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CRITERIA TO BE CONSIDERED IN THE EVALUATION OF GENETIC TOXICOLOGY TEST DATA

Richard H. McKee

It is generally agreed that a battery of tests which assess a variety of endpoints is necessary for a thorough genetic toxicity evaluation. This approach assures that agents which induce a limited spectrum of effects will not be judged inactive on the basis of insufficient data. However, the use of a test battery also increases the likelihood that one or more of the tests will produce positive responses which are irreproducible, artifactual or are elaborated only under in vitro testing conditions and have no relevance to the in vivo condition. The probability of spurious or trivial findings increases not only as the number of tests performed increases but also as the criteria required to draw the conclusion that a response is positive are relaxed to "improve" assay sensitivity. This situation becomes increasingly uncomfortable when "non-negative" results from single short-term studies are regarded as sufficient to "trigger" chronic bioassay requirements for Test Rule compliance (under Section 4 of the Toxic Substances Control Act) (Fed. Reg., 50, pp. 20662-20676, May 17, 1985). Therefore, it seemed appropriate to outline the issues which should be considered in drawing conclusions from genetic toxicology batteries, and the weight which should be ascribed to certain types of test data. Howev r, this document was not intended to be a thorough review of genetic toxicology testing procedures or an attempt to define rigid decision criteria. Rather, this was intended simply to summarize the general principles which should be considered in the evaluation of genetic toxicology test data.

The issues to be discussed below include the criteria for assay validation, criteria for assay evaluation, proper use of decision rules, the relative importance of different genetic toxicology endpoints, and the need to consider all available data before reaching a decision to proceed to chronic testing. Specifically:

1. Each test protocol should define the minimal criteria which must be met for an assay to be considered valid. Data from assays which do not meet these minimal criteria should not be used in any decision-making process.

2. The scientific method requires that the assay evaluation criteria should be established prior to study initiation. In those cases in which alternative criteria may seem preferable, as for example by methodological advances or

because accumulated experience indicates that early suggestions are not supported, sufficient documentation should be provided to justify the change. Additionally, each assay may produce artifactual responses under certain conditions. Examples of these testing artifacts include apparent increases in mutant frequency under conditions of low cell survival (Green and Muriel, Mutat. Res., 38, 1976, pp. 3-32) or perturbations in cellular physiology which may be induced by changes in osmolarity or pH (Brusick, 1985 Environmental Mutagenesis Society). To the extent possible, these conditions should be documented in the experimental protocols and should be given consideration in the data evaluation process. Positive responses produced only under unusual or heroic conditions should not be seriously regarded.

3. Criteria which are commonly utilized to evaluate biological test data include reproducibility, statistical significance, and evidence of dose response. Of thes , reproducibility is the most important and the most generally applicable. If consistency cannot be obtained (i.e., a series of tests produce mixed responses which are weakly positive or negative), the assay should be considered equivocal and should not be used to support a decision to conduct chronic testing. Statistical tests for significance and for dose-responsiveness should also be utilized when appropriate. However, generally accepted statistical methods are not available for many of the commonly utilized *in vitro* tests. If a statistical model is utilized, the assumptions of the model must be satisfied by the study design. Additionally, the methodology should be well documented and generally available to the scientific community.

There has been a tendency, particularly with the *Salmonella* assay, to move from arbitrary decision criteria (i.e., 2-3 times the spontaneous mutation frequency) to statistically based methods of analysis. Several investigators have suggested models which consider both increased mutant frequency and dose-response relationships in assessing mutagenic activity (McCann *et al.*, Mutat. Res. 134, 1984, p. 37.; Snee and Irr, Mutat. Res. 128, 1984, pp. 115-125.). These methods seem reasonably conservative, and, in fact, appear to be reasonably consistent with the most commonly accepted arbitrary criteria. However, other investigators (e.g., Carnes *et al.*, Mutat. Res. 147, 1985, pp. 15-21) have suggested methods which consider only revertant yields. Such methods probably underestimate the inherent variability in the experimental methodology and should not be used as a sole positive response criterion. Finally, if statistical methods are used, one should remember that statistical significance does not prove biological relevance. The conclusion drawn from any study must be biologically plausible. There e, good scientific judgment must always be used in reach a final decision.

Finally, in most assays, one should expect to demonstrate dose-responsiveness. Mutagenic elevations at single dose points should be regarded as artifactual unless the response is both substantial and reproducible.

4. Broad decision rules such as those provided in some of the Gene-Tox reports should be used as guidelines but should not supercede the evaluation methodology provided by the testing laboratory. It has been amply demonstrated that each assay is subject to "interlaboratory" variation (DeSerres and Shelby, Environ. Mutag. 1, 1979; DeSerres and Ashby, Results of the International Collaborative Program). The historical data base and accumulated experience of the testing laboratory provid s the most relevant framework within which to judge assay response.

5. In the evaluation of short-term test data one should look for consistency among endpoints. If initial studies suggest that a test chemical is a point mutagen or a clastogen, then those properties should be examined further using other short-term tests which measure those properties. Chemicals which induce effects in one assay (i.e., Salmonella) but not in other assays which address the same endpoint (i.e., point mutational assays in mammalian cells) should be of little concern.

6. If consistent results are obtained from in vitro tests, then one should look to limited in vivo tests if possible to confirm that the genetic activity is also elaborated under more biologically relevant conditions. As one example, test materials which cause clastogenic effects in vitro should be tested under in vivo conditions in assays for chromosome aberrations or induction of micronuclei. Point mutagens may be more difficult to confirm in limited in vivo tests; the relevance of in vivo assays for sister chromatid exchange or unscheduled DNA synthesis to point mutation is not established and more direct assays (for example, mutation in peripheral lymphocytes) have not reached the status of routine testing. However, relevant in vivo tests should be used whenever possible to separate biologically important compounds from laboratory curiosities. In particular, compounds which produce positive responses in single tests or weakly positive responses in several tests should be examined by further in vitro testing or under limited in vivo testing before chronic testing is initiated.

7. One should remember that the short-term data are intended only for screening purposes. The data may provide suggestive evidence about the biological properties of test chemicals. However, all of the results of the short-term assays should be considered before one draws a

conclusion about the likelihood that genetic activity would be expressed under in vivo conditions. In the evaluation of short-term test data, a hierarchal approach should be taken to data interpretation. For example, greater reliance should be placed on tests performed in vivo than in vitro, on tests in mammalian species than submammalian species, and on tests in eukaryotes than prokaryotes. The need to consider all of the data is amply demonstrated by the interim results of an ongoing NTP program to assess the predictive value of in vitro test batteries. Seventy noncarcinogenic chemicals have been identified (Shelby and Stasiewicz, Environ. Mutag. 6, 871-878, 1984). Each of these chemicals is under test in a battery of assays including the Salmonella and mouse lymphoma tests for point mutations, in vitro chromosome aberrations, and in vitro sister chromatid exchange (a test battery quite similar to the EPA "generic" Section 4 mutagenicity scheme, NewBerg-Rinn, Environmental Mutagenesis Society Meeting, February 1983, San Antonio, TX). To date, 34 of these materials have produced positive results in one or more of the in vitro assays, and none of the remaining 36 have been tested in all 4 of the assays. These data emphasize the need to consider all of the short-term test results along with any other relevant data (such as metabolism and pharmacokinetic studies, evidence of pathology from subchronic studies, and results of studies of structurally related materials) before a chronic bioassay should be initiated.

of 70 non-carcinogenic compounds [redacted] have been [redacted] & shown that 34 are positive in one or more in vitro. Others have not been tested in all four series

TRANSCRIPT OF PROCEEDINGS

ENVIRONMENTAL PROTECTION AGENCY

PUBLIC HEARING ON PROPOSED TEST RULE)
FOR DIETHYLINE TRIAMINE)

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ENVIRONMENTAL PROTECTION AGENCY

PUBLIC HEARING ON

DIETHYLENETRIAMINE PROPOSED TEST RULE

December 3, 1985

Washington, D.C.

PRESENT AT THE HEARING WERE:

Richard Troast, EPA

Andrew Gordon, EPA,

Raymond Lodie, EPA,

Lettie Cahaun, EPA,

R. Bruce Dickson of Paul, Hastings, Janofsky & Walker,

Dr. David Brusick, Hazleton Biotechnologies,

Alan Rautio, SOCMA,

John Gray, DOW Chemical Company,

William Cornelius, DOW Chemical Company,

Tipson Tyler, Union Carbide,

Vincent Johnkoski, Union Carbide,

Ron Grandon, PTCN

(The EPA public hearing on Diethylenetriamine proposed test rule came to order, Richard Troast presiding.)

MR. TROAST: I would like to call the meeting this morning to order.

My name is Richard Troast. I am section chief in the Petroleum Development Branch, and it is my section that puts out the final proposed rules on Diethylenetriamine, which is the subject of today's meeting.

For the Agency on my left is Raymond Lodie, who is the project manager for this chemical, and to my right is Andrew Gordon from our Office of General Counsel.

I think that for the Diethylenetriamine Producers/Importers Alliance, Mr. Bruce Dickson is going to be their opening spokesman to introduce those members up here for the record.

MR. DICKSON: Yes, I will. My name is Bruce Dickson. I am with the law firm of Paul, Hastings, Janofsky & Walker, and I am representing the Diethylenetriamine Producers/Importers Alliance.

On my left is Alan Rautio, who is the executive director of the DPIA.

To my right, and addressing the scientific issues today, is David Brusick with Hazleton Biotechnologies.

Dr. Brusick will introduce himself in a moment and summarize his comments on the scientific aspect of the proposed rule.

Also with us today is Dr. Tip Tyler with Union Carbide; Bill Cornelius, Dow Chemical; John Gray with Dow Chemical; and Vince Johnkoski with Union Carbide as well.

Our principal reason for requesting the hearing here today is to address the automatic trigger proposal under which the Agency has proposed that if certain short-term tests produce positive results, then there will be a required two-year oncogenicity bioassay for Diethylene-triamine.

This causes serious concern to the DPIA for scientific reasons, because it is our view that the science does not support those automatic triggers that have been required and also because it is our view that the legal framework in which EPA must promulgate test rules is not consistent with this automatic trigger approach.

Dr. Brusick will address the scientific issues, and I think by the end of the morning it certainly will be our conclusion -- and we hope it will be the Agency's -- that the current state of the science, state of the art is unsettled.

There are a number of issues as to which

cientists in the scientific community seriously disagree, nd it is simply not appropriate at this point in time for he Agency to fix its position on specific trigger tests fter which the Agency will require oncogenicity bioassays.

We think that in many ways is a generic issue hat ought to be addressed in a much broader scientific ymposium under which the basic underlying policy issues by hich the Agency selects specific tests and the issue as to he use of automatic trigger mechanisms for the onset of ncogenicity testing also ought to be considered in such a eneric symposium.

We can begin then, and I would like to ask r. Brusick to introduce himself and to proceed with omments on the scientific issues.

MR. TROAST: If I can interrupt one moment. I ould like to introduce one more party from the Agency, and iat is Lettie Cahaun on the far right, and Lettie has been ecently named as the senior health scientist in Test Rules anch.

MS. CAHAUN: Sorry I'm late.

STATEMENT OF DAVID J. BRUSICK, Ph.D., HAZLETON BIOTECHNOLOGIES

DR. BRUSICK: First I would like to indicate iat if you have any questions and feel like interrupting me

at any time, please feel free to do so.

My name is David Brusick. I am currently vice president at Hazleton Biotechnologies Company, a subsidiary of Hazleton Laboratories.

In this position I am responsible for genetic toxocology and a few associated other types of safety testing programs.

I have been with Hazleton now for about two or three months. Prior to that time I was with Litton Industries and Litton Bionetics, again a subsidiary company of Litton Industries, and was responsible for most of that time for genetic toxocology, in a short period during that time for all the toxocology testing with Bionetics.

I joined Litton Bionetics in 1974, set up their genetic testing program, and during the past eleven years have devoted attention to developing tests, applying tests to chemicals, and interpreting data.

Over the course of the eleven years that I have been involved in this type of business, technology science, we have had the opportunity to look at over 9,000 chemicals in one or more of these type of test systems, and in some cases we have had an opportunity to know something about the other toxocology of these chemicals in addition to their

:ocology.

So, I think this offers a perspective on the
: of these tests in various situations with the
:emicals that a lot of people do not have.

I would like to provide some of my education
: learned in the course of those eleven years about
:k these tests can tell us and what they can't

One of the major problems or facets of this
:en not the application of the test chemicals
:rpret the results that have been derived from
:; and, in fact, over the past two and a half
:ve worked with a subcommittee of the
:al Commission for the Protection Against
:al Mutagens and Carcinogens to look at the
interpretation of tests.

In doing so, this has been in conjunction with
: other scientists, some of which are from
:rom the academic environment, and some from the
sector.

We have tried to look at the problem, and the
initial conclusion is that one has to first of
several types of tests to make a judgment of the

extrapolation or the predictiveness of these types of tests to whole animals or to humans and that in some way this data has to be integrated into a final judgment that any single test probably is not sufficient for the vast majority of chemicals. In the deliberations, there may very well be ways to look at this data and crunch it, if you will, into some sort of assessment tha oes have a very powerful predictive value.

So, that is something that's coming down the line, I believe, in the near future, and we may have more to say about that.

With respect to Diethylenetriamine and this test rule as well as other test rules, my main concern is that at the present time if we misuse the technology, which I think is basically very good and significant technology in short-term testing, there is a possibility that there may unnecessary application and cost involved in testing and also the bigger problem is the issuer of credibility if the tests are not used properly. There are a number of errors, inaccuracies made over the course of the early test rules that this could shed a bad light, so to speak, upon the use of these tests. That, I think, is a very big problem. So, that credibility could be shaken considerably through

misuse.

Now, the underlying premise that was mentioned is that the automatic trigger is scientifically unsound, and I think it is scientifically unsound primarily based upon the data we have at hand in that if you look at unique positives, you will find that very few chemicals which are carcinogenic in animals and/or humans but which we have concern of toxicological harm are identifiable by only a unique positive result in a short-term test. It just does not work out that way.

One good example is that Mike Waters of EPA has done a study of human carcinogens and a constructive profile of short-term test results. If you look at these profiles, you will see that all of the tests merge in the direction of positive. There are a few occasional negatives, but there is no situation where one has a carcinogen that works in humans and a single positive short-term test is the only one that registers with the other tests being negative.

On the other hand, research that has been supported by the National Toxocology Program and to some extent by EPA has shown very clearly that if you look at noncarcinogens, particularly those which are of the more common

type of chemicals in industry today, and look at individual short-term tests, you find many instances of unique positives where one test registers positive and a whole other series of tests are negative.

So, just looking at this data base that we currently have, it would argue against the use of a single positive being used as a trigger.

The automatic trigger in this case then could be viewed as something of a hair trigger in that it could result in going off in the wrong direction and actually harming the whole intent of what EPA is trying to accomplish with the development of predictive tests and using those to stimulate or make a decision whether to go into oncogenicity tests.

Why are we faced with a situation where the data does not support the use of the single positive test as a trigger? There are many reasons. I would like to cover four or five of those in some detail, because I think they are important to be put in the record and have an impact on the scientific application of this concept as it might be in policy.

First of all, the approach using a single test doesn't really take into account the impact that a given

test -- now in the case of each test rule, there may be a series of different tests. In this case, there is in vitro and cytogenetics, but each test has a certain susceptibility to false positives, and that is not taken into account in looking at the results of these tests.

That probably is more important when we are talking about in vitro tests. In vitro tests, although there are false positives, tend not to be as susceptible, and among the in vitro tests there are different levels of susceptibility.

I want to give you some examples of some recent information that I think will support this application and would tend to allow one to make a statement, which I possibly shouldn't make, and that is that almost any in vitro test can be shown to be positive with almost any chemical if the test is not applied properly, because although these tests have a certain level of susceptibility to false positives, one cannot tell always in advance whether this chemical is going to be in the category that will produce a false positive in this test or another test or in any tests at all.

But recently our laboratory and a number of other laboratories have begun to look at how tests are

performed, in vitro tests in particular, and what the test conditions, the treatment conditions, are, how those might affect the outcome of the test.

What we found is that unlike whole animals or intact organisms, even a bacteria or a whole animal which have evolved mechanisms to modulate their internal environment with respect to rapid and abrupt changes in the external environment, cultured animal cells cannot do this.

They have been taken from the animal, put in the culture, and although they are able to control the nutrient flow in and out and other types of chemicals, they have evolved within an organism that adjusts the homeostatic levels to a very series of complex relationships between organs and the circulatory system, horemones, and other things of that sort.

So, now they are put in a very strange environment in a sense, and if you change rapidly the external environment to these cells, it can have serious problems to the internal environment, which in turn can produce toxicological effects, particularly pH and ions, and we have found that for in vitro cell assays, cytogenetics being one, mutation being another, cell transformation,

they are susceptible to ion and pH effects, reduction of pH in cultures down to levels which are totally compatible with cell growth and proliferation in vitro and normally occur under the conditions of treatment with chemical pHs of between 5.5 and 6.5.

You can have significant increases in mutation, chromosome abberation, and transformation which does relate to high levels, and these in fact are real effects. These are pH levels not compatible with normal survival of an intact organ but are in vitro.

The same is true for ion effects, sodium ions, potassium ions, at levels that exceed the normal homeostatic level which would be about 300 milliosmals per kilogram. If you go up to 400 or higher, you begin to see the same thing, chromosome breakage, gene mutation, cell transformation, the same types of phenomenon.

These are independent of the chemical that is associated with. It presents a real problem in that the way the protocols are set up, the study designs for in vitro tests look for the maximum dose set at a level which produces a toxic effect or the maximum level of soluability if it is nontoxic.

So, let's assume we have a material that is

:toxic and happens to be made into a sodium or potassium
.e to increase soluability. A lot of pharmaceuticals
: other types of chemicals which are used, salt is
:ed to enhance the soluability of the material.

So, now we are faced with a situation that
.ows us to go very high in dose because of the soluability
the material due to the salt. That then presents a severe
)blem for the interpretation of the test results. So,
: study designs in a sense not necessarily encourage but
:ilitate the false positives that are generated.

These false positives then can occur in testing
chemicals. One doesn't necessarily know under the
ıditions that you are testing whether you are going to
)duce false positives or not, but we do know that based
)n looking at a wide range of material - sodium chloride,
:assium chloride, sucrose -- which are erally considered
be innocuous materials, that in most of these tests we can
)duce very good positive results.

That really brings into question then what does
)ositive result in any one of these tests mean or even a
:ple of these tests and should they be done alone or put
conjunction with other tests which may not be susceptible.

So, if you are using then a group of tests and

allow a single test to trigger the oncogenicity, you are setting yourself up for a situation where the test environment could cause a trigger totally unrelated to genotoxicity of the material.

So, I think it is an important concept, and using a battery of tests in which all of the data, both positive and negative, are conducted well to use and make that judgment becomes very important to avoid these false positives.

A weight of evidence, battery-type concept allows for this to be built into the evaluation scheme.

The second is that one of the nice or convenient aspects of using a test battery is that you build into the system duplicity of mutagenic or genotoxic mechanisms again across different types of cell systems, but you use different types of cell systems, several of which or at least two or three of which looking at the same genetic mechanism.

This again allows you to avoid a problem of an unusual or unique response due to the organism itself and not to the mechanism or the chemical.

For example, if one can look at several different types of point mutation assays built into the system and one responds but the others do not, that should tell you

something. It may not be a property of the chemical. It may be some sort of strange interaction between the chemical and that particular organism.

Again, that kind of mechanism, that is, multiplicity of mutagenic mechanisms, is built into schemes that employ a battery and weight of evidence but are devoid in a single trigger approach.

A third point is the ability to look at what I will call a consensus response. There is no doubt that given any test system and a chemical and the assumption being that you are going to do the test right on this compound -- and we are not dealing with a false positive problem that we can see in advance -- there is a certain probability that at any point when you do a set test there is going to be a positive result just on the basis of probability on a single trial.

That is not necessarily very high, but if you again are going to do three or four tests or five tests, there is a chance that one of those may come up for whatever reason with a positive result that cannot be reproduced.

I'm sure if you look at enough short-term data or any kind of toxicological data, whether it be in vitro or using whole animals, you will find situations where for

whatever reason the first time test showed an effect that is not reproduceable.

Again, in some of these tests the chances are that you are not going to go back and reproduce these tests several times to see this. But if you have a series of tests, in a sense you reproduce it. You have multiplicity or redundancy of the same kind of testing built into a battery.

It is very similar to the genetic mechanism, but this allows one to look at a redundancy or reproduceability of the tests and to come up with some sort of consensus, which again is not present if you are looking at a concept that says any positive is going to be viewed as a trigger.

So, these I think are somewhat related, but it is important to have that power in a test battery, the breadth and the depth, breadth across different types of tests and the depth in redundancy, built into the types of testing to avoid the possibility of calling a material or sending a material into an oncogenetic process on the basis of a false positive response.

Another issue is that of philogenetic development. I think that's really a very important issue in developing the test battery interpretation concept. One of the very

underlying principles that this subcommittee I'm talking about has approached has been two concepts. One is the importance of the genetic end point as it relates to disease induction, carcogenetic damage, inheritable genetic damage, and this is a concept that I think is very important.

Some genetic end points are more important for initiation, so to speak, of carcinogenesis than others. I do believe that there is a relationship between the genetic event and the initiation process of carcinogenesis.

A few years ago this seemed to be something we woud say, well, we have it. We have mutation and carcinogenesis linked together by this initiation process. That is true, but as we learn more about oncogenes and how they operate, it appears that initiation is one step and that there are other very important steps.

So, just mutation or just genotoxic effects alone are not of sufficient weight to say that that is going to provide the one critical step in determining a carcinogen.

As I was getting at, the various types of end points. We know that mutation -- we look at oncogenes and how they are activated and how they initiate tumor development, respond to chromosomal abberations and gene mutation. Other types of end points, DNA repair

stimulation, and so on have different weight because those are not contributing 100 percent or directly to the process that transforms a cell into a malignant cell.

So, there is a very easy way of stratifying genetic end points from those which just interact with DNA to those which actually produce chromosomal abberation and gene mutation.

You have different steps along the way. So, you can stratify tests according to what end points these tests measure.

The other critical factor is what is the relationship of that cell type; how important is it from a predictive standpoint into an intact organism?

So, you also need to stratify on a philogenetic complexity basis. As you go up in complexity, the interpretation or the response that is obtained becomes more predictive.

These two factors together provide I think an inportant basis in evaluating these test data that an automatic trigger does not give. It does not take into account what is the philogenetic level of that positive which we have with respect to all of the other tests that were conducted that may not have shown a response.

If we have an in vitro test that is positive and a well conducted in vivo study of the same type of end points and both of them are highly weighted end points, the in vivo is negative, clearly negative, in vitro is positive, that needs to be considered because if you look at the predictive, that is, the positive result in a short-term test, how often that is going to be a carcinogen, in vivo positives much more often than in vitro positives are going to be true predictives for animal studies.

So, philogenetic relationships in these tests become very, very critical in the interpretation.

So, now we have gone down the line and looked at the false positive issues, redundancy, reproduceability, a consensus of response in genetic mechanisms, and also philogenetic relationships and how those impact on the interpretation of the tests.

I think that all of this is extremely important in the use of these tests in making a judgment of whether this is likely to be oncogenetic.

If one wants to get the most out of this type of technology, I think those factors have to be taken into account, because it is too easy for a single positive to be due to factors which have nothing to do with the chemical.

They have all to do with the selection of the wrong test, selection of the wrong test conditions, and that without looking at the other data come to the wrong decision, apply a policy, and that policy could then eventually reflect back on the use of this type of test situation.which then can seriously jeopardize credibility of these tests.

I think that is not good for anybody involved in the process.

The next issue is what can be done as an alternative. I think first of all EPA has invested and is investing considerably in the gene tox program. The gene tox program is partially complete but not entirely complete. It will be ongoing, as far as I know, and a continual update of the data that goes into the data bank.

But one of the critical problems or critical portions of the gene tox program was an assessment of what the data that has already been generated out of the first phase tells us. There are a number of papers that are being prepared that relate to test batteries, test battery construction, chemical specitiv test batteries, whether these are possible or not possible, the oncogenetic performance or predictiveness of these various tests either alone or in groups.

All of this information is coming out, and although it is somewhat delayed with respect to when it was initially planned to be available, it will be coming out, and I think that it would be at this point not wise to preempt what gene tox is going to be able to tell us.

Again, it would be good to wait and see what the information produced and use that information in forming policy for test rule production.

There are, as I mentioned, efforts, very serious and intense efforts, in developing schemes for evaluating multi-test data. It is the only way in my opinion we are going to be able to use the information.

I think that after eleven years in this business, the one thing I can say with a lot of confidence is that you cannot use any single test that will work for all chemicals under all circumstances with any kind of high degree of reliability. You have to use a group of tests and in some way take all that data into consideration.

When one does that, these tests become extremely powerful and the predictive value goes up considerably. They will never be perfect, I don't believe, but they will be considerably improved in their predictive value and reasonableness if they are used in that way.

So, I don't think at this point it is good to try to jump too far ahead of where the science is. The science is moving along, and I think there is a tremendous amount of support within the scientific communith now. That was not true two or three years ago, but now I believe there is a tremendous amoutn of support within the scientific community for the use of systematic evaluation test data in some sort of a system, whether it be the one that the committee I'm dealing with is using or others which are being developed, to evaluate data, but I think these have to be viewed and looked at.

I think that in conclusion, that the use of short-term tests as a trigger for oncogenicity can be substantially more cost effective by the Agency, certainly can be more reliable, and it will be more consistent with current trends in data assessment if a weight of evidence greater than a single positive test is used as a trigger for oncogenicity.

Thank you.

STATEMENT OF BRUCE DICKSON

MR. DICKSON: I would like to bring to focus at this point the proposed rule that's sitting before the Agency and to apply what we have talked about already this

morning to that specific rule.

As I understand it, the record before you contains the following data: It contains negative gene mutation data in bacteria. It contains negative gene mutation data in yeast. It contains negative gene mutation data in CHO cells in culture, and it contains negative dermal carcinogenicity data.

It also contains questionable results which the Agency has interpreted as positive -- but by no means is that without dispute -- in a sister-chromatid exchange assays and in an unscheduled DNA synthesis assay in mammalian cells.

By issuing the proposal that it has issued, the Agency has implicitly conceded that this current data base is insufficient to support an oncogenicity test rule for Diethylenetriamine. It is a proposed test rule based not upon the evidence in this record but based instead upon a hyphothesis; the hypothesis that at some point in the future there may be evidence from one of these three short-term assays that are being performed which may be positive.

In other words, the record before the Agency does not support a test rule, but the Agency is proposing to issue a final test rule based upon hypothesis or

supposition regarding future data in some future rule-making record.

Even if the law did permit findings based on hypothetical evidence of that sort, as Dr. Brusick explained, the Agency's hypothesis at this point is not in accord with the current state of the science.

The single positive hair trigger approach that Dr. Brusick referred to is not supported by data showing, as the Agency has suggested it shows, that there is a high correlation between the results of these assays and oncogenicity. That high correlation has not been shared with us. At least it does not appear on the record.

We understand this is one of the subjects being addressed currently by the Gene-Tox Program, so presumably there is data within the Agency, but if there is data showing a high correlation for each of these three assays, we have not seen it. It isn't on this record.

More importantly perhaps is consequences of misuse of these sort of short-term assays. We know that the Agency is going through a process of considering, a policy decision process, in terms of determining what the best use is of these short-term assays, and there certainly is very good use to be made of short-term testing. We are concerned

that by misusing data in the way that is proposed in this rule making, the Agench would adversely affect the ultimate use of those short-term tests.

Beyond that, though, our reading of the statutory framework in which the Agency is bound to promulgate rules tells us that the Agency should not be proceeding in the way it is.

The Toxic Substance Control Act requires that before an agency can issue a test rule, it must find that the activities with respect to this particular chemical may present an underlying risk. TSCA also requires that an agency's findings on this point have to be submitted with substantial evidence in the rule-making record taken as a whole.

We do not see that substantial evidence in this rule-making record taken as a whole. In fact, we find that the hair trigger approach violates this requirement by providing that all future negative data will not be considered, will be ignored, while a single positive will be determinative.

That absolute approach we think violates the provision of TSCA. We also think that the legislative history of TSCA makes it very clear that testing may only be

required under Section 4(a)(1)(A), which we have discussed today, for chemicals -- and I quote the legislative history -- "about which there is a basis for concern." Note the present tense "is". It does not provide that testing may be required for chemicals about which there may be a basis of concern at some point in the future when test data are completed.

We also see that the Administrative Procedure Act requires notice and opportunity to comment. In explaining what is meant by adequate notice, courts have said very clearly that the data, the information on which an agency bases its findings, must be available during the rule-making process to enable review and comment.

It does not provide that a final rule can be issued today based upon evidence that will become available at some point in the future but only evidence on which the industry or other interested parties will not have an opportunity to comment.

The cases are very clear on this. The Portland Cement Association against Rucklehaus case very clearly says that it cannot be done, that an agency cannot base regulations on data which is known only to the agency.

The case of United States against Nova Scotia

Food products very clearly says -- and the language on this is particularly pertinent -- "Unless the scientific data relied upon by the agency are spread upon the public records, criticism of the methodology used or the meaning to be inferred from the data is rendered impossible."

It seems obvious to us that if the data themselves have not yet been generated, interested parties cannot possibly comment on the significance to be attached to those data.

Yet by proposing that a final rule is issued contingent upon a positive result in a future test, a test to be conducted, the Agency is precluding an opportunity for commenting on the significance of those data.

You also ought to consider the Gulf South case, Guls South Insulation against CPSC. This was a case in which the Fifth Circuit ruled under a very similar statutory constraint that the agency had to consider facts of record that detract from its position as well as those it supported. The agency has to consider negative data as well as positive data before it can make a decision.

Yet, by issuing this rule, the Agency is saying it will not consider negative data but at the point of time in the future at which the positive data become available.

Finally, this proposed rule we think violates the due process caluse of the Constitution. The Supreme Court has repeatedly held that regulations based on an irrebuttable presumption of fact which do not afford affected parties an opportunity to comment or to demonstrate that the presumption is wrong violate the Constitution.

By issuing a decision today that a positive in the future will trigger an oncogenicity test requirement, the Agency is saying the industry will not have an opportunity to rebut that presumption, the presumption that a positive in a Drosophila test will lead to the finding that the substance may present an unreasonable risk of oncogenicity.

That presumption the industry and other affected parties cannot rebut under the scheme that is being proposed.

So, to summarize very briefly the points we made here and what we hope will be taken into consideration: There are currently insufficient data to support a test rule requirement. I think the Agency has conceded that. The Agency is relying instead upon theories based upon their view of the state of the art, the state of the science.

Yet, as Dr. Brusick has said, the state of the art is not yet sufficiently developed to allow these tests

to be used as hair triggers. EPA's own Gene-Tox Program has not yet reached the point of issuing its recommendation in this regard.

We understand that a symposium of some sort will be held this spring, within the next few months, to address these tier testing issues, and we urge that that take place. We also request that the rule-making record in this proceeding remain open so that the results of that symposium can be put on the record, can be considered by the Agency before a final decision is made in the case.

We also intend to submit supplemental written comments as a follow-up to the comments we have made and as a follow-up to Dr. Brusick's comments, and we request that the record remain open to receive those.

Finally, we urge that this proposed rule be abandoned. We urge that the results of the scientific discourse that is to take place within the next few months including the results of the Gene-Tox Program be considered when the Agency formulates its approach to tier testing, its approach to trigger tests.

We urge that the rule in this matter, if one is to be proposed, be reformulated and reproposed once the Agency has taken into account the views of the Gene-Tox

Program and the views of outside experts.

We will be happy to answer any questions that anyone has on this.

MR. TROAST: I don't know if anyone from the Agency has any questions. I think your points are very well spoken and give the Agency a lot of questions and though that need to be looked into before we can move forward.

As far as the symposium, we will have some sort of public announcement prior to this, and while it appears that as we have discussed in other times, there will be industry participation. Your points raised here will certainly be part of the program that should be considered.

MR. DICKSON: I think that would be very useful. It is of obvious relevance to this rule making. For that reason, it is very important that the views expressed in that hearing and any results of that symposium be put on this rule-making record. We really do feel that is important.

This is an issue which is in a state of flux. Positions are changing, even positions of the Agency are changing, and we feel to close off this rule-making record is a serious mistake.

MS. CAHAUN: I would like to thank you all for coming and keep in mind that there are major policy

we are going to have to consider. The
vant to get into is having people
; for bioassays, for instance.
ıld encourage you to keep your comments
ɔpen, and we will try to consider
s and the position and changing state of
things.

SON: Do you have a view for the timing

ST: As I understand it, Charles
the country until next week or the week
ts back and focuses on this effort, I
it. I know we are looking toward the
generally all I know. Just trying to
now is probably going to be the hardest
ing a time and then we will work towards

had a better answer to give you, but

SON: Can we expect that this record will
ɔugh to take into account the views
ymposium?

ST: I would expect so. I certainly

REPORTER'S CERTIFICATE

[redacted]

CASE TITLE: EPA HEARING ON DIETYYLENETRIAMINE PROPOSED TEST RUL

HEARING DATE: DECEMBER 3, 1985

LOCATION: WASHINGTON, D.C.

I hereby certify that the proceedings and evidence herein are contained fully and accurately on the tapes and notes reported by me at the hearing in the above case before the Environmental Protection Agency
and that this is a true and correct transcript of the case.

Date: December 13, 1985

Brenda Snowden

Official Reporter
ACME REPORTING COMPANY, INC.
1220 L Street, N.W.
Washington, D. C. 20005

DPIA
DIETHYLENETRIAMINE PRODUCERS/IMPORTERS ALLIANCE
1330 CONNECTICUT AVENUE N.W., #300, WASHINGTON, D.C. 20036-1702 • (202) 659-0060

BEFORE THE
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF TOXIC SUBSTANCES

Diethylenetriamine; Proposed Test Rule	OPTS-42012A

POST-HEARING COMMENTS OF THE DIETHYLENETRIAMINE PRODUCERS/IMPORTERS ALLIANCE

William E. Cornelius
Chairman
Diethylenetriamine Producers/
Importers Alliance

Of Counsel:

R. Bruce Dickson
Michael A. Wiegard

PAUL, HASTINGS, JANOFSKY & WALKER
Twelfth Floor
1050 Connecticut Avenue, N.W.
Washington, D.C. 20036
(202) 223-9000

February 11, 1986

BEFORE THE
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF TOXIC SUBSTANCES

Diethylenetriamine; Proposed Test Rule	)	OPTS-42012A

POST-HEARING COMMENTS
of the
DIETHYLENETRIAMINE PRODUCERS/IMPORTERS ALLIANCE

On December 3, 1985, a public hearing was held before the Environmental Protection Agency's Office of Toxic Substances ("OTS") on a proposed test rule for diethylenetriamine ("DETA"), 50 Fed. Reg. 21413 (May 23, 1985). The Diethylenetriamine Producers/Importers Alliance ("DPIA"), an association of producers and importers of DETA organized as a special project of the Synthetic Organic Chemical Manufacturers Association, discussed at the public hearing the substantial scientific and legal issues raised by the proposed rule. Counsel for DPIA stated that supplemental written comments would be submitted for the record.

DPIA hereby provides its supplemental written comments on the proposed test rule. These comments set forth in more detail for the record facts and arguments presented orally at the public hearing and supplement the comments filed by DPIA on July 22, 1985. The EPA officer presiding at the hearing indicated that the record would remain open to receive these supplemental comments.

Background

On April 29, 1982, EPA issued a proposed test rule for DETA under Section 4(a)(1)(A) of the Toxic Substances Control Act ("TSCA"). 15 U.S.C. § 2603(a)(1)(A). The proposal differed from the recommendations of the TSCA Interagency Testing Committee (ITC) regarding the need for oncogenicity testing. Although the ITC had recommended such testing, EPA concluded that "[t]he available data provided no sound basis for suspicion of DETA's ability to cause oncogenic...effects." 50 Fed. Reg. 21399.

In the absence of any data suggesting that DETA may cause oncogenic effects, the Agency in May 1985 reversed its earlier conclusion and issued a proposed rule to require oncogenic effects testing. The only explanation given for the reversal -- issued as it was at the same time as the final test rule was issued for other health effects and over three years after the original proposed rule was issued -- was that EPA had adopted the "general approach" of requiring tiered testing sequences which "usually include an automatic requirement for chronic oncogenicity bioassays" of substances exhibiting a positive response in one of several short-term tests. 50 Fed. Reg. 21413.

The current DETA proposal, if finalized, would require oncogenicity bioassays of DETA in both rats and mice if DETA exhibits positive test results in any one of the following short-term assays:

-the sex linked recessive lethal gene mutation assay in Drosophila melanogaster;

-the *in vitro* cytogenetics assay;

-the *in vivo* cytogenetics assay.

DPIA opposes the proposed rule as an unprecedented effort to promulgate a test rule on the basis of no data other than possible future short-term results. The rulemaking record does not contain sound scientific data to satisfy the substantial evidence requirement. Moreover, the proposal violates the legal standards governing the issuance of test rules and therefore should be withdrawn.

I. The DETA Proposal is an Unprecedented Effort to Promulgate a Test Rule Under Section 4(a)(1)(A) Solely on the Basis of Hypothetical Data

The proposal appears at first glance to be consistent in outcome with the "general approach" taken by the Agency with several other substances. However, upon closer scrutiny the DETA proposal represents a significant extension of that "general approach" far beyond the parameters of past rulemakings.

A review of the other test rules cited by the Agency as examples of the automatic trigger test scheme reveals that, while they contain many of the same scientific and legal flaws as the DETA proposal, there are substantial differences which make this proposed rule even more objectionable.

The "general approach" which the Agency cites was put forward in proposed test rules for ethyltoluenes, trimethylbenzenes and the C9 aromatic hydrocarbon fraction (48 *Fed. Reg.* 23088 (May 23, 1983)), mesityl oxide (48 *Fed. Reg.* 30699 (July 5,

1983)) and cresols (48 Fed. Reg. 31812 (July 11, 1983)). Two final rules have been published encompassing a similar automatic trigger test requirement -- the C9 aromatic hydrocarbon fraction (50 Fed. Reg. 20662 (May 17, 1985)) and mesityl oxide (50 Fed. Reg. 51857 (December 20, 1985)).

The C9 aromatic hydrocarbon fraction test rule was issued under TSCA Section 4(a)(1)(B), based upon a finding of substantial exposure. Therefore, unlike the DETA proposal, the Agency did not make a finding (required under Section 4(a)(1)(A), but not under Section 4(a)(1)(B)) that the C9 fraction may present an unreasonable risk of oncogenic effects.

The cresols proposed test rule was also based upon Section 4(a)(1)(B) findings, although the mutagenicity and oncogenicity testing requirements were proposed to be based additionally on Section 4(a)(1)(A). The data base upon which the proposal was premised did contain positive results for a cresol mixture in some of the same tests as were proposed as triggers. Thus, there were in existence at the time of the proposed rule for cresols data showing a positive response in trigger tests.

As explained in the final test rule for mesityl oxide (50 Fed. Reg. 51861), EPA has taken the position that when Section 4(a)(1)(B) findings are made,

> "there is a presumption that testing of the substance for oncogenicity is needed, and the question before the Agency is whether the weight-of-evidence from the mutagenicity testing shows an absence of oncogenic potential such that EPA can reasonably predict that the expected exposures to the substance

> will not present an unreasonable risk of oncogenicity. In contrast, where testing is being required under section 4(a)(1)(A) alone, EPA must consider whether all of the relevant data available to the Agency after completion of the required mutagenicity tests provide evidence that the substance may present an unreasonable risk of oncogenicity."

Unlike the C9 fraction and cresols, the mesityl oxide rule was based upon Section 4(a)(1)(A) alone. As explained by EPA, the finding of potential unreasonable risk of oncogenic effects for mesityl oxide was based not only upon the hypothetical mutagenicity test results, but also upon structure-activity relationships. 50 Fed. Reg. 51863.

The DETA proposal differs from all of these earlier test rules. Because the proposed rule is based upon Section 4(a)(1)(A) findings, the "presumption" made for the C9 fraction cannot be made for DETA. Moreover, unlike cresols, the record does not already contain positive gene mutation data. Unlike mesityl oxide, the Agency has not found DETA to be a potential oncogen on the basis of structure-activity relationships. On the contrary, the DETA record contains two negative dermal bioassays with DETA,[1/] studies showing similar biologic activity with DETA and ethylenediamine (EDA) and another study showing no oncogenic activity for EDA, a lower molecular weight structural analogue of

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1/ It is noteworthy that the Agency has not placed more weight on the negative dermal bioassay results, in view of its having stated that "the Agency's major exposure concern is via the dermal route." 47 Fed. Reg. 18386, 18389 (April 29, 1982).

DETA. See 50 Fed. Reg. 21400. Thus, if the Agency considers structure-activity relationships for DETA, as it purported to do in making its conditional findings for mesityl oxide, and the negative oncogenicity data for DETA, it would be unable to find that the existing rulemaking record, taken as a whole, supports a "may present" finding. In the absence of the hypothetical data that might possibly be generated in the short-term assays to be performed with DETA, the record does not satisfy the substantial evidence standard mandated by TSCA.

Thus, the question squarely and uniquely presented by the DETA proposal is whether an oncogenicity test rule under Section 4(a)(1)(A) may be based solely upon hypothetical future test results in the three short-term assays listed above. DPIA believes that the test rule cannot properly be issued on this basis, because of the lack of scientific justification and because of the substantial legal objections -- as discussed at the public hearing.

II. The Proposed Rule Is Scientifically Unsound

A. The rulemaking record contains no evidence to support EPA's proposed "may present" finding.

The DETA proposal is unique in that it was issued solely on the basis of EPA's current policy regarding tier testing. Indeed, the proposal was apparently issued as an afterthought, following an earlier Agency decision not to propose an oncogenicity test rule. EPA conceded in its original proposal

and again in the final test rule for DETA that the "available data provided no sound basis for suspicion of DETA's ability to cause oncogenic or age-related effects." 50 Fed. Reg. 21399; see 47 Fed. Reg. 18386 (April 28, 1982).

Moreover, data received since the original proposal was issued further demonstrate that there is no basis for a "may present" finding regarding oncogenicity. As noted above, the data submitted include two negative dermal carcinogenicity studies, pharmacokinetics and metabolism studies and data regarding structural and biologic activity relationships between DETA and ethylenediamine. In addition, negative gene mutation data using bacteria, yeast cells and Chinese hamster ovary cells were submitted. Questionable results obtained in a sister-chromatid exchange assay and in an unscheduled DNA synthesis assay in mammalian cells were also presented to EPA. EPA implicitly concedes in the preamble to the proposed rule that the current data do not provide substantial evidence upon which to premise an oncogenicity test rule. See 50 Fed. Reg. 21414.

B. The hypothesis that a single "positive" short term assay will justify a "may present" finding is scientifically unsound.

The proposed test rule in fact is not based upon evidence in this record. It is instead premised upon the hypothesis that if at some point in the future one of the required short-term tests produces "positive" data, the record will then support a "may present" finding. The preamble states

that EPA "believes" that positive results in such short-term tests show a "strong correlation" with oncogenicity in animal bioassays, but there is no evidence in the record to validate such a correlation.

EPA refers to the C9 aromatic hydrocarbon rulemaking to justify its belief of a strong correlation. There the Agency cited the percentage of carcinogens which tested positive in the short-term tests being used as triggers. (See 50 Fed. Reg. at 20672.) The Agency stated that 88.2 percent (67 of 76) of the known carcinogens tested were positive in the Drosophila sex-linked recessive lethal assay. Of a total of ten known carcinogens tested in the in vivo cytogenetics assay, nine (90 percent) were positive. The in vitro cytogenetics assay has produced positive results for 17 of 22 known carcinogens tested (77.3 percent). Id.

The Agency fails to recognize that these data tell nothing about the utility of the individual assays for predicting whether a substance is likely to be a carcinogen. Positive results produced with known carcinogens are irrelevant if an assay also produces positive results with non-carcinogens. EPA has presented no data regarding the specificity of these assays, i.e., their ability to detect chemicals not likely to be carcinogens. Most "correlation" data have been generated in tests with known carcinogens. If the in vitro cytogenetics assay is considered, one review noted that 49 out of 54 carcinogens were positive. However, when 136 chemicals which tested positive in

this assay were examined, only 54 were shown to be carcinogens. Thus a chemical with a positive short term assay has less than a 50 percent chance of being a carcinogen.[2/] The "correlation" data cited by the agency are clearly inadequate to support the finding that a single positive result with DETA will constitute evidence that DETA may present a risk of oncogenicity.

As Dr. David Brusick of Hazelton Biotechnologies Company pointed out at the public hearing, *in vitro* tests are particularly subject to false positives caused by the treatment environment used in exposing the chemical to the target cells. Variations in ion concentration and pH from normal homeostatic levels, for example, can produce chromosome breakage, gene mutation and cell transformation. Such "positive" results would, of course, have no direct bearing upon the question of a possible carcinogenic effect, *in vivo*. Moreover, the data base for using the *in vivo* cytogenetics assay as an automatic trigger test is limited in scope, based as it is on results with only ten known carcinogens.

Dr. Brusick also pointed out that organism-specific responses may occur and are difficult to intepret and extrapolate since they may represent a unique activity of an agent in a given

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2/ Ishidate, M., Jr., Sofuni, T. and Yashikawa, K., "Chromosomal aberration tests in vitro as a primary screen tool for environmental mutagens and/or carcinogens," in "Mutation, Promotion and Transformation in Vitro," N. Inui, *et al.*, eds. *Gann Monograph on Cancer Research*, 27:95, 1981.

organism. For example, nitrosamenes are typically much more active in Salmonella than in animals.

An assessment of short-term results intended to predict the likelihood of carcinogenicity must take into account the mechanistic relationship between the genetic endpoint being considered and oncogenicity. It must also consider the relevance of the particular cell system being used to determine oncogenicity.

EPA has cited no scientific justification for the reliance on a single positive result in the presence of several negative studies, a practice which is inconsistent with the requirements of TSCA described below. In fact in the recent IPCS collaborative study, several chemicals that were positive in a mammalian bioassay, but negative in the Ames test, were tested in several short-term tests.3/ None of the carcinogens were positive in only a single test. The consensus in the scientific community appears to be in favor of the use of a battery of assays, the results of which are all considered in the evaluation, regardless of their outcome.4/

3/ Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr., Margolin, B., Matter, B. and Shelby, M., "Overview and conclusions of the IPCS collaborative study on in vitro assay systems," in "Evaluation of Short-Term Tests for Carcinogens," Ashby, J. et al., eds., Progress in Mutation Research, Vol. 5:117, 1985.

4/ See, Grice, H.C. (ed.), "The use of short-term tests for mutagenicity and carcinogenicity in chemical hazard evaluation," Current Issues in Toxicology, Springer Verlag, New York, 1984, p. 187.

Agency representatives indicated at the public hearing that a symposium on tier testing sequences and on trigger testing (Continued)

Dr. Brusick advised that a battery of assays should be used to determine whether there is a consensus response. With a clear consensus response one can make accurate predictions regarding oncogenicity. A single positive simply cannot be used as a "hair trigger" with any reasonable expectation of predictability.

Thus the DETA proposal is premised upon a factual record noteworthy for its total lack of data showing that DETA may present an unreasonable risk of oncogenic effects and containing nothing more than the highly speculative theory that a future record containing data from one or more short term tests might support a Section 4(a)(1)(A) finding. As discussed in Dr. Brusick's testimony, even if a positive test result is placed in the record in the future, the available data simply do not substantiate the claim that a single positive result is evidence that a substance may present a risk of oncogenicity.

III. The Proposed Rule is Legally Unsound

Even if there were a sufficient correlation, supported by a substantial body of data from carcinogens and non-carcinogens, to allow the Agency to use positive short term results as substantial evidence of potential oncogenicity, the

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would be held within the next few months. DPIA urges the Agency to hold such a symposium so that test rules, including the DETA proposal, will have the benefit of the current state of scientific thinking in this area. The rulemaking record for this proposal should remain open to include the results of such a symposium.

proposal to premise a test rule on hypothetical data is legally unsound. It violates the clear provisions of TSCA, as well as the Administrative Procedure Act and the United States Constitution.

A. The Language and Legislative History of the Toxic Substances Control Act Make Clear That Findings Under Section 4(a)(1)(A) Must Be Supported by Substantial Evidence in the Rulemaking Record.

TSCA and its legislative history clearly provide that test rules must be based upon evidence presently found in the record. Hypothetical future data may not be used as the basis for a rule.

As noted above, EPA issued the proposed rule under Section 4(a)(1)(A) of TSCA, 15 U.S.C. § 2603(a)(1)(A). Under this provision, the Agency must first find that

> "the manufacture, distribution in commerce, processing, use, or disposal of a chemical substance or mixture, or that any combination of such activities, may present an unreasonable risk of injury to health or the environment . . ."

15 U.S.C. § 2603(a)(1)(A) (emphasis added). EPA has acknowledged that TSCA requires substantial evidence of a potential risk of oncogenicity before it can lawfully order oncogenicity testing. 50 Fed. Reg. at 21415 (May 17, 1985).

The judicial review provision of TSCA specifically states:

> "[T]he court shall hold unlawful and set aside such rule if the court finds that the rule is not supported by substantial evidence in the rulemaking record . . . taken as a whole." Section 19(c)(1)(B) (emphasis added).

The legislative history of TSCA confirms that testing may be required under section 4(a)(1)(A) only for chemicals "about which there is a basis for concern," H.R. Cong. Rep. No. 1679, 94th Cong. 2d Sess. 61 (1976) (emphasis added). EPA's findings must be based on scientific evidence, not on "mere conjecture or speculation." H.R. Rep. No. 1341, 94th Cong. 2d Sess. 18 (1976).

The legislative history indicates that Congress purposefully chose the "substantial evidence" standard of review for Section 4(a) test rules to ensure "a searching review of the Administrator's reasons and explanations for the Administrator's conclusions." H.R. Rep. 1341, 94th Cong. 2d Sess. 55-56 (1976). More specifically, the conferees expressly stated their intent that the reviewing court "focus on the rulemaking record to see if the Administrator's action is supported by that record." H.R. Rep. No. 1679, 94th Cong. 2d Sess. 96 (1976).

Thus, it is clear that Congress intended that the Agency justify rules promulgated under TSCA on the basis of existing evidence in the rulemaking record. A test rule cannot be based on hypotheses or prospective test results where the requisite substantial evidence of potential unreasonable risk does not now appear in the rulemaking record. The DETA proposal is in direct

violation of these requirements in that it is based upon data that will not exist until long after the final rule is issued.

It also is not in compliance with the requirement that the record taken as a whole be considered. By refusing to consider any negative data that may be generated, the Agency is basing its test requirement on a single element of the rulemaking record to the exclusion of everything else in that record. Such an approach was specifically rejected in TSCA.

B. The Courts Have Rejected Efforts to Regulate without Regard for the Evidentiary Record.

Under similar statutory provisions, the courts have held that an agency cannot act without considering data that are inconsistent with its position. In Gulf South Insulation v. United States Consumer Product Safety Commission, 701 F.2d 1137 (5th Cir. 1983), the court construed and applied provisions of the Consumer Product Safety Act ("CPSA") which, like TSCA, requires that agency action be supported by substantial evidence regarding an unreasonable risk of injury. The court in Gulf South vacated an agency rule after concluding that it was not supported with the requisite substantial evidence. The court noted that under the substantial evidence requirement, the agency must consider facts in the record that detract from the agency's position, as well as those that support it. Id. at 1142-43.

It is clear that in this case EPA has failed to comply with the substantial evidence requirement. By relying upon a

single positive result, the Agency has simply dismissed in advance all evidence which does not support the proposed rule.

Essentially, EPA is shifting the burden to the industry to prove that DETA is not a carcinogen. It is taking an approach to Section 4 testing that is similar to the approach that was taken by OSHA in promulgating the benzene standard reviewed by the Supreme Court in Industrial Union Department v. American Petroleum Institute, 448 U.S. 607 (1980). There, OSHA had assumed that a positive result in a carcinogenicity bioassay was substantial evidence of a significant risk. It refused to evaluate that evidence beyond a determination that the bioassay was positive. It left industry with the burden of proving that the risk was not significant. This approach was rejected by the Supreme Court, which said:

> "[T]he burden was on the Agency to show, on the basis of substantial evidence, that it is at least more likely than not that . . . exposure . . . presents a significant risk. . . ."

Similarly, the burden here is on EPA to show, on the basis of substantial evidence in the rulemaking record taken as a whole, that "it is at least more likely than not" that DETA may present an unreasonable risk. The Agency is ignoring this basic precept of the law. It is proposing to regulate on the basis of a single positive, regardless of the number of negatives. As noted above, the state of the science is such that EPA cannot

presume that a single positive is evidence that DETA may present an unreasonable risk.

In short, the proposed rule is not supported by evidence in the record and therefore violates the basic statutory standards by which test rules are to be issued.

C. The "Automatic Trigger" Provision Denies Interested Persons Meaningful Notice and Opportunity to Comment Upon The Proposed Test Rule.

1. TSCA and the Administrative Procedure Act require that interested persons receive notice and an opportunity to comment on proposed rules.

The Administrative Procedure Act provides that federal agencies shall give interested persons notice and an opportunity to submit "written data, views, or arguments" in all rulemaking proceedings. 5 U.S.C. § 553(c). This requirement is augmented by the TSCA requirement that interested persons also be given an opportunity for the oral presentation of data, views or arguments. 15 U.S.C. § 2603(b)(5).

It is a fundamental principle of administrative law that the data and information upon which a regulation is based must be made available during the rulemaking process so as to allow a meaningful opportunity for review and comment by interested persons. Connecticut Light and Power Co. v. NRC, 673 F.2d 525, 530-31 (D.C. Cir.) cert. denied, 459 U.S. 835 (1982); Portland Cement Association v. Ruckelshaus, 486 F.2d 375, 394, 402 (D.C. Cir. 1973), cert. denied, 417 U.S. 921 (1974). As the D.C. Circuit explained in the Portland Cement case:

> "it is not consonant with the purpose of a rulemaking proceeding to promulgate rules on the basis of inadequate data, or on data that, [in] critical degree, is known only to the agency." 486 F.2d at 393.

Indeed, in United States v. Nova Scotia Food Products Corp., 568 F.2d 240 (2nd Cir. 1977), the court held that a regulation had been promulgated in an arbitrary manner and was invalid because the agency had failed to allow interested parties a meaningful opportunity to review and comment upon the scientific data and methodology upon which it relied. The court stated:

> "unless the scientific data relied upon by the agency are spread upon the public records, criticism of the methodology used or the meaning to be inferred from the data is rendered impossible." 568 F.2d at 251.

The court in Nova Scotia provided a detailed explanation as to why failure to allow comment upon the underlying scientific data in an informal rulemaking proceeding is improper:

> "When the basis for a proposed rule is a scientific decision, the scientific material which is believed to support the rule should be exposed to the view of interested parties for their comment. One cannot ask for comment on a scientific paper without allowing the participants to read the paper. Scientific research is sometime rejected for diverse inadequacies of methodology; and statistical results are sometimes rebutted because of a lack of adequate gathering technique or of supportable extrapolation. Such is the stuff of scientific debate. To suppress meaningful comment by failure to disclose the basic data relied upon is akin to rejecting comment altogether . . . The

inadequacy of comment in turn leads in the direction of arbitrary decisionmaking." Id. at 252.

2. The "automatic trigger" provision does not allow meaningful comment on the proposed requirement for oncogenicity testing.

The "automatic trigger" provision in the proposal operates to deprive interested persons of an opportunity to comment meaningfully on the central issue -- whether the results from the specified short-term assay, in conjunction with the existing negative evidence and any additional data which may have been developed by the time the assay results are available, constitutes "substantial evidence" that DETA may present an unreasonable risk of oncogenic effects.

The automatic trigger provision mandates in advance that a "positive" result in any one of the specified mutagenicity tests will constitute such substantial evidence, regardless of the currently available negative evidence on oncogenicity and regardless of any additional negative evidence which may by then have been developed. By denying interested persons an opportunity to comment upon the interpretation and evaluation of these test results after reviewing the actual test data but before a final test rule decision is made, the automatic trigger provision prevents the public from commenting upon the central issue of whether there is the requisite substantial evidence to support required oncogenicity testing. This is contrary to the well-established principle that

"factual or methodological information which is critical to a proposed rule should be available in such a way as to provide adequate opportunity for comment." Sierra Club v. Costle, 657 F.2d 298, 397 n.484 (D.C. Cir. 1981).

In essence, EPA is proposing to require chronic oncogenicity tests based entirely upon the results of mutagenicity tests to be conducted in the future. Interested persons cannot now comment meaningfully upon the evaluation and interpretation of test results which do not yet exist. By relying upon such "prospective" evidence as the basis for requiring oncogenicity testing, the automatic trigger provision deprives interested persons of their statutory right under TSCA and the Administrative Procedure Act to comment on the data upon which the proposed rule is based.

3. The proposed test rule creates an irrebuttable presumption of fact, which violates the Due Process Clause of the United States Constitution.

The Supreme Court has held that regulations based upon irrebuttable presumptions of fact violate the Due Process Clause of the Constitution. Stanley v. Illinois, 405 U.S. 645 (1972); Vlandis v. Kline, 412 U.S.441 (1973); Cleveland Board of Education v. La Fleur, 414 U.S. 632 (1974).

The proposed rule would create the irrefutable presumption that a "positive" result in any one of the three required mutagenicity tests would establish that DETA may present an unreasonable risk of oncogenicity, regardless of the currently

available negative evidence or any additional negative evidence regarding oncogenicity which has become available by the time the positive result is found. Interested persons are precluded from rebutting the presumption following a review of the short-term results or following the receipt of other negative data.

CONCLUSION

The proposed oncogenicity test rule for DETA represents a significant departure from past Agency practice involving trigger testing for oncogenicity bioassays. The rule is being proposed -- not merely in the absence of data otherwise supporting the finding of potential unreasonable risk -- but in the face of a substantial rulemaking record showing no reason for concern.

It is against this background that EPA has issued its proposal premised solely on the hypothesis that if one of three short-term assays produces a positive result, then DETA may present an unreasonable risk of oncogenic effects. This hypothesis itself is scientifically unsound, as Dr. Brusick explained, since it is based upon correlation figures that are limited to known carcinogens. The Agency has shown no data regarding the number of false positives -- a figure of obvious relevance if positives are to function as automatic triggers for long-term bioassays.

Even if the "hair trigger" approach were scientifically sound, the DETA proposal violates the clear mandates of TSCA and the Administrative Procedure Act. EPA has conceded that there is no evidence supporting the rule in the rulemaking record, and if

the record as a whole is considered, as it must be, there is considerable evidence against the rule. The only evidence which might support the rule is not now in existence and therefore is not available for comment. Finally, a final rule would create an irrebutable presumption that is prohibited by the Due Process Clause of the United States Constitution.

For these reasons and for the reasons set forth at the public hearing and in previous comments, DPIA urges EPA to withdraw its proposed test rule for DETA.

Respectfully submitted,

William E. Cornelius
Chairman
Diethylenetriamine Producers/
Importers Alliance

Of Counsel:

R. Bruce Dickson
Michael A. Wiegard

PAUL, HASTINGS, JANOFSKY & WALKER
Twelfth Floor
1050 Connecticut Avenue, N.W.
Washington, D.C. 20036
(202) 223-9000

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WEIGHT-OF-EVIDENCE SCHEME FOR EVALUATION AND INTERPRETATION OF SHORT-TERM RESULTS

David Brusick, John Ashby, Fred de Serres, Paul Lohman, Tai Matsushima, Bernie Matter, Mort Mendelsohn, Mike Waters
A Committee 1 Report, under auspices of the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC) Medical Biological Lab.-TNO, Lang Kleiweg 139, PO Box 45, 2280 AA Rijswijk, The Netherlands

INTRODUCTION

Common practice in conducting an assessment for genetic toxicity of a chemical is to evaluate the agent in a series (battery) of tests measuring genetic activity. The rationale behind this approach is that (a) no single test is adequate to detect genotoxic events from all possible mechanisms of action and (b) target cells of different phylogenetic levels are necessary to provide a "net" that will detect genotoxic activity among diverse chemical classes.

The selection of tests for inclusion in a test battery is generally determined by availability of test methods, intended use of the chemical and financial resources allocated for the genotoxic assessment; however, most test batteries attempt to achieve a balance across genetic endpoints, phylogenetic diversity and in vitro and in vivo bioassays.

Once the data have been collected, the remaining effort involves data analysis and hazard evaluation. Presently, this activity is limited by a lack of information in several critical areas. For example, test methods employed in genetic toxicology, with some notable expectations (e.g., Ames test, Drosophila SLRL test) do not have adequate data bases on which reliable determination of their performance can be made. In addition, the ability to confidently extrapolate qualitative test responses laterally across similar test methods or vertically over different phylogenetic levels is also limited by a small information base.

JUSTIFICATION FOR DATA MERGER

The decision to develop a data analysis using all elements of the test battery was derived from the assumption that each test was intentionally included to provide some essential unit of information. Consequently, the results from each test, positive or negative (assuming the data is scientifically acceptable) should be used in generating the overall assessment of the chemical being evaluated.

This approach also assumes that each bioassay has an inherent value as a predictor of hazard (if positive) or lack of hazard (if negative). This inherent value is a function of many factors such as endpoint detected, resolving power of the test, phylogenetic level of the target organism, documented performance for a wide range of chemicals with known toxic properties. In an analysis of the results for a battery, each of the test results (positive or negative) would contribute to the overall assessment in proportion to its intrinsic value. In addition, the potency (i.e. dose of the agent which produces a recognizable response in the bioassay) will contribute a separate factor relevant to the judgment of hazard.

RESPONSE ANALYSIS

After each score is calculated and plotted for a given in vitro or in vivo battery, a mean score of all the components of the battery is calculated and graphed (Figures 1 and 2). Table 1 lists the specific classes identified by number in the Figures.

The final calculation requires averaging the in vitro (S_f) and the in vivo (S_f) to form the agent scores (S_{a1} or S_{a2}). The S_{a1} scores represent an approximate mean of all test scores biased by the number of test classes and by weighting for the family scores (S_f). The S_{a2} scores also represent an approximate mean of all scores but not biased by S_f since all classes are weighted equally irrespective of family. It is hoped that the S_a scores can eventually define the concern for genotoxic hazard.

To anchor the method it must be calibrated against some recognized "truth." The primary calibration will be set against ranked in vivo cancer activity. A subcommittee focused on experimental carcinogenicity has been established

to develop an animal carcinogenic activity standard to calibrate the system with chemicals having both adequate cancer data as well as genotoxicity data. The cancer ranking will represent a combination of potency and other factors affecting confidence of extrapolation.

TABLE 1

	IN VITRO FAMILY (S_{f_1})		IN VIVO FAMILY (S_{f_2})
CLASS 1:	PROKARYOTIC- PRIM. DNA DAMAGE	CLASS 1:	MAMMALIAN- UDS
CLASS 2:	LOWER EUKARYOTIC- PRIM. DNA DAMAGE	CLASS 2:	INSECT- DROSOPHILA-GENE MUTATION
CLASS 3:	MAMMALIAN CELL- UDS	CLASS 3:	MAMMAL- SOMATIC SPOT TEST
CLASS 4:	PROKARYOTIC- GENE MUTATION	CLASS 4:	MAMMAL- SPECIFIC LOCUS ASSAY
CLASS 5:	LOWER EUKARYOTIC- GENE MUTATION	CLASS 5:	MAMMAL- SOMATIC SCE
CLASS 6:	MAMMALIAN CELL- GENE MUTATION	CLASS 6:	MAMMAL- SOMATIC MICRONUCLEI
CLASS 7:	LOWER EUKARYOTIC- ANEUPLOIDY	CLASS 7:	MAMMAL- SOMATIC CHROM. ABERRATION
CLASS 8:	MAMMALIAN CELL- SCE	CLASS 8:	MAMMAL- DOMINANT LETHAL
CLASS 9:	MAMMALIAN CELL-ABERRATIONS	CLASS 9:	MAMMAL- HERITABLE TRANSLOCATION
CLASS 10:	MAMMALIAN CELL- TRANSFORMATION	CLASS 10:	MAMMAL- GERM CELL CHROM. ABERRATION
		CLASS 11:	MAMMAL- SPERM MORPHOLOGY

RESULTS

Some results have already been obtained using information entered for approximately 50 chemicals. They were encouraging and suggest that the process is moving in a productive direction. In addition, adjustments of internal analysis of the data can be used for optimizing the proposed scoring method.

Among the 50 chemicals contained in the original data base, 38 met the criteria established for use in analysis. In addition, 9 chemicals among the 38 were also ranked for carcinogenic activity in rodents.

The performance of test classes was analyzed by using the deviation of class score from agent score across the set of 38 chemicals. The results showed significant deviation of some classes. Among in vitro classes, bacterial

Figure 3

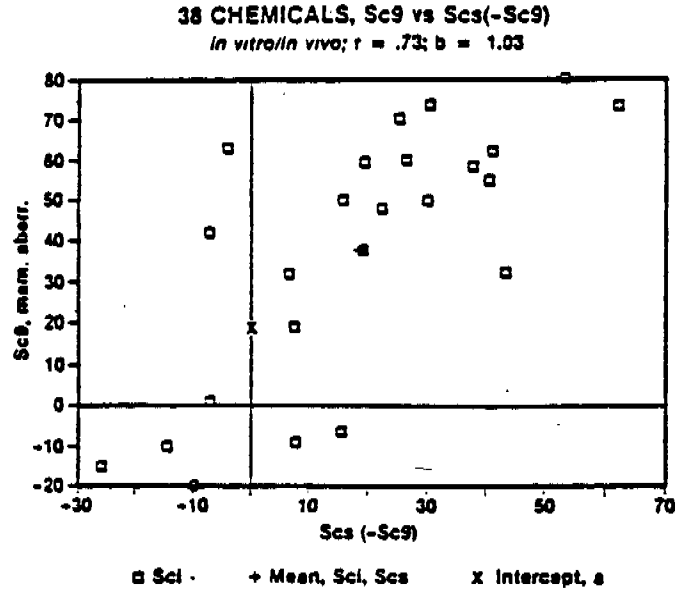
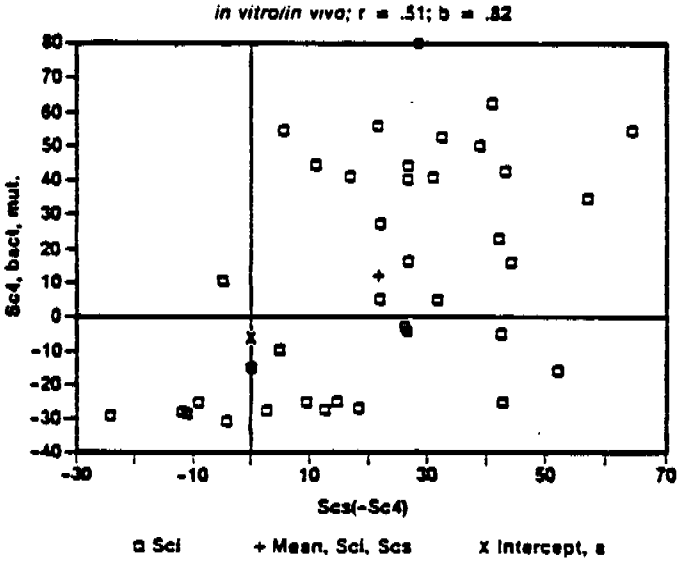


Figure 4



CONCLUSIONS

In its present form, the data assessment approach appears to satisfy many of the general criteria stated at the outset. The output of the system also seems to simulate intuitive processes generally employed when multitest data are evaluated.

At this point, a specific interpretation of the Agent Score would be premature since there has been no comparable external yardsticks developed for segmenting the range of S_a scores into specific categories of concern. It is anticipated that such specificity will be possible if (a) sufficient chemicals can be evaluated in the scheme which also have human or animal carcinogenicity results and (b) a consensus is reached on the reliability of the benchmark data used to calibrate the levels of concern.

The value of the system rests with the possibility that it provides a quantitative, potentially objective way to compare and use genotoxicity data. It also has the potential to progressively improve its assessments as more data are put through the system, by adjustment of various weightings in the light of experience.

HUMAN MUTAGENIC RISK: THE NEW FRONTIER IN REGULATORY TOXICOLOGY

Author : Richard H. McKee

DRAFT

Summary

The purpose of this document is to review the evidence for induced mutation in humans and to critique the EPA guidelines for mutagenic risk assessment. It is clear that mutation does occur in humans, and there is evidence for genetic changes in humans following occupational or clinical exposures to certain mutagenic agents. However, there is no evidence of chemically-induced heritable effects in humans; the available data suggest that the level of concern for human mutagenic risk may be excessive. The biological and mathematical tools which the EPA proposes to use for mutagenic risk estimation are not validated and could lead to overly conservative risk estimates. It is concluded that there is currently no justification to attempt to assess the risk of heritable disease in humans.

Introduction

The Environmental Protection Agency recently published proposed mutagenicity risk assessment guidelines (1). The EPA asserted that there was a need for these guidelines because various statutes administered by the Agency provide the authority to regulate chemicals on the basis of mutagenic properties. It has been argued that the promulgation of mutagenicity guidelines is premature, particularly as there is no epidemiological evidence that humans exposed to mutagenic agents exhibit elevated frequencies of heritable disease. The purpose of this document is to review the available data on mutagenic effects, particularly the evidence of induced mutation in humans and to discuss the limitations of the toxicologic tools and mathematical models which have been proposed for assessing human risk of heritable effects.

1. Relevance of mutation to human health

There is little doubt that mutation has a profound influence on human health. The EPA estimates that at least 10% of human disease is related to specific genetic states (1). One might question the assumptions which led to the 10% estimate (described in reference 2), nevertheless, it is clear that a substantial fraction of human disease is genetically derived. Further, many genetically-based diseases such as Down's Syndrome, achondroplasia and sickle cell anemia are extremely serious and may profoundly influence the health of those affected. The Mutagenicity Subcommittee of the American Industrial Health Council (AIHC) has acknowledged that mutagenesis is an important

toxicological endpoint and that there is at least the theoretical possibility that exposure to environmental mutagens could induce genetically-related disease (3,4,5). In short, there is no question that mutations arise in humans and that the effects of these mutations may be very serious. The current debate centers on whether or not occupational exposure to mutagenic agents makes any contribution to the overall genetic disease burden.

2. Evidence for chemically-induced mutagenic effects in humans

There are several lines of evidence which suggest that chemical exposure may induce gene and chromosomal mutations in both somatic and germinal cells in humans. Albertini and coworkers (reviewed in 6) have developed a technique to isolate 6-thioguanine resistant (TGr) lymphocytes from peripheral blood. Patients undergoing radiation treatment or chemotherapy for cancer or other diseases have exhibited elevated frequencies of the TGr variants. The thioguanine-resistance assay currently poses both technical and theoretical difficulties; nevertheless, it would appear that the induction of gene mutation has been demonstrated in somatic cells of mutagen-exposed humans.

Another line of evidence for human somatic mutation is the elevated incidence of cancer in workers exposed to certain mutagenic agents (e.g. benzene, vinyl chloride). Somatic mutation has been generally accepted as the causal event in malignant transformation, and it is certainly plausible that the development of cancers in humans exposed to agents such as benzene and vinyl chloride is the result of induced somatic mutation in exposed individuals. However, it should be noted that the evidence for the somatic mutation hypothesis is primarily inferential and the molecular events in neoplastic transformation are not known (7).

There is also data which suggests that exposure to elevated levels of certain clastogenic agents under occupational or clinical exposure conditions may produce chromosomal mutations in circulating lymphocytes. Benzene (8) and vinyl chloride (9) are examples of chemicals associated with elevated chromosome aberration frequencies in occupationally-exposed workers. In related studies, industrial exposure to ethylene oxide (among other compounds) has been shown to induce dose-related increases in the frequency of sister chromatid exchange in peripheral lymphocytes (10,11).

A final category of studies have examined genetic changes in germinal cells following occupational exposure to mutagenic agents (reviewed in 12). Double Y-body (a fluorescence-based test thought to detect Y-chromosomal

nondisjunction) has been detected in the sperm of workers exposed to dibromochloropropane (13), and there are also reports of sperm abnormalities in workers exposed to various materials (summarized in 12).

In summary, there is both direct and circumstantial evidence for chemically-induced mutation in both somatic and germinal cells of humans occupationally exposed to certain chemicals. However, the transmission of mutagenic changes to the offspring of exposed individuals has not been demonstrated. Thus there is no direct evidence that exposure to environmental mutagens presents the hazard of heritable disease.

3. Epidemiological studies of humans exposed to mutagenic agents

There have been few epidemiological studies of transmissible genetic effects in exposed populations. Due to the rarity of mutations and limited number of detectable genetic end-points, there have been few exposed populations large enough to provide sufficient statistical power to justify such investigations. However, two large studies have been conducted and neither has produced compelling evidence of induced heritable effects (14,15). In the first of these studies, birth cohorts from Hiroshima and Nagasaki were examined to assess the genetic effects of ionizing radiation. There was some indication of elevated mutational frequency which was directionally consistent with the expectation that heritable genetic damage would be associated with exposure; however, statistical significance was not achieved. A comparison of the estimated doubling dose in humans to that determined experimentally with the Mouse Specific Locus (MSL) test suggested that humans were less sensitive to radiation-induced mutagenesis than predicted from laboratory studies in rodents (14).

A second study examined the offspring of patients who had undergone radiation or chemotherapy treatment for childhood cancer (15). In a preliminary investigation there was no evidence of elevated frequencies of genetically-related disease. In fact, a greater percentage of major birth defects was found in the control population (11%) than in the exposed group (8%). Additionally, none of the defects observed to date has been a sentinal phenotype (16).

Neither of these can be considered as a definitive negative epidemiology study because neither had sufficient statistical power. Nevertheless, these studies do suggest that the current level of concern for heritable effects may be excessive. Additionally, questions were raised about the utility of the MSL assay as a means of estimating the potential for human mutagenic risk (14). Finally, it should be noted that other toxic endpoints (including

cancer) which have been associated with exposure to genotoxic agents are identified in the exposed populations.

4. Use of experimental models to identify potential germ cell mutagens.

Much of our understanding of mutagenic mechanisms has been derived from studies in submammalian test systems. Within the last 10 years, research in this area has been stimulated by the theoretical association of in vivo mutation and neoplastic transformation (7) and the concordance of mutagenic activity in bacteria with carcinogenic activity in mammals (17).

It has become a common practice to use in vitro techniques to identify potential carcinogens, and, more recently it has been proposed to use a similar approach to identify agents which may pose genetic hazards (18). Unfortunately, the predictive capacity of submammalian assays for mammalian germ cell mutation is tenuous (19,20), and better experimental methods are needed to identify potential germ cell mutagens. It is evident that if quantitative germ cell mutagenesis data are desirable, then these data can only be obtained from in vivo studies (21). Five in vivo assays are considered appropriate for quantitative mutagenesis data; namely the MSL, heritable translocation (HT), dominant lethal, dominant mutation, and cytogenetic translocation assays (4).

Of these, the EPA has designated the MSL and HT tests as the assays to be used to produce quantitative gene and chromosomal mutation data respectively (1). The majority of agents tested in these assays have been identified as potent mutagens in submammalian systems; however, few chemicals other than potent alkylating agents have produced positive responses in germ cell mutagenesis assays. Thus the applicability of these assays to a wide range of chemical classes is unknown.

In summary, it is evident that the science of testing mammalian germ cell mutagens is at an early stage. It is difficult to predict chemicals which are likely to be germ cell mutagens. Current experience suggests that the majority of materials identified as potential germ cell mutagens by the schemes suggested by the EPA (1) and the National Research Council (18) are likely to produce negative results in the MSL and HT assays. It is probable that the only chemicals likely to be positive in either of these assays would be potent alkylating agents.

5. Mathematical models for risk extrapolation

The EPA has asserted that it "will strive to use the most appropriate extrapolation models for risk analysis and will be guided by the available data and mechanistic considerations in this selection." (1). The mathematical models were not listed in the risk assessment guidelines, but it is considered likely that a linear extrapolation with no threshold would be used to assess the risk of point mutations (22).

The appropriate mathematical model for risk extrapolation has been an extremely controversial topic for many toxic endpoints. It has been argued that toxicologic dose-response relationships tend to be exponential with respect to administered dose because of the dependence of biological responses on kinetic processes (23). Thus one should either incorporate kinetic parameters into risk assessment models or, alternatively, utilize a "biologically effective dose" (e.g. the fraction of chemical bound to DNA) as the dose unit. In a study of ethylnitrosourea-induced mutation in mice, the mutational yield obtained at low doses was significantly lower than predicted by the linear extrapolation model despite the fact that DNA alkylation was linearly associated with administered dose over the range used (24). Certainly few studies have examined the dose-responsiveness of heritable effects in mammals. However, the available data suggest that mutational induction in mice may not be linearly associated with either the administered or the "biologically effective" dose and may, in fact, exhibit threshold behavior.

A second controversial feature of the risk assessment proposal is the assumption that no thresholds exist for mutagenic effects. An expert review panel was unable to reach a conclusion about the existence of thresholds for mutational effects (25). The preponderance of available data were consistent with the hypothesis that thresholds did not exist; however, the majority of the cases were studies of alkylating agents in submammalian systems. As noted above, the data of Russell et al (24) suggest threshold behavior in the MSL assay.

There are two other features of the risk assessment models which may also be excessively conservative. Specifically, these are the extrapolation from mouse to man and the use of acute experiments to model chronic exposure situations. The atomic bomb data discussed earlier suggested that humans are less sensitive to mutagenic effects than predicted by the MSL; although the degree of difference has been difficult to precisely determine (19). The extrapolation from experiments involving acute high dose exposures to occupational settings of chronic low dose exposure also presents difficulties. The yield of mutants following chronic radiation exposure was approximately one-third that of an

equivalent acute exposure (cited in 14).

Similarly, fractionated doses of both ethylnitrosourea (26) and procarbazine (27) produced significantly reduced mutational yields as compared to single dose studies.

Conclusions

It is evident from the literature that a substantial fraction of human disease results from heritable effects. It is also clear that humans are exposed to mutagenic agents. Occupational exposure undoubtedly contributes to the total exposure to mutagenic materials. However, the greatest contribution to mutagen exposure may be due to other sources including naturally occurring genotoxins (28). In vitro assays are useful for mutagen identification; however, they have limited predictive capacity for in vivo germinal mutagens. Assessment of in vivo genotoxic potential should only be made from in vivo test data (21). Because it is difficult to identify potential germinal mutagens from submammalian assays (19,20), there is a need for limited in vivo assays which are predictive of germinal mutation.

The need to assess human mutagenic risk is, in itself, controversial in light of epidemiological data (14,15) which suggest that exposure to mutagens presents little if any risk of inducing heritable effects. Additionally, it seems likely that most of the materials selected for in vivo testing by the strategy endorsed by the EPA would also be carcinogenic and thus be regulated on the basis of toxic properties other than mutagenesis. In fact, it was this line of reasoning which led the EPA to defer testing of methyl chloride in the MSL and HT assays despite demonstrated activity in in vitro, submammalian, and in vivo mutagenesis assays (29).

Finally, if quantitative in vivo mutagenesis test data are obtained, the mathematical models used for risk estimation should be carefully considered. The use of a linear extrapolation model with no threshold may be overly conservative (30) and could lead to an overestimate of risk by several orders of magnitude (23). In addition to the conservatism presented by the assumptions of no-threshold behavior and linearity, the models used to extrapolate from mouse to man and from acute to chronic exposure situations may also be overly conservative.

It appears that measures to assess human mutagenic risk are not clearly justified at the present time. It is reasonable to utilize in vitro and limited in vivo assay systems to evaluate genotoxic potential, and chemicals which are in vitro mutagens should be considered for carcinogenesis testing. Because of the limitations of the

MSL and HT assays, quantitative in vivo mutagenesis assays for germ cell mutagenesis should be limited to those chemicals which exhibit in vivo mutagenic activity, are not carcinogenic, and present substantial exposure opportunities.

BIBLIOGRAPHY

1. Environmental Protection Agency (1984). Proposed guidelines for mutagenicity risk assessment; Request for comment. Fed. Reg. 49 : 46314-46321.

2. J. Drake (1978). Some guidelines for determining maximum permissible levels of chemical mutagens. In: Advances in Modern Toxicology vol. 5 (W. Flamm and M. Mehlman, eds.), Hemisphere Publishing Company, Washington, DC., pp. 9-26.

3. J. Irr (1981). An evaluation of epidemiological methods of monitoring human populations for increased genetic risk. Regulat. Tox. Pharm. 1: 84-89.

4. J. Brewen (1981). Human genetic risk assessment for chemical substances. ibid. 78-83.

5. P. Buffler and J. Aase (1982). Genetic risks and environmental surveillance: Epidemiological aspects of monitoring industrial populations for environmental mutagens. J. Occ. Med. 24: 305-314.

6. R. Albertini (1982). Studies with T-lymphocytes: An approach to human mutagenicity monitoring. Banbury Report 13: Indicators of Genotoxic Exposure. Cold Spring Harbor Laboratories. pp. 393-410.

7. H. Rubin (1980). Is somatic mutation the major mechanism of malignant transformation? J. Nat'l Cancer Inst. 64: 995-1000.

8. A. Forni, A. Cappellini, E. Pacifido, and E. Vigliani (1971). Chromosome changes and their evolution in subjects with past exposure to benzene. Arch. Environ. Health 23: 385-391.

9. M. Kucerova, Z. Polivkova and J. Batora (1979). Comparative evaluation of the frequency of chromosomal aberrations and the sister chromatid exchange numbers in peripheral lymphocytes of workers occupationally exposed to vinyl chloride monomer. Mutat. Res. 67: 97-100.

10. S. Galloway, P. Berry, W. Nichols, S. Wolman, K. Soper, P. Archer, and P. Stolley (1985). Cytogenetic study of 61 employees exposed to ethylene oxide and of a large control population. Fourth International

Conference of Environmental Mutagens. Stockholm, Sweden, p 95(A).

11. S. Wolman, S. Galloway, W. Nichols, S. Norman, K. Soper, and P. Stolley (1985). Ibid, p 278 (A).

12. A. Wyrobek and R. Bruce (1978). The induction of sperm shape abnormalities in mice and humans, in Chemical Mutagens, vol. 5 (A. Hollaender and F. De Serres, eds.), New York, Plenum Press, pp 257-285.

13. R. Kapp Jr., D. Picciano, and C. Jacobson (1979). Y chromosome nondisjunction in DBCP exposed workmen, Mutat. Res. 64: 47-51.

14. W. Schull, M. Otake and J. Neel (1981). Genetic effects of the atomic bombs: A reappraisal. Science 213: 1220-1227.

15. J. Mulvihill (1985). Reproduction in cancer patients. Fourth International Conference on Environmental Mutagens. Stockholm, Sweden, p 97(A).

16. J. Mulvihill and A. Czeizel (1983). A 1983 view of sentinel phenotypes. Mutat. Res. 123: 345-361.

17. J. McCann, E. Choi, E. Yamasaki, and B. Am s (1975). Detection of chemicals as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat'l. Acad. Sci. 72: 5135-5139.

18. J. Crow (1983). Chemical mutagen testing: A committee report. Environ. Mutag. 5: 255-261.

19. W. Russell (1985). Positive genetic hazard predictions from short-term tests have proved false for results in mammalian spermatogonia with all environmental ch micals so far tested. Fourth International Environmental Mutagenesis Society Meeting, Stockholm, Sweden, 41(A).

20. L. Russell (1985). Heritable effects of chemicals in mammalian germ cells: Relation to results from other test systems. Ibid, 118 (A).

21. J. Ashby, H. Bartsch, U. Ehling, T. Kada, H. Malling, A. Naratayan, G. Obe, H. Rosenkranz, L. Russell, J. Schoneich, A. Searle, E. Vogel, J. Wassom, F. Zimmerman (1983). Screening strategy for chemicals that are potential germ-cell mutagens in mammals. Mutat. Res. 114: 117-177.

22. M. Christian and P. Voytek (1982). In vivo reproductive and mutagenicity tests. EPA-600/d-82-318. National Technical Information Service, Springfield, Va.

23. D. Hoel, N. Kaplan, and W. Anderson (1983). Implications of nonlinear kinetics on risk estimation in carcinogenesis. Science 219: 1032-1037.

24. W. Russell, P. Hunsicker, G. Raymer, M. Steele, K. Stelzner, and H. Thompson (1982). Dose-response curve for ethylnitrosourea-induced specific locus mutation in mouse spermatogonia. Proc. Nat'l. Acad. Sci. 79: 3589-3591.

25. U. Ehling, D. Averbeck, P. Cerruti, J. Friedman, H. Greim, A. Kolbye Jr., and M. Mendelsohn (1983). Review of the evidence for the presence or absence of thresholds in the induction of genetic effects by genotoxic chemicals. Mutat. Res. 123: 281-341.

26. W. Russell, P. Hunsicker, D. Carpenter, C. Cornett, and G. Guinn (1982). Effect of dose fractionation on the ethylnitrosourea induction of specific locus mutations in mouse spermatogonia. Proc. Nat'l. Acad. Sci. 79: 3592-3593.

27. U. Ehling (1980). Induction of gene mutations in germ cells of the mouse. Arch. Toxicol. 46: 123-138.

28. B. Ames (1983). Dietary carcinogens and anticarcinogens (oxygen radicals and degenerative disease). Science 221: 1256-1264.

29. Environmental Protection Agency (1985). Chloromethane; Withdrawal of proposed health effects test rule. Fed. Reg. 50: 19213-19216.

30. F. Sobels (1985). Personal comments on "Environmental Protection Agency" proposed guidelines for risk assessment. Mutat. Res. 147: 211-216.

DRAFT

A REVIEW OF REGULATORY POLICY AS IT RELATES TO GENETIC TOXICOLOGY TESTING REQUIREMENTS

INTRODUCTION

It is difficult to provide a short review of the regulations governing genetic toxicology test data.* Most, if not all, of the developed countries require that mutagenicity data be collected for some regulatory purposes and that short term in vitro assays are to be employed as a first step in the data collection process. In general, the testing requirements are designed to assess both point mutations and chromosomal aberrations. Additionally, most regulations either suggest or absolutely require that bacterial assays for point mutations such as the Ames Salmonella test and cytogenetic assays utilizing either cells in culture or intact mammals, should be included in the initial stages of genetic toxicology testing.

There is no concensus with regard to additional tests which need to be performed, the triggering points at which additional test data should be obtained, or the regulatory actions to be taken on the basis of the test results. The test requirements vary between countries, regulatory agencies, and the types of products to be regulated. The situation is further complicated because in some situations mutagenesis test data is intended for carcinogen detection; whereas, in other cases, the induction of heritable effects in human populations is the potential health hazard of concern.

This paper will review the current state of government regulations and requirements. The review will be limited to those laws and regulations which apply to the commodity and specialty chemical business and to petroleum products. There are regulations which govern the testing of food additives, pesticides, drugs, cosmetics, and animal feed additives. (A more complete listing, compiled by Berry and Litchfield, is included in the Appendix). An attempt was made to include the most relevant national and international regulations; however, due to the complexity of these regulations and to the fact that they are under essentially constant revision and amendment, this task was quite difficult. The reader should be aware that this document reviews the regulations as they exist in mid 1985, but in the future the regulations may be substantially different.

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* For purposes of this review, "genetic toxicology" will be used as a generic term which encompasses point and chromosomal mutation and it will also encompass other end points such as transformation, sister chromatid exchange, and unscheduled DNA synthesis which are evaluated primarily through in vitro testing and are presumed to result from purturbations in DNA but have not been well described at the molecular level. The US EPA, among other regulatory bodies, utilizes these "ancillary" tests in determinations of mutagenic potential (ie. hazard) but not for risk assessment.

I. The U.S. Toxic Substances Control Act (TSCA)

The Toxic Substances Control Act (1) which was signed into law in 1976, is the primary U.S.legislative act which addresses the toxicity of industrial chemicals and petroleum products. This act authorizes the Environmental Protection Agency to require that industry document production levels, uses, health effects, and other matters concerning chemical substances and mixtures. Specifically, as outlined in TSCA Section 2, it is the policy of the United States that adequate data should be provided to predict the effects of chemical substances and mixtures on health and the environment and that it is the responsibility of those who manufacture and process such chemical substances and mixtures to provide the necessary data.

A) Section 4: Testing of Chemical Substances and Mixtures

Section 4 of TSCA was enacted by Congress in response to the concern that, in many cases, the effects of chemical substances on health and the environment were not adequately understood. This section of TSCA directs the administrator of the EPA to ensure that sufficient toxicity test data is provided to reasonably define the health and environmental effects of potentially hazardous chemicals. Specifically, if the EPA makes certain findings about a chemical, the Agency must adopt a rule requiring the manufacturers and/or producers to test that chemical for the effects of concern.

For promulgating a new rule, the EPA must find:

(i) either that the chemical may pose an "unreasonable risk" of harm to health or the environment; or that the chemical is or may be produced in "substantial" quantities which may result in substantial or significant human exposure or environmental release; and

(ii) that insufficient data exist about the health or environmental effects of the chemical to reasonably predict the impact of manufacturing, processing, use or disposal of such chemicals, and

(iii) that testing is needed to develop such data.

Section 4.b.2.A specifically lists mutagenesis as a h alth effect for which testing can be required. This section specifies that testing can include epidemiological studies, in vitro tests, and whole animal tests. If the EPA administrator determines that a chemical substance or mixture may present a "significant risk of serious or wide spread harm to human beings from cancer, gene mutation, or birth defects," the Agency is required under section 4(f) to take regulatory action under TSCA sections 5,6, or 7 to minimize these risks.

Prior to 1983, the Agency concentrated efforts on developing a list of priority materials. However, within the past two years the Agency has taken a more active stance towards developing toxicology test data. As an initial approach to developing test data, the Agency met with industry representatives to formulate "voluntary" or "negotiated" programs. However, the "voluntary" approach was successfully challenged by environmental groups and has been replaced by a more formal procedure. At present the agency prepares "test rules" which are published in the Federal Register and which specify both the toxicologic endpoints which must be assessed and the testing guidelines which must be followed.

Mutagenesis has been a major concern of TSCA. A survey of ITC* recommendations between 1977 and 1982 indicated that 71% (42/59) proposals requested genetic toxicity data (3). The agency has also published a "generic" mutagenicity test proposal which specifies a mutagenicity testing proposal leading to either chronic bioassays or mutational assays in mammals. (4, included in the Appendix).

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* The ITC is the Interagency Testing Committee. The ITC was established under TSCA section 4(e) to advise the EPA on chemicals which should be given priority consideration under section 4(f).

The Agency has published several variations of the "generic" mutagenicity testing proposal, but all share two elements which are particularly disturbing:

(i) the use of positive results from single in vitro tests to trigger two year bioassays for oncogenicity, and

(ii) the requirement that in vivo mutagenicity tests including the heritable translocation and specific locus tests in mice be conducted to provide data suitable for risk assessment.

Additional discussion of the "generic" mutagenicity testing proposal is given below in III (A).

2) Section 5: Premanufacturing Notification (PMN)

Section 5 requires that notice be filed with the EPA prior to manufacture or import of a new chemical substance intended for commercial purposes. Section 5(d)(1) requires that all data in the possession or control of the submitter, which are related to effects on health or the environment, must be provided to the Agency. However, Section 5 does not specify any toxicity testing requirements. An early guidance statement from the EPA (5) recommended (but did not require) that the OECD "base set" be the starting point for premanufacturing testing. (The OECD minimum data set requirements will be discussed in greater detail in another section of this report -see VI :International Requirements. However, the OECD minimum data set does include genotoxicity screening tests, and the number of tests required increases with increasing production volume.) Some companies in the US do include genotoxicity test data as part of the PMN process; however, others do not routinely provide such data (in fact, as of 1984, approximately half of all PMN submissions contained no toxicity data). There have been indications from time to time that the EPA would like to see more toxicity test data in PMN submissions, and it has been suggested that if additional data were not forthcoming, TSCA section 5 might be amended to include requirements for toxicity testing. One research program currently under consideration by the Agency would compare predictions from structure-activity relationships to toxicity test data for a series of chemicals which the Agency has reviewed under Section 5. There is speculation that the toxicity tests in that proposal (genetic toxicity, acute toxicity, subchronic toxicity, and skin sensitization) would form the basis for a PMN data set should the SAR approach prove to be ineffectual (which is the most likely outcome of the study).

If available data suggest that the new chemical may exhibit toxicity which has not been adequately evaluated, then the EPA can take action on a PMN to either require such testing under a section 5(e) order or cause the PMN to be rejected.

3) Section 8: Reporting and Retention of Information

Section 8 requires submission of health and safety studies under several sets of circumstances. In particular, under Section 8(d), the Agency may request companies to supply all toxicity test data on specified chemicals, and, under section 8(e), companies are required to report to the Agency any findings which suggest significant new human health hazards. Most relevant to this analysis is the fact that the EPA considers heritable mutation to be as significant as carcinogenicity and reproductive toxicity as a basis for section 8(e) filings.

II. U.S. Occupational Safety and Health Act of 1970 (OSHA)

The Occupational Safety and Health Act grants to the U.S.

Labor Department broad powers for controlling hazards in the workplace. Although OSHA does not specifically address heritable mutation (as does TSCA), the Labor Department has been asked to consider mutagenesis data in some instances. Two sections of the OSHA Act are relevant to this regulatory analysis:

A) Section 5(a)(1) - "General Duty Clause" which states that it is the duty of an employer to furnish a place of employment which is free from recognized hazards which would pose a serious threat to the employees.

B) Section 6(b)(5) - "Health Standards Clause" which grants the power to promulgate health standards on specific substances.

Under the "Health Standards Clause", OSHA has considered mutagenicity data in connection with two chemicals, benzene and ethylene oxide. Specifically:

i) The induction of chromosomal aberrations in rodents exposed by inhalation to benzene at levels approaching the current permissible exposure limit, and

ii) The induction and persistance of sister chromatid exchanges in autoclave workers exposed to ethylene oxide.

Both of these data sets pose a challenge to OSHA because the clinical significance of these genotoxic effects is uncertain. Even the proponents of cytogenetic monitoring recognize that these effects demonstrate exposure but cannot be directly extrapolated to risk of cancer or heritable effects except in a statistical sense.

C) OSHA labelling requirement - Late in 1983, OSHA issued a hazardous communication act that requires chemical manufacturers and importers to assess the hazards of the chemicals which they make or import and to inform workers of the hazards associated with the chemicals in their work areas (6).

Under this Act, chemicals are presumed to pose a health hazard if they are listed under Subpart Z of the OSHA standards for hazardous and toxic substances or if they are listed as carcinogens by the National Toxicology Program (NTP) or the International Agency for Research on Cancer (IARC). In addition to the above, corrosives, irritants and sensitizers as well as chemicals that are toxic or highly toxic or exhibit target organ effects are considered to be health hazards. At present this law does not specifically address mutation; however, inasmuch as a labelling proposal has been developed by the EEC (IV : European Regulations), it is conceivable that labelling for mutagenesis may be required in the future. (In addition to the U.S. (OSHA) and Europe (EEC), Canada has also developed a proposal to label hazardous chemicals. A proposal to establish consistent labelling criteria throughout the regions is included in the Appendix.)

III. OTHER U.S. ACTIONS RELEVANT TO REGULATORY AFFAIRS

A. EPA "GENERIC" MUTAGENICITY TESTING SCHEMES

A mutagenesis testing scheme, similar, if not identical to the "generic' proposal of Newburg-Rinn has been included in several recent TSCA 4 test rules including the C9 Aromatics proposal. In specifying that results of single in vitro assays were sufficient to trigger chronic testing, the EPA has essentially ignored both prevailing scientific opinion, as well as the results of government-sponsored research programs.

Most investigators believe that the results of all short term assays as well as other relevant information (ie. pharmacokinetic and metabolism data as well as evidence of pathology from subchronic bioassays) should be considered on a "weight of evidence basis" in determining the need for chronic tests (for example as listed in the AIHC Mutagenicity Subcommittee comments on the generic mutagenicity testing proposal). None of the short term assays are considered to be sufficiently reliable to be used as a sole basis for determining the need for chronic testing.

An alternative to the use of single test triggers is a tiered or hierarchal testing program in which positive results from in vitro assays are followed by limited in vivo tests to determine if the genotoxic activity is elaborated in intact mammals. Variations of this strategy have been proposed by a number of prominant scientists in the field. This alternative approach was specifically rejected by the Agency in its response to the API comments to the proposed test rule on C9 aromatics (7). The Agency proposed that industry sponsor an in vitro cytogenetics assay, and, if th assay were positive, to conduct a year chronic bioassay and a dominant lethal assay in rats. If the in vitro cytogenetics test was negative, the Agency proposed that industry should then conduct an in vivo assay for chromosomal aberrations in rat bone marrow. The API responded that the in vivo assay should be considered the "definitive" endpoint and that positive results in the in vitro assay should not be considered to be biologically significant if the results could not be reproduced in intact animals. Therefore, the API suggested that industry sponsor an in vivo assay only and that chronic testing be conducted only if the in vivo assay was positive. The Agency rejected that proposal, asserting that a positive in vitro cytogenetics assay was sufficiently predictive to justify chronic testing regardless of the in vivo test results. (The Agency reached this conclusion on the basis of data from 10 chemicals evaluated in the USEPA Gene-Tox program.) But the Agency has also ignored a more recent government-sponsored study by the National Toxicology Program in which chemicals tested for carcinogenicity by the NTP were evaluated for genotoxic potential in a battery of in vitro assays (8,9). Of 70 chemicals tested which showed no evidence of carcinogenic potential in vivo, 34 have been active in one or more of the genetic toxicology tests specified in the C9

Aromatics Test Rule. Of the remaining 36, none has been completely tested, and, thus, none of the 70 "noncarcinogens" identified by the NTP would be "cleared" by the EPA's generic mutagenicity testing scheme.

B. MUTAGENIC RISK ASSESSMENT

The Agency has published proposed guidelines for mutagenicity risk assessment (10). The Agency has proposed that in vitro tests be used for hazard identification purposes (ie. to detect mutagenic materials) and that in vivo assays, specifically the mouse specific locus test and the heritable translocation test, be used to develop mammalian mutagenicity data which could be used for risk assessment purposes. These guidelines have recently been approved by the EPA Science Advisory Board and should be considered as final. The C9 aromatics test rule mutagenicity testing scheme is consistent with the data gathering strategy outlined in the risk assessment guidelines. The mathematical model for risk assessment was not specified, but it is anticipated that a linear, no-threshold, extrapolation model, similar to the carcinogenic risk assessment model, would be utilized (11).

The degree of risk of heritable mutation associated with exposure to genotoxic chemicals is a matter of considerable controversy. The EPA proposal is not supported by epidemiologic evidence of chemically-induced heritable effects (12,13), and, additionally, no other country has proposed to regulate chemicals on the basis of mutational risk. Therefore, it has been argued that any attempts to assess the risk of heritable mutation in humans is premature. However, the EPA has asserted that the specific mention of mutagenesis in TSCA and the requirement that mutagenic risk be minimized demonstrate that intent of Congress intended that mutational risk should be assessed and that chemicals should be regulated in part on that basis.

C. HEALTH EFFECTS DOCUMENTS

The Agency has also issued a series of health effects guidelines which are, in effect, protocols for the various toxicity tests required by the Agency (14). The publication of these methods was required by a provision in TSCA 4, that the Agency must provide standards for the development of test data. These standards are generally compatible with those utilized by other regulatory groups, particularly OECD.

D. Toxicological Testing Programs for Genetic Toxicology

The majority of government sponsored toxicological research is done under the auspices of the National Toxicology Program (NTP), the Department of Energy (DOE), the National Institutes of Health (NIH), and the EPA.

Research in genetic toxicology has been directed primarily at the development and validation of in vitro methods of carcinogen detection. Major programs have included:

1) An expansion of the mutagen/carcinogen data base through an evaluation of a set of chemicals previously tested for carcinogenic properties in a battery of genotoxicity assays. Preliminary results of this program suggest that the more tests that are run, the more likely it is that there will be a positive result (8,9).

2) A correlation of mutagenic and carcinogenic potency of human lung carcinogens (coal tar pitch, coke oven emissions, and cigarette smoke condensate). All of these materials were tested for dermal carcinogenic activity in vivo and for genotoxic properties in a variety of short term assays. The goal of the program is to identify short term assays which quantitatively predict carcinogenic potency (15).

3) The development of in vitro assays for tumor promoters. Current interest is focusing on aneuploidy (16) and cell-cell communication (17).

4) The development of in vitro assays using human cells, tissues, or activating enzymes derived from human tissue (18).

5) The correlation of the mutagenic and carcinogenic potency of human dermal carcinogens. This program has been conducted under DOE sponsorship and has been aimed at assessing the carcinogenic hazards of synthetic fuels. Little future work is expected in this area because it has been shown that for coal- and shale-derived liquids, the mutagenic activity is associated primarily with aromatic amines; whereas, the carcinogenic activity is associated primarily with neutral polycyclic aromatic hydrocarbons (19,20).

6) The development of in vivo assays for mutagenic risk assessment. Most of the effort in this area is being conducted by scientists at Oak Ridge National Laboratory. Studies over the past several years with the mouse specific locus assay suggest that 1) only extremely potent mutagens are likely to produce genotoxic effects in this model; 2) there is evidence of repair at low doses suggesting that the linear, no-threshold model for mutagenic risk assessmemt is overly conservative; and 3) there is little correlation between in vitro and in vivo mutagenicity results (21-23).

7) A proposed study to compare toxicity test data with predictions based on structure-activity relationships. The proposed tests include Salmonella, in vitro sister chromatid exchange, mouse lymphoma, acute oral toxicity, 28 day oral toxicity, eye and skin irritation, and skin sensitization. It is possible that if the SAR approach does not appear to be satisfactory, that the EPA will propose these tests as a minimum toxicity data base for premanufacture notification (TSCA 5).

IV. EUROPEAN REGULATIONS

The European Economic Communities Directive relating to the Classification, Packaging and labeling of Dangerous Substances offers guidance in its Annex VI Part 2D for the labeling of dangerous substances within the EEC. Finalized in November 1984, the criteria in this directive have been already incorporated into the national legislation of several EEC member countries. Labeling requirements of this directive and of the pending and separate "Directive on Dangerous Preparations" are intended to provide a primary means by which the general public and persons at work are given essential information about the inherent danger of the material and to draw attention to the comprehensive ifnformation of the safety and use of the product available in the associated data sheet.

The labeling takes account of all potential hazards which are likely to be faced in the normal handling and use of dangerous substances and preparations in the form in which these are marketed though not necessarily in the form of final use (e.g., diluted). The most severe hazards are highlighted by symbols and these and other dangerous porperties are specified in standard risk pharases (R) and safety phrases (S).

In regard to health effects, the hazard classification is based on both acute and long term effects whether resulting form a single exposure or repeated or prolonged exposure. If effects other than acute effects are indicated (e.g., carcinogenic, mutagenic, teratogenic, allergenic, subacute or chronic) the substances are classified according to the magnitude of these effects. Thus, for mutagenicity, three classifications are applicable:

Category 1

Substances known to be mutagenic in man.

- there is sufficient evidence to establish a causal association between human exposure to a substance and heritable genetic damage.

- requires R46 `may cause heritable genetic damage' and either `toxic' or `very toxic'

Category 2

Substances which should be regarded as if they were mutagenic to man.

- there is sufficient evidence to provide a strong presumption that human exposure to the substance may result in the development of heritable genetic damage, generally on the basis of appropriate animal studies or other relevant information.

- requires R46 `may cause heritable genetic damage' and

`harmful'.

Category 3

Substances which cause concern for man owing to possible mutagenic effects.

- requires R40 `possible risk of irreversible effect' and `harmful'.

The manufacturer is required to apply a provisional (minimum) label of `R40' and symbol X and to submit a document summarizing relevant information (including a bibliography and any published/unpublished data) to the member state in which the substance is placed in the market.

V. CANADIAN REGULATIONS

Canadian activity regarding toxic and hazardous substances has been directed towards the development of a national standard for a Workplace Hazardous Materials Information System (WHMIS). The strategy envisioned by Labour Canada includes three elements; labels, material safety data sheets, and worker education programs. Specifically, the labels are intended to alert workers and to provide basic information for immediated use in emergencies. The Material Safety Data Sheets are to provide more comprehensive information about possible health effects and protective measures, and the worker education program is intended to explain the available information to the workers and to relate this information to specific workplace conditions.

A. Hazardous Materials Classification

Development of a definition for "hazardous material" and a classification system for "hazardous materials" were the necessary first steps in the WHMIS process since these would determine the materials to which the system would apply. As a fundamental principle it was established that no part of WHMIS would require additional toxicological testing.

The hazardous materials classification system includes six general categories:

(1) compressed gas
(2) flammable and combustible material
(3) oxidizing material
(4) poisonous and infectious material
    - very toxic material
    - toxic material
    - biohazardous/infectious material
(5) corrosive material
(6) dangerously reactive material

The toxic and very toxic subclasses include materials

which:

(1) are acutely l thal in animal assays.
(2) elicit chronic toxic effects in animal assays.
(3) are teratogenic or embryotoxic in animal assays.
(4) are listed as carcinogenic by specific internationally accepted references.
(5) produce respiratory tract sensitization in humans following occupational exposure.
(6) cause acute dermal irritation/corrosion in animal assays.
(7) cause acute eye irritation/corrosion in animal assays.
(8) cause skin sensitization in humans following occupational exposure.
(9) cause adverse effects in humans following occupational exposure.
(10) cause adverse reproductive effects in humans following occupational exposure or in animal assays.

On the subject of mutagenicity, the three groups involved (Government, Labour, and Industry) agreed that WHMIS should include hazard criteria for mutagenic materials; however, no concensus could be reached regarding the specific criteria which should be utilized. Therefore, a decision on the appropriate mutagenicity criteria for WHMIS has been deferred pending futher discussion and review.

VI. OECD RECOMMENDATIONS

The OECD (Organization for Economic Cooperation and Development) is an intenational organization of major Western industrialized countries which was formed to facilitate trade and economic development among its members. The OECD has recommended that the toxic hazards of industrial chemicals be assessed. The proposal, similar to that to TSCA section 5, recommends that the manufacturers should obtain certain toxicity test data before chemicals nter the marketplace. The OECD has also developed testing guidelines, similar to those published by the US EPA, which specify the methods by which the testing should be conducted. Although the EPA did not include PMN testing requirements in TSCA, the OECD recommended Minimum Premarketing Data set (MPD) was largely adopted by EEC. The minimum requirements include tests for acute and subchronic toxicity, skin and eye irritation, subchronic toxicity, mutagenicity, and ecotoxicity; and these become more complex as production volumes increase. Most EEC member states require that chemicals be assessed in two mutagenicity tests, one for gene mutation in bacteria and a second for chromosome aberration which can be satisfied by one of several in vitro or in vivo tests. (However, individual member states may have somewhat different requirements. Holland, for example, requires three tests and recommends that the battery include a test for gene mutation in mammalian cells). The recently passed Annex 8 stipulates additional requirements for materials produced in excess of 10 tons. Two additional tests are required to confirm in

vitro activity and to begin to extend the evaluation to the in vivo situation. The choices available include yeast, mammalian cells, drosophila, and somatic cells in vivo. At present the test results are to be used for labelling purposes; there is no current plan to attempt to assess human mutagenic risk.

BIBLIOGRAPHY

1. Toxic Substances Control Act. Public Law 94-469, 90 Stat. 2003 et. seq., 1976.

2. Fed. Reg. 50, 20662-20677, May 17, 1985.

4. Newburg-Rinn. Environmental Mutagen Society Satellite Meeting, San Antonio, Texas, March 2, 1983.

5. Fed. Reg. 46, 8986-8993, Jan. 27, 1981.

6. Code of Federal Regulations, 29, 1910.1200.

7. Comments of the American Petroleum Institute In Re Proposed Test Rule on Ethyltoluene, trimethylbenzene, and the C0 Aromatics Hydrocarbon Fraction, September 1, 1983.

8. Shelby and Stasiewicz, Environ. Mutag. 6, 871-878, 1984.

9. Zeiger and Tennant, 4th International Conference on Environmental Mutagens, Stockholm, Sweden, June 19, 1985.

10. Fed. Reg. 49, 46314-46321, November 23, 1984.

11. Christian and Voytek, Report Number EPA-600/D-82-318, 1982.

12. Schull, et. al. Science 213, 1220-1227, 1981.

13. Mulvihill, 4th International Conference on Environmental Mutagens, Stockholm, Sweden, June 19, 1985.

14. EPA 560-83-001.

15. Lewtas, 4th International Conference on Environmental Mutagens, Stockholm, Sweden, June 19, 1985.

16. Resnick, 4th International Conference on Environmental Mutagens, Stockholm, Sweden, June 19, 1985.

17. Elmore, et al, 4th International Conference on Environmental Mutagens, Stockholm, Sweden, June 19, 1985.

18. Oglesby, et al, 4th International Conference on Environmental Mutagens, Stockholm, Sweden, June 19, 1985.

19. Holland, et al. J. Appl. Toxicol. 4, 117-123, 1984.

20. Pelroy, et al. In Synthetic Fossil Fuel Technologies, Results of Health and Environmental Studies, Butterworth Publishers, Boston, 267-302, 1984.

21. Russell, Proc. Nat. Acad. Sci. 79, 3589-3591, 1982.

22. Russell, Proc. Nat. Acad. Sci. 79, 3592-3593, 1982.

23. Russell, 4th International Conference on Environmental Mutagens, Stockholm, Sweden, June 19, 1985.

Barry and Litchfield, In: Progress in Mutation Research, volume 5 (Ashby and de Serres, eds.), Elsevier Science Publishers, Amsterdam. pp 727-740, 1985.