably as much as ten percent of all of the fluid in the blood actually goes through that filter. The cells don't come through, the large proteins like the plasma, albumin, which is a normal part of the plasma, and the immuno proteins that we were talking about, all those large proteins don't go through. This is a rather convoluted tube, but I'm just going to draw it straight. And it is surrounded by circulation. And what happens is that all of the water, most all of the water, probably 99.9 percent of all of the fluid that went through the filter is brought back out. But in the process, many of the materials that we don't want to bring back are left in that fluid. And most of the materials that we don't want to get rid of like glucose, you know, we have lots of glucose

in the blood. Normally about 100 million grams per 100 milliliters, and that's going to come through here, too, because it is a small molecule. So it goes down here and we reabsorb it back out again if we are normal. Some people can't do that very well, but we recover all of that glucose, unless we've just eaten a candy bar or something like that, there may be too much to recover, and some of it will escape. And then there are some other cells that actually have the function of secreting certain things into the urine, ammonia and so forth. But anyway, in essence, it is a filter followed by a scavenging unit which brings most of the fluid and valuable stuff back in, and those thousands of units all converge in the center of the kidney. And then from the kidney there is a tube that goes to the bladder. So what we have here is a filter, and it deals -- if we are talking about a toxic substance or any kind of a foreign substance, if it is at all soluble in water, it is delivered to this point and hopefully left in the urine and disposed of.

Q Now, the thing that you have drawn, the mass of blood vessels at the top is called what?

A It is called the glomerulus. I had nothing to do with naming these.

Q All right, and the tube is called the tubule?

A This is the kidney tubule, and it has a proximal

segment, actually it comes in kind of an S shape, and this is the distal segment and there are different functions in different lengths of that tube, but --

Q All right. Now, how many of those operating units, let's say, the glomerulus and the tubule with its reabsorption collar, and that sort of thing, how many of those are in the kidney?

A Quite a few million.

Q So the blood is coursing through this thing?

A These are little bitty vessels here, you see, the kidney circulation has divided into an enormous number of vessels. This thing is only one cell in thickness, you know, the wall of this tubule is only one cell. So there is really a collar of cells, you might say. In other words, if you were to -- took a tube from the end, it would look something like this, you see. So it is a very, very small structure.

Q And so the blood courses through the kidney, does the kidney receive its own blood supply for energy and life for the cells?

A Well, that's right. It happens, you see, all of these tubular cells receive their circulation from the same blood flow that is bringing, it is a very efficient system, we don't have to have a whole separate plumbing system in order to supply nutrient to the kidney itself. The same

circulation that brings fluids to the kidney and carries wastes in here and comes back down here, the same circulation it brings out the fluid that we don't want to dispose of, provides the energy resources for those cells.

Q All right. Now, when Dr. Kociba studied the kidney, how many occasions did he do it?

A I'm not sure what you mean, how many.

Q How may separate studies were there in which this was observed?

A Well, they didn't find -- he had done two studies, one thirteen week study that I think I mentioned yesterday, and then the long term, lifetime study that they did.

Q Was one of them in 1976?

A The short one was reported in 1976. The longer one was reported in 1978, and they had a substantial number of animals at each experimental level, of course.

Q Now, what did the 1976 study show with respect to kidney effects in animals?

A It didn't show any kidney pathology that I recall.

Q Were there any blood chemistry results?

A Oh, yes. They did a variety of blood chemistry, and they didn't find, except -- I believe at the higher dose they found some elevations in levels that would suggest that might be interpreted upon the kidney, but there could

also have been effects upon the liver, too.

Q Now, you say the higher doses?

A They used a maximum dose in that study, in the 1976 study, of one microgram per kilogram per day.

Q All right, was that high enough to cause death in some of the animals?

A It certainly did.

Q And at that high dose, there were some blood chemistry results, is that right?

A Yes.

Q Did they do any kidney pathology on that occasion?

A Yes.

Q Did they find any pathological?

A To my recollection they found no pathology at all. They found no change in the architecture or the shape of the kidney cells.

Q All right, so that in 1976 Kociba did not show that TCDD destroys kidney cells?

A That's right, and the same outcome emerged in the study reported in 1978 where they had maintained it for two years.

Q All right, now the 1978 was a chronic study for the lifetime of the animal?

A Yes.

Q And how did the kidney effect in the 1978 study relate between the dosed and the undosed animal?

A Well, I think I mentioned a short time ago that the normal incidence of degenerative kidney ailments, all, particularly rats, as they age, have a substantial incidence of kidney damage. The nephron here, this unit that we have been talking about will just simply fail, and there will be areas in the kidney where is scar tissue and a lot of the number of functional units decreases with age.

Q That naturally occurs?

A That occurs in any, it occurs in all of us, of course, but it is quite visible and easily quantifiable in experimental animals because we are examining their pathology all of the time. And the interesting thing in that particular study was that the incidence of that kind of degenerative image decreased at the highest dose, and decreased to an extent that was statistically significant.

Q Decreased in the animal that had received the highest doses of TCDD?

A Yeah, I'm afraid so.

Q Well, is there any evidence in the animal data that the kidney is adversely affected by the 2,3,7,8 TCDD exposure?

A Well, really only at doses that have caused the animal to collapse, and sometimes not then. That's where it is

seen, is at very, very high doses where the animal is probably mortally affected.

Q At below that high dose?

A It appears that there isn't an appreciable impact on the kidney.

Q All right, sir. Now, Dr. Dost, I'd like to talk next about cancer. I wonder if you would define cancer for us.

A Cancer is a condition in which cells have lost their regulation, usually have regressed in their differentiation. By that, I mean that in all of our cells begin the same, and as we develop, cells become, we use the term differentiation, and that I think is self-explanatory. Eventually we have to have a liver and we have to have bones and we have to have kidneys, and as the animal develops, cells begin to take direction toward the formation of the various organs, and so as the embryo develops, the cells become more and more and more different until finally we have a complete organism with arms and fingernails and eyes and all of the structures, but they all started with similar cells. In the case of tumor cells, there is a tendency to go back part way down that ladder and lose the differentiation. Now, they don't go all the way back to the point where they are just like initial embryonic cells, but cancer is a collection then of cells that is reproducing by itself and forming usually a tumor

that has some form, and it has been removed pretty much from the formal regulation that the body exerts on itself. These cells have become autonomous. They are self-governing to a large extent, so that they are no longer controllable and they grow and grow and grow at a slow rate. But they grow all by themselves, without regulation by the rest of the body.

Q Now, when you say regulation, how is it that the body regulates a cell's growth?

A Well, we are really a very well-organized system and not only does the body regulate each cell's growth, but the companions, all of the cells in the liver have a common mission, all of the cells of a given type, and they exert regulation on one another. When they lose that regulation, well, cells that have become a cancer cell may start to multiply, and it's going to form a larger and larger colony of cells that eventually it is independent of the cells that are around it. But we regulate our cells, we have hormones that turn cells on and off. If we want to cause sugar to be absorbed by cells, we release insulin and that tells cells to absorb the sugar. And that is a form of regulation that arises from a central point. That particular phenomena doesn't have anything to do with cancer except when you have a cancer of the organ that produces insulin, and there might very well be for a while a great deal of insulin produced so that we have

caused a problem elsewhere in the body. Generally, the problem with cancer is it reaches a point where it invades and interferes with the function or some of those cells may break off and go to other parts of the body and continue to reproduce by themselves without regulation.

Q What's the name of the latter condition you just described?

A That's a phenomena known as metastasis, m-e-t-a-s-t-a-s-i-s, and it simply means the movement of the cells from their origin to some other location.

Q All right. Now, when you are talking about cells developing, each cell nucleus contains, you mentioned a library of material?

A Yes.

Q Called what?

A DNA.

Q Now, but you say it starts, the human embryo starts from the joining of two cells, right?

A That's right, it starts from the fertilization of an egg, and we have one cell, it divides into two identical cells, and they divide. Early on, you have a structure that looks rather like a raspberry that's comprised of a substantial number of cells that are all identical.

Q Now, is the DNA in the nucleus in each of these cells

the same?

A Yes.

Q Now, what about when the differentiation begins to occur, when cells start to become part of the bone or part of the foot or skin or hair or whatever it is?

A All of the information is there, but by some process that isn't really well understood, cells only utilize part of the information, the part that's important to that function. So if we have a liver cell, that is all the same information that kidney cells have, but they don't use the part that's related strictly to the kidney cells, you see.

Q And the kidney cell doesn't use the part of the DNA cell that relates to what the liver parts use?

A Exactly.

Q So the DNA is the same in every cell?

A Yes, it is a question of which book we take off the shelf.

Q Now, what does DNA have to do with cancer?

A Cancer is generally believed to start with some kind of an injury or a lesion on DNA, so that DNA is changed and not repaired, and that that change remains and somehow it remains part of the genetic equipment in the cell, and ultimately is triggered to develop into a tumor cell. It is a very long, complex process. It doesn't just happen.

Q Have you develop a chart which would assist you in explaining this?

A I have a chart I use in lectures for that very purpose.

(Defendant's Exhibit 1285 was marked for identification.)

Q Dr. Dost, let me show you what's been marked as Defendant's Exhibit 1285 and ask you to examine that and identify it for me, please.

A It is a -- it is the chart that I brought with me that I use in describing the process. It is a general description of the process by which a cell eventually can become a tumor. And as it points out, it is hypothetical steps, this is generated not from a specific body of research data, but it represents the present understanding of the process. All right --

Q Would you step up and use it, please? I am interested in the term "hypothetical."

A Well, no one has determined fully the process by which cancer is caused. That probably is the most, probably it is the most heavily investigated problem in biology at the present time is to try to establish just how cancer is caused. There is a great deal known about how it is caused, there is a great deal known about intermediate steps. But unfortunately

the process seems to vary with certain kinds of cancer, and it also, and also there are some very specific and important pieces of information that simply are not yet known, so it would be presumptuous to say that this is the way cancer happens. It does, I think, represent quite properly the general scope and sequence of the process. So these are all, these are generalizations. They do not relate to some specific tumor type, nor do they relate to any specific pattern that someone has laid out. But the first thing that happens, you spoke of DNA, and the first thing that happens is apparently a mutation of some other damage to DNA in the cell, and that affects some part of the genetic apparatus. Now, there is a concept of the oncogene that has crept into the popular press, and it is a very important issue of discussion among cancer researchers, and that is the idea that there are segments of DNA, genes, that by some mischance with very minor changes can be converted into a gene that actually leads to cancer. In other words, the idea is that we have in all of our cells genes that require very little modification to touch them off and cause them to take control of the cell, so to speak, and cause cancer. In any case, there still has to be some kind of a change in the DNA to start this whole process. And that step probably is irreversible. But other things have to happen for it to be expressed, and when I say, by express, I mean other things have

to happen for it to take effect. Now, one of the things that apparently have to happen is that the cell has to divide, and in dividing it, copies of its DNA for the second generation cell. So that there is two identical sets of DNA. And if that happens, the speculation is that probably it takes two cell divisions in order to do that for that to be copied. So at the same time I want to step back for just a moment. We have talked about a DNA lesion, that is irreversible. There is damage occurring to DNA all of the time in the cell, naturally, and as a result of everything that we do in our environment. And this lesion has to survive this repair process, and I probably will have an opportunity to discuss the idea of DNA repair a little later.

Q Dr. Dost, excuse me, when you talk about our environment, what are you referring to specifically?

A Diet, everything we do, our ultraviolet radiation from the sun, our environment is kind of hostile place, and we are taking in all of the time a lot of genetically active material in our diet. If you were to go through everything that you eat and identify all of the substances that are known to have some kind of genetic activity as determined in microbial mutation tests and so forth, you would soon be searching trying to find out what in the world you could eat that will sustain you without creating a problem. Apparently,

we are not creating problems by eating our daily meals because our life span is increasing, but the fact remains that there are lots and lots and lots of materials in the natural environment, both physical and chemical, that has capacity to at least theoretically cause injury. Not only that, within the cell, the intercellular environment is also very violent and there are lots of very reactive molecular species created in the normal metabolism in the cell. Perhaps I can talk about that a little later, too.

Q When you talk about genetically active, what do you mean?

A I mean it has the ability to interact with DNA.

Q Now, we've heard talk here about DNA and RNA, and messengers and all that sort of thing. What does all that mean? Is DNA a ribbon or is it a piece of --

A DNA is, okay, DNA is the primary source of information. The RNA that we have talked about, there are a number of kinds of RNA, but the only number of RNA that we have particular concern about is that is made as a copy of a segment of DNA and goes out into the cell to the ribosone or to endoplasmic reticulum to form a pattern for the synthesis of some protein. DNA is a very, very, very long molecule. There are probably -- it is comprised of pairs. I may have to stop and go back now and then because this is a little complex, but DNA, you

may have heard of the double helix,which was a book that Watson and Crick wrote about thirty years ago when they finally discovered what the configuration of DNA was.

Q And won a Nobel prize?

A And they won a Nobel prize. The DNA, I guess the best way to describe it is to think of a zipper, an ordinary zipper. And you know that a zipper has teeth and they come together like so. Well, DNA is set up as pairs of bases, and I don't need to describe what these bases are, but there are four kinds in DNA. And I guess we could say that those four bases are the letters of the genetic alphabet, and you can imagine that there is an unimaginable -- that's not very good language -- there is an enormous number of possible combinations because these molecules are very, very long. They carry an enormous amount of information. And if we think of this as being, as three, four letters in this alphabet, and there are words in the DNA and they are all three letters long and each of those words codes for an amino acid. Now, when we go through this process of inducing, which we've talked about, the DNA opens up and we form RNA as I've just mentioned. And that RNA carries the same code, this series of three letter words. It is very long and it codes for these thousand or two thousand amino acids that come together to make this protein.

Q So it unzips it?

A Unzips, half of it comes apart and the RNA is copied off the other half, or it comes apart and RNA is copied off of one half. But if you think about this zipper, for every 10 teeth it has taken one twist, this thing is twisted, and I mention the number of bases. There are in the cell about, between 10^8 and 10^{10} base pairs. In other words, in each cell there are, let's say, 10^8 words or letters, how would I put that? No, we will divide it into three and make words. We will say there are 3 times 10^7 words of information in each cell. That's 30 million, probably more than that, but the point is that in these rather interesting three letter words are the codes for all of the amino acids. And so it is the sequence on the DNA that governs the sequence of synthesis out in the cell. All right, if there is an error, if there is an error in that sequence, then incorrect information is going to be transmitted. Now, that may not make any difference, it may be a serious error or a big chunk of DNA is lost and the cell dies. That, fortunately, is not an unhappy circumstance except in the nervous system, but it is happening all the time. In this situation we have inverted some kind of an incorrect message. All right, it may stay that way forever and nothing ever happen, but if the cell divides before it is repaired, that lesion may be thick in the gene so that it stays there and the cell then is hearing that message. Well then,

there are a number of processes that go forward that come into the category of promotion and enhancement. Some of these words are used interchangeably, sometimes you find progression used here and sometimes translation. This question of cell transformation falls up here aways, but let me read this to you. "It is assumed that specific physiological or biochemical phenomena are required to facilitate the expression of the initiating event." Now, that may be cell injury, there are certain chemicals that are known to promote this effect. I've mentioned the phorbol esters the other day when we were showing that chart on the difference between promotion and initiation. TCDD, phenolbarbitol, smoke condensates having been suggested as promoters. And the promotion process appears to be reversible at least in its early stages, in other words, if it does take place before some of the other events going on, why then that cell may just revert back to normal. It also is clearly dose dependent and clearly there is argument about whether it is a threshold or not, but it is very clearly dose dependent. Then, the next step is what is called cell transformation, and the cell changes its whole genetic makeup in some fashion and it does different things than it used to do. It becomes juvenile, if you will. It reverts back to a less developed form. And one of the problems that it may or may not be recognizable as a strange cell, as a different cell, and if it

is recognizable, then the immune system will clean it up. And this is probably going on in our bodies all the time. From this point forward, those cells when they reproduce they carry all of these bad characteristics with them, and they begin to reproduce. If they do evade the immune surveillance and they could very well repeat characteristics of the normal cell that are sufficient that the immune, the cellular immune cells, the killer cells don't recognize them as being tumor cells or nearly tumor cells. Well, so there is a progression here where we go from a genetically injured cell to a cell that has to be further stimulated in some fashion. And then from that we go to cells that are producing, changing their character and eventually becoming a sufficient population of cells that either they form a tumor that can be seen or they may form a tumor in the bone marrow that is recognized as, say, leukemia or something like that, which is a circulating tumor in effect. There is no mass, no hard tumor. I don't know whether I have made that sufficiently clear, it is a very muddy process, and there a lot of different -- there is a lot of alternative descriptions that can be inserted.

Q I'm interested in the initiating event that you've got there at the top. I'm not sure I understand how this occurs. Is this something that has to come in from the outside, or is it something that naturally occurs in the cell

because you talked about DNA repair and errors in the cell before.

A Well, DNA is, to start with, we have an enormous amount of material that has to be manufactured, and we considered that the manufacture of DNA in the cell is a very accurate process. But the error rate is about one in every million base pairs. Well, I've already talked about a situation in which we've got what, 10^8, that's 100 billion, I beg your pardon, a 100 million base pairs in the cell. And if we are, actually the number is higher than that, it is probably about 300 million. And if we have, if we have an error rate of one in a million, that means that for the growth of a single cell there will have been 300 errors just in that cell alone. Just in manufacturing DNA. And that has to be corrected. There is a repair process that inspects DNA. It is fascinating. It literally inspects it, like going over a string of beads and find defective spots, slices them out and fixes them. Now, that is not a perfect process. Some repair may have been missed or there might be a mistake made in the repair process, but if you consider that there are 300 errors in a given cell and if, oh, in the liver there is something in excess of one billion cells per gram, so let's say 10^9 cells per gram for a gram of liver, which is maybe would be a piece that size, perhaps, there would be 300 billion errors committed in the process of making the DNA for those cells. So this process is going on all the time of error in synthesis.

The other thing that is happening is that, I'll just write that down, the other thing that's happening is, as I said, the cell is a very violent place and there are some very violent reactions going on in the cell in the process of generating energy. There are some very reactive oxygen atoms that are generated in the cell, and most of them are trapped and are, don't cause any harm, but enough of them get away that there is a very significant and measurable amount of DNA damage by some of these very reactive oxygen species inside the cell. And peroxide, hydrogen peroxide is made inside the cell, and there are enzymes that get rid of that stuff. There are traps that collect it, but some of it inevitably escapes and the rate of injury of cellular DNA can be measured by the collection of some of the products, because as I said, they slice out pieces of DNA and bring them, they are excreted into the blood and into the urine and they are disposed of. People in Bruce Ames' lab at Berkley have done some interesting work on this, and what they find is those products emerge at a rate that indicates cell damage to the extent of 320 DNA damaging events per cell per day. And when you multiply that by the number of cells in the body, which has been estimated, there are various estimates, but the one I generally use is 6 times 10^{13} cells, which is 60 trillion cells in the body. Then we have an enormous number of events taking place in the cell every day in the body, every day that can

be potentailly leading to genetic damage of some kind. Virtually all are repaired. Now, if the system was absolutely perfect, we might not have cancer. But unfortunately the system is not absolutely perfect and there are inevitably some errors in DNA, some damage to DNA that persists. Now, also, external functions. I've talked about some of the mutogens that are in natural substances, black pepper, broccoli, and when I say broccoli, I don't mean to isolate it, I think we can say that about most vegetables, don't worry about it. Carcinogens, bena'a'pyrene in automobile exhaust, or gasoline, benzene, lots of chemical carcinogens that can also result in this DNA damage that serves as the initiating step. If the concentration is high enough it can cause these kinds of events, too. So we have all kinds of things happening, very high repair rate that presumably is not absolutely perfect, so you can see, there is an incredible amount of activity going on in the cell all of the time that relates to the potential for DNA damage in this initiating event.

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

MR. HEINEMAN: Yes, sir, it will be fine.

THE COURT: We will take a short break at this time. The admonishments that I gave you earlier will apply during this break also.

(At this time Court was in recess.)

(Defendant's Exhibit 1286 through 1289 were marked for identification.)

BY MR. HEINEMAN:

Q Dr. Dost, for the record, I have had these sheets marked. Defendant's Exhibit 1286 is the calculation of the gram molecular weight of dioxin.

A That's correct.

Q Showing of Avigodril's number at the bottom?

A Yes.

THE COURT: What was that, 1286?

MR. HEINEMAN: 1286, your Honor.

Q 1287 is the calculation of the total number of cells.

A Total number of molecules.

Q I'm sorry, total number of molecules, and what?

A Well, I made an error when I calculated the number. First, we are talking about the number of molecules in one microgram, and then the number if we are talking about a thousandth of a microgram, and then I carried that calculation to the number of molecules that there would be in a 70 kilogram person and I had made an error in the exponent and I had a thousand times more than it should have been, so it should have been 132 trillion rather than 132 thousand trillion. This is not like Senator Dirksen's famous saying, "A billion here and a billion there and you are talking real money." We need to be a little more precise.

So the number is 132 trillion, not 132 thousand trillion.

Q Okay, and that's exhibit 1287?

A If you want, I will put per 70 kilogram person.

Q All right. Then I have marked as Defendant's Exhibit 1288 the drawing you did of the--

A Kidney tubule.

Q Kidney tubule. You referred to it as a nephron?

A A nephron, yes, that's the word for the whole unit.

Q And 1289 is the calculation you did for what, sir?

A For the number of natural errors in the formation of the DNA in cells as cells grow, as tissue grows, and then a statement of Dr. Bruce Ames' finding of about 320 DNA or finding the residue that has arised from the repair of about 320 damaging events per cell per day, and the number below is the number of cells in the estimated in the human body, 60 trillion. Perhaps I should identify that number so in the future--

Q All right, thank you.

MR. HEINEMAN: Your Honor, at this time we would offer into evidence Defendant's Exhibits 1283 through 1289.

(Defendant's Exhibits No. 1283 to 1289 were offered into evidence.)

MR. CARR: The Plaintiffs have no objection to these exhibits, your Honor.

THE COURT: Fine, they are all admitted without

objection. Thank you.

(Defendant's Exhibits 1283 to 1289 were admitted into evidence.)

Q Dr. Dost, if you would resume your place there by your chart, discussing the mechanisms of cancer, you did some detail on the initiation, and to make sure I understand it, the initiation can be an internal event?

A It is an internal event in the cell.

Q All right. But it can be caused perfectly naturally without any outside intervention at all?

A It can be caused, well, we assume that all of the events that take place in the cell that damage DNA also have the capacity to cause a DNA mutogen. Now, how does that relate to the event of initiation that you are describing?

A Well, first I should point out that a cell has initiated does not behave any differently in the organism than a cell that has not been initiated. So its function has not changed, its function hasn't changed, and it appears that even when it divides, while that initiating lesion has stayed in the cell and then carried to the next cell, the daughter cells also will not function any differently than the normal cell. So we have got a cell here whose nature hasn't really been changed in terms of what it does in the organism. Other events are necessary to make that happen.

You asked about mutation. Mutations are changes in the genetic material of a cell such that the cell in either its next generation or possibly some subsequent generation will be altered. Generally when we are speaking at the cellular level, we are talking about a change that appears in the next generation. If we are speaking about the whole animal, then we would be discussing a change that is induced in germinal tissue in a reproductive organ, and then again there is some complex genetics involved here. It could very well be that offspring of the parent with damage might be similarly affected or if it is a recessive change of some kind, it may appear several generations down the family tree, in an adult organism. Now, we use, it is thought and again pretty universally thought, that mutation, the event that leads to mutation are rather similar to the events that lead to the initiation of cancer. And it is possible for us to study those kinds of events in the laboratory. Microorganisms are very handy for this sort of thing, cultured cells. So there are a variety of laboratory, of fruit flies, there are a variety of systems available in the laboratory for learning whether a chemical has the capacity to interact with DNA, it happens that there is a good correlation between the ability of a chemical to interact with bacteria and other bench-top kinds of tests and the ability of chemical to cause cancer. Generally chemicals that do not cause mutation does not cause the initiating

kinds of events.

Q Well then, as I understand mutation or mutogenicity is not the same thing that you are talking about here?

A The initial event is probably very similar, just the initial event. The only difference is that it's likely that in mutogenosis there is sufficient change that the next generation of the cell is changed, you see, and imaginably we could have cells in, let's say, the liver that had a minor mutation, and they functioned a little differently, and we may accumulate a few cells of that sort in the liver, but it wouldn't matter because it isn't transmitted to the next generation, and those cells don't necessarily make a tumor, they are just a little bit different from the cells, the normal cells of the liver, and that presumably happens frequently.

But the carcinogenic event may be sufficiently specific that it only can be transmitted in this fashion to the next generation of cells. The point about mutogenicity, though, is that apparently the damage in the cell is about the same, and there is an empirical correlation, by that I mean the experimental evidence shows that chemicals that are mutogens are frequently carcinogens. Okay, now, precisely what is happening at the level of DNA in the two cases to link them is not clearly known, but we know that DNA damage takes place in both instances, and that there is a correlation. Chemicals

A That has been demonstrated by again, Dr. Poland and Dr. Gunther. Poland has used, has done experiments in which he has used labeled TCDD and has found no evidence of binding to DNA, much evidence of binding to protein and other structures, but none to DNA.

Q Now, Dr. Silbergeld in her testimony here stated that TCDD--

MR. CARR: What page, counsel, and day?

MR. HEINEMAN: April the 12th, Pages 120 and 149.

Q States that TCDD causes cancer itself. It is the most powerful chemical carcinogen identified, and is a complete carcinogen. Do you recall her testimony in that regard?

A Yes, I do.

Q Do you agree with that, sir?

A No, I don't agree that it is a complete carcinogen.

Q And on what basis do you disagree?

A I've already discussed really the evidence that shows that TCDD is in fact a promoter and it does not interact with DNA either by direct chemistry or by any ability to cause a significant mutation incidence, and also by studies that have been done that show that in organisms that are treated with TCDD there is no increase in the DNA repair activity, which is a measurable, a measurable factor in cells.

Q Now, at the same time in her testimony she suggested

that the Kociba studies show quite clearly that exposure to TCDD alone causes cancer, and that's why it is a complete carcinogen. Do you agree with that statement?

A No, I don't. What the Kociba studies show in that light is that at high doses, at very high doses up at a point where the animal is suffering really substantial injury, there is a lot of effects on the liver, particularly in those animals, that the incidence of a variety of tumors changes, a variety of tumors, and when I say change, I mean just that. There were increases in the number of hepatocellular carcinomas and also nodules in the liver that probably are reversible by the definitions of most pathologists. There were many kinds of tumors. Now, you must understand that in any population of animals there is going to be a high population of tumors, a high incidence of tumors of any kind. We all know unfortunately in our population that most cancers appear in older people. The same is true in laboratory animals. It is interesting that in those experiments the incidence of many kinds of tumors, particularly those of the endocrine system of the glands, actually it decreased statistically. In other words, it wasn't so much an increase in total numbers of tumors, but an alteration in the whole tumor pattern. In another study that was done in the National Toxicology Program, there was an increase in some tumors in the thyroid at the highest dose.

When the National Toxicology Program study was done, instead of feeding TCDD in the diet, they introduced it by stomach tube once a week. Long term experiment, they gave the rats their treatment once a week on an exact basis with a stomach tube. Those rats did not get the somewhat--the slight increase in tumors of the palate and the nasal passages and the upper respiratory tract that Kociba's rats did. The reason for that is because the material was not in contact with those areas. Rats when they eat tend to inhale quite a bit of food, particularly when it is in that powder form as is used in these kinds of experiments. The fact that these tumors occurred at the site of contact is suggestive of promotion, particularly when we compare with the studies that were done on the skin, TCDD has also been tested as a skin carcinogen, again in the National Toxicology Program, and the only tumors that they found were at high doses again, and they were a tumor called a fibrosarcoma of the skin. It is a connective tissue tumor, and there were no other tumors anywhere in the animals. That, too, is suggestive of a promoting response rather than initiation, and promoting at the site of application.

MR. HEINEMAN: At this point, we would move the admission of Defendant's Exhibit 1290.

(Defendant's Exhibit 1290 was offered into evidence.)

MR. CARR: No objection, your Honor.

THE COURT: Okay, 1290 is admitted without objection. Thank you.

(Defendant's Exhibit 1290 was admitted into evidence.)

Q Now, Dr. Dost, I'd like to talk to you again about some of Dr. Zahalsky's testimony, and in that connection, I'd like to show you what's been marked as Plaintiff's Exhibit 253. Have you seen that before, sir?

A I've seen pictures of it.

Q All right. Or a picture of it?

A Yes.

(Plaintiff's Exhibit 1291 was marked for identification.)

Q I'll now show you what's been marked as Defendant's Exhibit No. 1291 and ask if that's a blow-up of a photograph of Plaintiff's Exhibit 253.

A Yes, it appears to represent it.

Q Now, Dr. Dost, you have read, have you not, sir, the testimony of Dr. Zahalsky in which this chart was discussed?

A Yes, I have.

Q All right. Do you agree with that testimony and this chart?

A No, I don't, really.

Q All right. Would you please come up and explain to the Jury why you disagree with the chart and the testimony relating to it.

A This as I recall appeared in Dr. Zahalsky's testimony at a time when he was discussing effects on the immune system, and if I remember correctly began talking about this representation, and I guess that I would ignore that and take for granted his representation that this acheme represents the way that dioxin causes cancer, and it is clearly incorrect. There is, the schematic that we're dealing with here--

MR. CARR: Mr. Heineman, what would be the date the testimony was taken and the page number that he is referring to?

MR. HEINEMAN: It starts at 101, I think, and following that, I think is where he starts talking about it.

MR. CARR: What date?

MR. HEINEMAN: May the 3rd.

A Well, the scheme that's represented here is clearly a scheme that is apparently related through the induction of microsomal enzymes, the P450 enzymes. And what we have here, this looks very much like a diagram that Dr. Eisen published at one time or a part of that diagram, but I'm not prepared to say that it was adapted from it directly. When TCDD or any of a number of other polyaromatic hydrocarbons, and we've discussed this earlier, I believe, when it moves into the cell in order to

cause the induction process, it has to have a way of starting the process, and just by itself in the cell it can't do that. It attaches to a receptor. I don't like the term recognizer, because that implies that this is so specific that it identifies each molecule that comes into the cell. The fact is that this receptor will bind a very large variety of substances. It happens that it binds TCDD more intensely than it does any other, and I think that's just an accident of the very flat structure of TCDD. It turns out to have provided us with an excellent tool, however, for studying this process of induction, and it has led to an understanding of the way that all of these complex chemicals cause induction. The two join and they form a complex between TCDD and the receptor, and that receptor moves into the nucleus of the cell. Now, this receptor is not out on the surface of the cell, it is free within the cytosol or the body of the cell. That's why they call it a cytosolic receptor. It is in the cytosol of the cell. And it is correct that it does move from the cell body into the nucleus of the cell. But it starts from in here. The TCDD comes into the cell and joins with the receptor to form this complex, and the complex then moves into the nucleus. There is a whole series of these, and I suppose that we should bring them all up one by one.

Q Let me hand you, sir, what's previously been marked as Plaintiff's Exhibit 254, 255, 256, and 257, and ask you if you have

seen those documents before.

A These represent the photographs that I have seen and apparently they are in the sequence that they originally appeared.

(Defendant's Exhibits 1292 to 1295 were marked for identification.)

Q Now, sir, would you compare these photographs by number with the Plaintiff's exhibit to be sure I've got them right? Defendant's Exhibit 1292 is a photograph of what, sir?

A Of Plaintiff's Exhibit 254.

Q All right.

A And this is the next step.

Q And Defendant's Exhibit 1293 is a copy of what, sir?

A Let's see, is a copy of Plaintiff's 255.

Q All right, sir. And Plaintiff's Exhibit 12--I'm sorry, Defendant's Exhibit 1294 is a copy of--

A Plaintiff's 256.

Q And Defendant's Exhibit 1295?

A Plaintiff's 257.

Q Now, would you like to go through these one at a time?

A Maybe in order to maintain sequence I will put this over on this easel. This complex does in fact go into the nucleus and it interacts with the genetic material in the nucleus. I don't know what really is meant by a critical target here, because what is interacted with is a segment of DNA, and this complex, I

presume when he says nuclear complex does not act as a message. The message, what it does it cause in some fashion, causes the DNA in a particular part of the cell, in a particular part of the genome to go through the process of opening up and making a copy of messenger RNA. Now, it is a fact, yes, that it does result in the making of messenger. We are talking here about a form of nucleic acid called RNA, and it is very similar to the DNA. Then the messengers, the messenger RNA goes to the structures in the cell that manufacture protein, the endoplasmic reticulum, and they provide instructions to make certain protein, and in a sense, I suppose we could say this is correct, except that as he has it depicted here and as he states it here, they are making new receptors. If I understand that, he means recognizer as representing the receptor or TCDD. That does not happen. What is made are the group of enzymes that we have discussed at various times, the P450 enzymes, the other non-P450 enzymes that we saw illustrated on that chart of Dr. Eisen's, I think yesterday, but we do not, the cell does not make additional receptors, they do not go to the outside lining of the cell or the cell wall. I am not sure what is meant, and they don't react with dioxin except that those are the very enzymes that are responsible for changing dioxin, metabolizing it so that it can be disposed of. They are not a new set of receptors that are here in the cell body which interact with TCDD. There is a clear limit to the number of those

receptors. There are a few compounds that are known to be able to cause the formation of additional receptors to a limited extent, TCDD is apparently not one of them. And these proteins that we are talking about that are formed do not go to the outside of the cell. They go--they are attached to this endoplasmic reticulum, where they are synthesized. They stay there and are held in place. These are what are known as membrane bound enzymes as differentiated from some of the enzymes that I mentioned on another occasion that are soluble that can actually leak out of the cell. These P450 enzymes can't leak out of the cell because they are stuck on larger structures that can't leave. Now, apparently Dr. Zahalsky believes that these TCDD, these new TCDD receptor complexes go back through the same cycle again, according to this diagram, but then they do something different. According to his description, they react and form reactive intermediates. There are some real problems with that because to begin with the metabolism of chemicals that might or might not take place and might proceed to "reactive intermediates" takes place out in the cell body on that endoplasmic reticulum. There are no such reactions taking place in the nucleus. The nucleus has other kinds of work to do. So somehow or other we have two cycles described here that start out exactly alike and then are presumed to end up differently with a different function in the second cycle. So this part of the

description is really completely incorrect. We don't have formation of new receptors. There is a great deal of binding of TCDD to the proteins in the liver cell, particularly the endoplasmic reticulum, but it is nonspecific. It is where most of the TCDD that is stored in the liver ends up and it is just stuck there, it is not really doing anything in particular, and it can be taken back out in a biochemistry lab so we can tell where it is. There is just a very small amount of receptor. Measurements that have been made indicate that no more than one percent, and probably much less than that, of the TCDD in the liver cell actually has bound to a receptor. The rest of it is all bound nonspecifically to other proteins. So we don't have reactive intermediates formed in the nucleus, and this complex between the receptor and dioxin has nothing to do with the metabolism of dioxin except as that receptor causes the formation of the enzymes that eventually can break dioxin or it can alter dioxin so that it can be disposed of.

The last chart says that now the reactive intermediates bind to critical targets. We know that TCDD and its metabolites do not bind to DNA. The implication here is that it has bound to DNA inside the nucleus of the cell. This has been demonstrated not to occur. It has also been demonstrated that we do not have a problem of reactive intermediates that are thrashing around in the cell, which is 3-methylcholanthrene or dimethyl bez anthracene

the reactive intermediates of those substances do a great deal of harm and they do bind anywhere they can find a point in their path. The implication is this is what results in toxicity, and we've discussed this I think sufficiently to show that there isn't a relationship between reactive intermediates and their binding to toxicity because we don't have reactive intermediates as part of the system. The implication is also that this initiates the process by which cancer cells, cancer is caused, starting with the initiation in the nucleus of the cell, and it also results in transformation. And I am not sure what the relationship of these two events is. We would have to concede that TCDD is capable of the promotion process which, depending on definition, may or may not include transformation. But it has nothing to do with the initiation process. So the representation that this scheme is a description of the way that TCDD causes cancer is, I think, erroneous, entirely erroneous. I don't know where it arose.

THE COURT: Gentlemen, could I see you at the bench for a minute, please?

(At this time an off-the-record conference was held at the bench.)

THE COURT: Ladies and gentlemen, we are going to break for the day at this time. We will resume again tomorrow morning at 9:30. I would remind you as I do for any overnight break that you are not to read, listen to, or watch anything about this case

in particular or subject matter in general in any of the media, print or electronic. Thank you for your attention and cooperation. Court is adjourned. We will start at 9:30 tomorrow morning.

(At this time Court was adjourned for the day.)

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

I, Patricia A. Gandy, CSR, RPR, Official Court Reporter in and for the Twentieth Judicial Circuit, and the Official Court Reporter who transcribed the above-styled cause had on November 5, 1985, do hereby certify that the foregoing transcript of proceedings is a true, correct and complete transcript of the proceedings had on said date.

DATED this 10th day of November, 1985.

Patricia A. Gandy

PATRICIA A. GANDY, CSR, RPR
Official Court Reporter

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

I, RICHARD P. GOLDENHERSH, Circuit Judge in and for the Twentieth Judicial Circuit, hereby certify that the above is a true and correct transcript of the proceedings had in the case captioned: FRANCES E. KEMNER, et al., v. MONSANTO COMPANY, Cause No. 80-L-970, heard on November 5, 1985.

DATED this _____ day of November, 1985.

ENTER:

RICHARD P. GOLDENHERSH, Circuit Judge