January 5, 1978

From: Harry L. Skalsky, Ph.D.

To: Dr. E. C. Irby - EXO

cc: H. M. Cole - GOB

Subject: CARCINOGENIC MATERIALS REGULATION, OSHA

I have reviewed the carcinogenic identification and classification system proposed by the Occupational Safety and Health Administration (42 F.R. 54148) and have found it scientifically deficient.

1. It fails to address the subject of DNA repair.
2. It fails to recognize a threshold level for carcinogens.
3. It fails to consider that use of high dose testing can lead to unusual biotransformations.
4. It fails to demonstrate how animal tests can be designed for extrapolation to man.
5. It fails to distinguish between in vivo and in vitro tests, by giving them equal regulatory emphasis.

It is to these points that the following rebuttal and recommendations are submitted.

Harry L. Skalsky, Ph.D.

HLS/mm

Att.

SUMMARY

A need has been expressed by O.S.H.A. for the identification, classification and regulation of materials constituting a potential carcinogenic risk in the workplace. O.S.H.A. states emphatically that its present staff is not able to cope with the volume of cases before them. "At the outset, O.S.H.A. recognizes that some 1500 to 2000 agents have been identified by the National Institute for Occupational Safety and Health (N.I.O.S.H.) as being 'suspect carcinogens' ... Yet, O.S.H.A. has completed regulatory activity for only 17 of those substances since its creation on April 29, 1971." (42 FR 54148) To facilitate this rule-making procedure, O.S.H.A. has presented its present classification scheme for public review. O.S.H.A. claims and/or intends that their proposal is based on the best scientific evidence available. It is to this point the following rebuttal is submitted:

1) O.S.H.A. fails to address the subject of DNA repair.

DNA repair is one of the most pertinent areas of cancer research today. Deoxyribonucleic Acid (DNA) is the basic "blueprint" of the cell. It contains the directions of how each area of the cell is to be built and run. It is believed that the primary events leading to cancer are related to a direct modification of the structure of DNA, resulting in a change in the normal pattern of cell development. A variety of chemicals (carcinogens) have been shown to modify the structure of DNA and lead to the formation of cancer. O.S.H.A. has failed to consider the other side of the story - that each cell of the body has a variety of mechanisms that repair damage to DNA and maintain the integrity of the genetic material.

Testing policies of F.D.A. and O.S.H.A. were designed on the premise that there is no minimum level of a carcinogen. Animal experiments with

carcinogens continually demonstrated a "no effect" level as a dose below which no tumors were formed. This "no effect" data was ignored because there was no scientific basis for it. It was feared that it was the result of too small a sample of test animals. However, now that we have established the existence of a defense mechanism in the body that protects us against exposure to low levels of chemical carcinogens, these ideas must be re-evaluated. By failing to address the subject of DNA repair, O.S.H.A. cast serious doubt on the scientific basis of their proposal.

2) O.S.H.A. does not consider a threshold level for carcinogens.

Threshold is defined by Webster as the point at which a physiological or psychological effect begins to be produced. This is a universal principle of biology, whether it is the degree of stimulation of a nerve which just produces a response, the concentration at which glucose in the blood just begins to pass the barrier of the kidneys and enters the urine, or the dose at which a chemical just elicits a toxic response. Thresholds exist for every biological phenomena. O.S.H.A., as a matter of policy (42 FR 54165), and F.D.A., as required by law ("Delaney Clause"), do not recognize a minimum level of a carcinogen. However, recent advances in cellular biology indicate that this position should be modified. With the demonstration of an error-free repair system (excision repair) in mammalian cells, there is now a scientific basis for a no-effect level of a carcinogen. A scientific group of The World Health Organization called for the consideration of the existence of a threshold for carcinogens in 1974. Why, in 1978, has O.S.H.A. failed to even discuss this matter?

3) O.S.H.A. fails to consider that use of high dose testing can lead to unusual biotransformations.

The adherence by F.D.A. and O.S.H.A. of the use of high dose testing is based on the premise that there is no minimum dosage of a carcinogen. Testing of this type uses the Maximum Tolerated Dose (MTD) or the dose at which the animal is just able to survive. MTD doses are justified to maximize the possibility of detecting a carcinogen with a small number of test animals. Establishment of a threshold concept for carcinogens requires discarding the rationale of high dose testing. When O.S.H.A. accepts DNA repair, it must no longer recognize testing at the maximum tolerated dose.

MTD testing can also be criticized on other grounds. In any physiological system, a biochemical pathway may become overloaded and a secondary system will be activated. Unless specific effort is made to establish that the test compound is being metabolized in the same manner at both low and high doses, it can be assumed that the maximum tolerated dose effect is an artificial system with no extrapolative value.

4) O.S.H.A. fails to demonstrate how animal tests can be designed for extrapolation to man.

These two subjects must be treated as one. There is no reason to test a particular animal species if the results cannot be extrapolated to man. O.S.H.A. has stated that, "rodents are the animals of choice for carcinogenesis tests because of their convenience, comparatively short life span and proven susceptibility to a broad range of carcinogenic agents" (42 FR 54160). This arbitrary choice of a rodent model on the basis of convenience has led to an impasse concerning the extrapolation of animal data to man. Zapp (J. Tox. & Environ. Health, 2:1425, 1977) states that

every extrapolation involves a conjectured knowledge of an unknown area by inferences based on an assumed parallelism between it and what is known. In choosing proper animal models for man, O.S.H.A. should justify their choice by: (1) demonstrating that their model deals with carcinogens in a parallel fashion to man, and (2) indicating the "biological distance" between the parallel lines. Much of this type of comparative information is in the literature and needs only to be found and interpreted.

A proper animal standard must be similar to man in the biotransformation of carcinogens and rate of DNA repair. Rodents cannot always be the animal of choice.

5) O.S.H.A. fails to distinguish between in vivo and in vitro tests, by giving them equal regulatory emphasis.

"O.S.H.A. proposes that the combination of positive results in short-term tests and a positive result for carcinogenesis in a single dioassay in a mammalian test species should provide sufficient evidence for classifying a substance as a Category I toxic material" (42 FR 54167). This regulatory decision by O.S.H.A. is at variance with that of the F.D.A. which has taken the position that in vitro methods are not an appropriate basis for regulatory action (41 FR 26842). The proposed classification scheme equates an in vitro or "test tube" assay with an in vivo or whole animal test. This is scientifically unsound. Various reviewers and the National Cancer Advisory Board (42 FR 54167) have stated that in vitro methods are effective pre-screening procedures and suggest that long-term animal bioassay follow any in vitro positive result. It is suggested that in vitro testing be used only as a pre-screening technique and/or as a tool to define the genetic and biochemical properties of test animals.

Introduction

A need has been expressed by O.S.H.A. for the identification, classification, and regulation of materials constituting a potential carcinogenic risk in the workplace. O.S.H.A. states emphatically that its present staff is not able to cope with the volume of cases before them. "At the outset, O.S.H.A. recognizes that some 1500 to 2000 agents have been identified by the National Institute for Occupational Safety and Health (N.I.O.S.H.) as being 'suspect carcinogens' ... Yet, O.S.H.A. has completed regulatory activity for only 17 of those substances since its creation on April 29, 1971." (42 FR 54148) To facilitate this rule-making procedure, O.S.H.A. has presented its present classification scheme for public review. O.S.H.A. claims and/or intends that their proposal is based on the best scientific evidence available. However, they have conspicuously ignored an area of research pertinent to carcinogenesis, i.e., DNA repair. It is accorded one minor citation of the National Cancer Advisory Board (42 FR 54167). The failure to recognize the importance of DNA repair casts some doubt on the scientific soundness of the proposal as a whole. All present research knowledge must be utilized in the selection of appropriate models. It is to this and other points that the following comments are addressed.

REPAIR

O.S.H.A. clearly states (42 FR 54167) that its reliance upon short-term mutagenic tests is based upon the assumption that cancer can be related to genetic alterations and that detection of such changes is indicative of carcinogenicity. While O.S.H.A. supports the use of tests that detect chemically-induced genetic alterations, it fails to recognize the fact that cells have the ability to repair this genetic damage. A brief overview of DNA repair will add a degree of balance to this issue. Complete reviews on the subject of DNA repair may be found elsewhere (Beers et al., "Molecular and Cellular Repair Processes", John Hopkins Press, 1972; Howard-Flanders, Brit. Med. Bull., 29:226, 1972; Setlow, Ann. Rev. Biophys. Bioeng. 1:203, 1972; Cleaver, Adv. Radiat. Biol., 4:1, 1974; Hanawalt and Setlow, "Molecular Mechanism for Repair of DNA", Plenum Press, New York, 1975; Hart and Trosko, "Cellular Aspects of Aging: Concepts and Mechanisms", Karger, Switzerland, 1976; Maher et al., "In Vitro Metabolic Activation in Mutagenesis Testing", Elsevier/North Holland Biomed. Press, Amsterdam, 1976; Roberts, "Scientific Foundations on Oncology", Heinkenan Med. Book, Ltd., London, 1976; Van Laneker, Current Topics in Path., 64, 1977).

Historically, repair processes have been studied in prokaryotes for over a decade (Boyce and Howard-Flanders, Proc. Nat. Acad. Sci., 51:293, 1964; Setlow and Carrier, Proc. Nat. Acad. Sci., 51:226, 1964; Pettijohn and Hanawalt, Mol. Biol., 9:395, 1964). Intensive investigation of repair processes in mammalian cells is more recent. This upsurge of interest was the result not only of a general movement toward the study of eukaryotic organisms, but also the recognition that cells from patients with the cancer-predisposing disease xeroderma pigmentosum (XP) showed decreased or absent

DNA repair of ultraviolet radiation damage (Cleaver, Nature, 213:652656, 1969). XP patients are characterized by extreme susceptibility to multiple carcinomas of the skin on areas exposed to sunlight. The link between defective DNA repair and the clinical manifestations of the XP disease was confirmed (Robbins et al., Ann. Internal Med., 80:221-248, 1974; Cleaver, Proc. Nat. Acad. Sci., 63:428-435, 1969; Cleaver, "Advances in Radiation Biology", Academic Press, New York, 1974; Burk et al., J. Lab. Clin. Med., 77:759-767, 1971; Kleijer et al., Mut. Res., 20:417-428, 1973), and subsequently established the importance of DNA repair in the prevention of human carcinomas.

DNA repair, though a simple concept, is not a simple process. Oftentimes, the term DNA repair is misleading due to its simplicity. There are at least three general types of repair of DNA damage in mammalian cells: (1) excision repair; (2) strand-break repair; (3) post-replication repair. A fourth form of repair which is specific for the monomerization of UV-induced cyclobutane dimers (Sutherland, Nature, 248:109-112, 1974; Sutherland et al., Proc. Nat. Acad. Sc., 72:103-107, 1975) has also been demonstrated. In addition to the types of repair, the concept of error must also be considered. Errors introduced into DNA not only by physical damage but also during the repair process itself are important. These are extensively reviewed by Hart and Trosko (1976); Maher et al., (1976); Roberts (1976); and Vanlancker (1977); (all cited P.2).

Excision repair involves the removal of damaged parental DNA by a complex of enzymes. The damaged segment is replaced by DNA templated off the opposite strand. This process is capable of repairing most lesions that produce large distortions in the double helix. Thymine dimers caused by

UV-radiation and chemical adducts such as acetylaminofluorene and the epoxides of the polycyclic hydrocarbons (benzo(a)pyrene, dimethylbenz(a)-authracee, 3-methylcholantrene, etc.) are dealt with by this system. Excision repair is the most extensively studied DNA repair mechanism in mammalian cells. It is the predominant error-free process in human cells treated with either radiation (UV and ionizing) or chemicals (Maher et al., "In Vitro Metabolic Activation in Mutagenesis Testing", E/N.H.B. Press, Amsterdam, 1976; Lohman et al., Mut. Res., 46:95-164, 1977).

Strand-break repair is characterized by the ability to rejoin single-strand breaks. It is also capable of nucleotide insertion in which the "patch" size is much smaller than after damage that induces excision repair. Low molecular weight alkylating agents (methylating or ethylating) produce damage which is thought to be repaired by this system. These agents produce a variety of base adducts, chiefly on guanine and adenine moieties. These alkylated bases are removed from the DNA strand by either chemical depurination and/or enzymatic recognition and repair via the strand-break system. Due to experimental difficulties, little is known of the relationship of error to repair in this system.

Post-replication repair is the least understood of the various types of repair in mammalian cells. This system is thought to deal with damaged DNA (pyrimidine dimers, etc.) that may remain after excision repair. During DNA replication (S phase), it is assumed that the replication complex copies the parental strand until blocked by a lesion on one template strand and that replication of the complementary strand continues past the point of the lesion (Higgins et al., J. Mol. Biol., 101:417-425, 1976). Lehman (J. Mol. Biol., 66:319, 1972) proposes a gap-filling mechanism for post-

replication, repair is associated with recombinational events and appears to be error-prone. Recombinational events have not been clearly demonstrated in mammalian cells; thus it is assumed that their post-replication repair system differs from that in bacteria. One interesting feature of post-replication repair is the curious behavior of caffeine which apparently inhibits post-replication repair in rodent cells but not in most human lines.

It is necessary to view DNA repair as a dynamic process in which various repair mechanisms can co-exist in a given cell. The phase of the cell cycle (G vs. S) as well as the state of differentiation of the tissue in which cell damage occurs might favor the competitive edge of one repair system over another. Regardless of the complexities involved, one fact is undeniably true -- the more effective a cell is in the repair of genetic damage, the less sensitive it is to the deleterious effect of environmental agents. This is a major point O.S.H.A. has failed to address.

Recent findings on rates of removal of different alkylated bases from DNA of rats treated with nitroso alkylating agents in vivo strongly indicate that various DNA repair processes at the molecular level do occur in the intact animal (Goth and Rajewsky, Proc. Nat. Acad. Sci., 71:639-643, 1974; Margison and Kleihues, Biochem. J., 148:521-525, 1975; Nicoll et al., Nature, 264:624-627, 1976). These findings have obvious implications for the existence of defense mechanisms in the body against exposure to very low levels of chemical carcinogens and mutagens.

Testing policies of F.D.A. and O.S.H.A. were designed on the premise that there is no minimum level of a carcinogen. Animal experiments with carcinogens demonstrate a "no effect" level as a dose below which no tumors were formed (Bingham, Studies for The Aluminum Association, 1974). This

"no effect" data was ignored because there was no scientific basis for it and because it was feared to be the result of too small a sample of test animals. However, now that we have established the existence of a defense mechanism in the body that protects us against exposure to low levels of chemical carcinogens, these ideas must be re-evaluated. By failing to address the subject of DNA repair, O.S.H.A. casts serious doubt on the scientific basis of their proposal.

THRESHOLD

Threshold is defined by Webster as the point at which a physiological or psychological effect begins to be produced. This is a universal principle of biology whether it is the degree of stimulation of a nerve which just produces a response, the concentration at which glucose in the blood just begins to pass the barrier of the kidneys and enters the urine, or the dose at which a chemical just elicits a toxic response. Thresholds exist for every biological phenomena. O.S.H.A., as a matter of policy (42 FR 54165), and F.D.A., as required by law ("Delaney Clause"), do not recognize a minimum level of a carcinogen. However, recent advances in cellular biology indicate that this position should be modified. With the demonstration of an error-free repair system (excision repair) in mammalian cells, there is now a scientific basis for a no-effect level of a carcinogen. It is conceivable that the large amount of negative data that has been generated in the animal testing of carcinogens and the presence of "no effect" levels could be the result of the repair phenomena. A scientific group of The World Health Organization called for the consideration of the existence of a threshold in 1974 (Wld. Hlth. Org. Techn. Rep. Ser.,

1974, No. 546). Why, in 1978, has O.S.H.A. failed to even discuss this matter?

HIGH DOSE TESTING

The adherence by F.D.A., E.P.A., and O.S.H.A. of the use of high dose testing is based on the premise that there is no minimum dosage of a carcinogen. Testing of this type uses the maximum tolerated dose (MTD) or the dose at which the animal is just able to survive. MTD doses are justified to maximize the possibility of detecting a carcinogen with a small number of test animals. Establishment of a threshold concept for carcinogens requires discarding the rationale of high dose testing. When O.S.H.A. accepts DNA repair, it must no longer recognize testing at the maximum tolerated dose.

MTD testing is also criticized on other grounds. In any physiological system, a biochemical pathway may become overloaded and/or the secondary system will be activated. Young and Gehring (Toxicol. Appl. Pharmacol. 33:183, 1975) demonstrated a dramatic increase in the excretion of unchanged dioxane in the expired air as a function of dose, suggesting that the hazard from a high dose of dioxane may be disproportionately greater than from a lower dose. The pharmacokinetic and metabolic fate of 1,4 dioxane was later shown to be dose dependent (Young et al., Toxicol. Appl. Pharmacol., 37:138, 1976). As the intravenous dose of dioxane to rats was increased (10 to 1,000 mg/kg) the percentage of dose excreted as hydroxyethoxyacetic acid (HEAA) (major urinary metabolite, Braun and Young, Toxicol. Appl. Pharmacol. 39:33, 1977) decreased from 92% of a 10 mg/kg dose to 60% of a 1,000 mg/kg dose, while excretion of

dioxane increased from 5 to 38%. Young et al. concluded that the decreased percentage of the dose excreted as HEAA with increasing doses of dioxane and the reduced plasma clearance indicated that the primary mechanism for nonlinear elimination of dioxane was the saturation of its metabolism to HEAA, rather than saturation of an excretory pathway. Thus toxicity of dioxane occurs only when doses are sufficient to saturate the metabolic pathway for its detoxification.

The possibility of changes in the pharmacodynamics or metabolism of a compound has been ignored in MTD testing. With the amount of toxic stress on a test animal at the MTD, logically, changes in metabolism would be expected. That these parameters have not been measured discredits any data from MTD testing. Therefore, unless specific effort has been made to establish that a test compound is metabolized in the same manner at both low and high doses, it cannot be assumed that data obtained at the maximum tolerated dose has any extrapolative value.

PROPER ANIMAL MODEL - EXTRAPOLATION TO MAN

These two subjects must be treated as one. There is no reason to test a particular animal species if the results cannot be extrapolated to man. O.S.H.A. has stated that, "rodents are the animals of choice for carcinogenesis tests because of their convenience, comparatively short life span and proven susceptibility to a broad range of carcinogenic agents" (42 FR 54160). This arbitrary choice of a rodent model on the basis of convenience has led to an impasse concerning the extrapolation of animal data to man.

Zapp (J. Tox. & Environ. Health, 2:1425, 1977) states that every

extrapolation involves a conjectured knowledge of an unknown area by inferences based on an assumed parallelism between it and what is known. In choosing proper animal models for man, O.S.H.A. should justify their choice by: (1) demonstrating that their model deals with carcinogens in a parallel fashion to man, and (2) indicating the "biological distance" from "mouse to man". It is unreasonable to assume that a proper animal model can be chosen without some scientific measure of this distance. Much of this type of comparative information is in the literature and needs only to be found and interpreted. For example, in vitro methods can be used to choose in vivo models. There are in vitro methods currently available that could provide valuable insight into the comparability of an animal model with the human situation, both on the basis of metabolism and the ability to repair genetic damage.

Weekes and Brusick (Mut. Res., 31:175, 1975) demonstrated that in vitro mutagenesis techniques are useful tools for investigating the genetic and biochemical properties of different animal species. They compared the relationship between target organ susceptibility for dimethylnitrosamine (DMNA) induced tumors and in vitro metabolic activation of DMNA to a mutagen. In mice, DMNA induces tumors predominantly in liver and lung tissue with lower frequencies in kidney and only rarely in the spleen or gonads (Takayama and Oota, GANN, 54:465, 1963 and GANN, 56:189, 1965; Terracini et al., Brit. J. Cancer, 20:871, 1966; Clapp et al., Cancer Res., 31:197, 1971). The activation of DMNA to a mutagenic intermediate by microsome preparations from these organs of BALB/cJ, RF/J and C57Bl/6J mice show the same relationship (liver > lung > kidney > spleen > testes) suggesting that for this carcinogen, the level of metabolic activa-

tion of a tissue may be directly related to the susceptibility of that organ to tumor induction. While the liver and lung tumor susceptibilities of most mouse strains to DMNA are roughly similar, there are striking differences with respect to the incidence of renal tumors. C3H and Swiss mouse strains exhibit renal tumor incidences of 16 and 11%, while BALB/c and RF mice are resistant to renal tumors with frequencies lower than 4%. When microsomal preparations from the kidneys of these four mouse strains were compared (Weekes, J. Nat. Cancer Inst., 5:1199, 1975), the microsomes from the susceptible C3H and Swiss strains produced the highest level of mutagen. Mutagen production by liver and lung microsomes from all four strains were similar. Weekes examined the kidney size and the protein content of the microsomes. No substantial differences were found and it was assumed that the metabolic activity of the microsomal enzymes obtained from the kidney cells of the susceptible and resistant strains were in some way different.

Along similar lines, deSerres (Mut. Res., 1976) used the defined carcinogen 2-acetamidofluorene (AAF) to compare microsomal preparation from six animal species.

Comparative Mutagenesis for AAF[a]
Using Activation Tissues (9000 x g Fractions)
from Six Mammalian Species

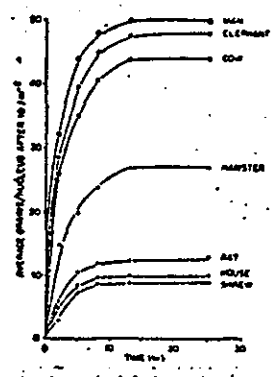
Species[b]	Strain	Organ	Revertants of TA-1538 per 10^8 surviving cells (X 10^{-3})[c]
MOUSE	ICR random bred.	Liver	27.4
		Kidney	587.5
		Lung	3.2
RAT	Sprague-Dawley	Liver	39.9
		Kidney	3.5
		Lung	1.0
GUINEA PIG	Strain 13 Inbred	Liver	272.3
		Kidney	43.5
		Lung	5.1
RABBIT	New Zealand White	Liver	222.1
		Kidney	3.3
		Lung	0.5
DOG	Mixed Breed	Liver	160.37
		Kidney	11.15
		Lung	5.81
MONKEY	Rhesus	Liver	11.0
		Kidney	14.3
		Lung	0.7

[a]AAF concentration for all assays was 800 µg/ml. Treatment was at 37°C for 60 min.
[b]All animals were males.
[c]The spontaneous frequencies were subtracted from each value.

Among the six species tested, four (mouse, rat, dog, and rabbit) were susceptible to the carcinogenic effects of AAF. Monkeys fed AAF for up to eight years failed to develop tumors. No lesions suggestive of neoplasia were found at autopsy (O'Hara and Adamson, "Pathology of Simian Primates", Karger, New York, 1972, Page 190). Guinea pigs probably form a mutagenic intermediate (other than N-hydroxy-AAF; Weisburger and Weisburger, Pharmacol. Rev., 25:2, 1973) that has no carcinogenic properties.

Recently, Grasso et al. ("The Mouse and Carcinogenicity Testing", B.I.B.R.A. Carshalton, England, 1977) cast strong doubts on the wisdom of using mice for carcinogenicity studies. He states that differences in metabolism between mouse and man have generally been ignored in conducting bioassay programs. The example of AAF mentioned previously points out that rodents would not serve as a good predictor of the affects of AAF on primates. It also demonstrates an in vitro method that can be used to choose the proper in vivo model.

In determining the proper in vivo model, the parameter of DNA repair must also be considered. Clearly, an animal that is efficient at repair would not be as susceptible to a low dose of carcinogen as an animal that was repair deficient. Hart and Setlow (Proc. Nat. Acad. Sci., Vol. 11, No. 6, 1974) compared the rates of excision repair in fibroblasts from seven species.



The average amount of unscheduled synthesis as a function of time after 10 J/m² for seven different species. Each point is obtained from the average of the individual distribution curves as shown in figure.

It was concluded that the rate of unscheduled DNA synthesis was approximately proportional to the logarithm of the life span of the DNA repair. Comparative rates of repair not only for different species, but also for different organs within a species, could be accomplished through tissue culture techniques. Magee (J. of Toxicol. and Environ. Health, 2:1415, 1977) mentions the use of fresh tissue slices and other methods for comparing metabolism. However, the point must be made that there are sufficient variety of in vitro techniques to aid in the choice of a "proper" in vivo model for a particular test compound. The best scientific evidence available should be used to demonstrate that an in vivo model is similar to man in the biotransformation of the test compound and in the rate of DNA repair.

SHORT-TERM TESTS

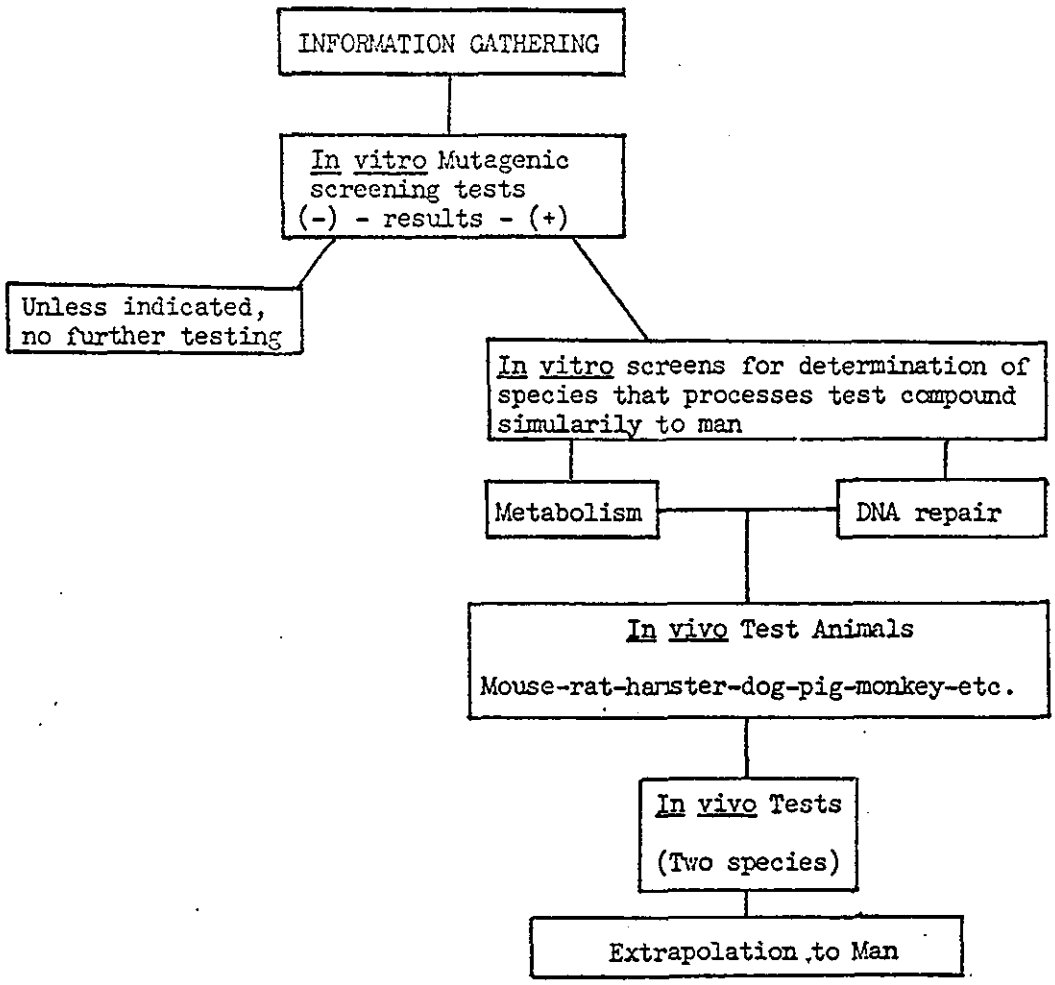
"O.S.H.A. proposes that the combination of positive results in short-term tests and a positive result for carcinogenesis in a single bioassay in mammalian test species should provide sufficient evidence for classifying a substance as a Category I toxic material". This regulatory decision by O.S.H.A. is at variance with that of the F.D.A., which has taken the position that in vitro methods are not an appropriate basis for regulatory action (41 FR 26842). The proposed classification scheme equates an in vitro assay with a whole animal test. This is scientifically unsound. The route of exposure in the in vitro system does not simulate human exposure and there is no assurance that the metabolic activation in vitro accurately represents in vivo activation and detoxication. There is, also, no way to evaluate comparative pharmacokinetics or dose levels in the

in vitro system. Various reviewers (Brusick, Clin. Toxicol. 10(1):79, 1977; Rosenkranz *et al.*, "*In Vitro* Metabolic Activation in Mutagenesis Testing", Elsevier/North Holland Biomed. Press, Amsterdam, 1976) and the National Cancer Advisory Board (42 FR 54167) have stated that *in vitro* methods are effective pre-screening procedures and suggest that long-term animal bioassay follows any *in vitro* positive result. It is suggested that *in vitro* testing be used only as a pre-screening technique and/or as a tool to define the genetic and biochemical properties of test animals.

RECOMMENDATIONS

1) Perhaps the most critical problem in carcinogenesity testing is how to choose an appropriate animal model for a particular test compound. It is imperative that we be able to extrapolate this data to man. Thus, a system is needed to determine how to choose the proper animal test system. The Interagency Toxicology Committee should provide a wide enough data base to solve such a problem. The I.T.C. was established to provide continuity between O.S.H.A., F.D.A., E.P.A., and C.P.S.C., all of which draw information from N.C.I., N.C.T.R., and N.I.E.H.S. Thus, the I.T.C. provides a wider data base than O.S.H.A. and is perhaps more able to solve such a difficult problem.

2) It is also suggested that the following general scheme be used in the decision process of choosing the proper animal for a particular compound:



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Two points that must be emphasized: (1) In vitro tests are used as a screening procedure and to define the best animal species. (2) At least two animal species must be used for final assessment. With the extensive biochemical definition of a test species and its relation to man, safety assessments would have a more scientific basis and it could help to explain a negative test in one species while the same compound gives a positive result in another.