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SHORT-TERM BIOASSAY OF COMPLEX ORGANIC MIXTURES: PART II, MUTAGENICITY TESTING*

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INTRODUCTION

The feasibility of using short-term mutagenicity assays to predict the potential biohazard of various crude and complex test materials has been examined in a coupled chemical and biological approach. The principal focus of the research has involved the preliminary chemical characterization and preparation for bioassay, followed by testing in the *Salmonella* histidine reversion assay described by Ames (1). The mutagenicity tests are intended to (a) act as predictors of profound long-range health effects such as mutagenesis and/or carcinogenesis, (b) act as a mechanism to rapidly isolate and identify a hazardous biological agent in a complex mixture, and (c) function as a measure of biological activity correlating baseline data with changes in process conditions. Since complex mixtures can be fractionated and approached in these short-term assays, information reflecting on the actual compounds

responsible for the biological effect may be accumulated. Thus, mutagenicity tests will (d) aid in identifying the specific hazardous compounds involved and in establishing priorities for further validative testing, testing in whole animals, and more definitive chemical analysis and monitoring.

Our work has emphasized test materials available from the developing synthetic fuel technologies (2). However, the procedures are applicable to a wide variety of industrial and natural products, environmental effluents, and body fluids. The general applicability of microbial test systems has already been demonstrated with, for example, the use of the assay as a prescreen for potential genetic hazards of complex environmental effluents or products, e.g., tobacco smoke condensates (3), natural products (4, 5), hair dyes (6), soot from city air (7), fly ash (8), and, in our work with synthetic fuel technologies, oils and aqueous wastes (9, 10).

BIOASSAY METHOD

For the study of application of mutagenicity testing to environmental effluents and crude products from the synthetic fuels technology we performed preliminary screening with the highly sensitive Ames histidine reversion strains known to respond to a wide variety of known mutagens/carcinogens. The working hypothesis was that sensitive

detection of potential mutagens in fractionated complex mixtures could be used to isolate and identify the biohazard. In addition, the information could be helpful in establishing priorities for further testing, either with other genetic assays or carcinogenic assays.

The Salmonella strains used in the various assays are listed below. All strains were obtained through the courtesy of Dr. Bruce Ames, Berkeley, California.

Salmonella typhimurium Strains

TA1535 hisG46, uvrB, rfa (missense)

TA100 hisG46, uvrB, rfa (missense plus R factor)

TA1537 hisC3076, uvrB, rfa (frameshift)

TA1538 hisD3052, uvrB, rfa (frameshift)

TA98 hisD3052, uvrB, rfa (frameshift plus R factor)

In the screening of fractionated materials, the two strains TA98 and TA100 were generally employed. Standard experimental procedures have been given by Ames et al. (7). Briefly, the strain to be treated with the potential mutagen(s) is added to soft agar containing a low level of histidine and biotin along with varying amounts of the test substance. The suspension containing approximately 2×10^8 bacteria is overlaid on minimal agar plates. The bacteria undergo several divisions with the reduced level of histidine, thus forming a light

lawn of background growth on the plate and allowing the mutagen to act. Revertants to the wild-type state appear as obvious large colonies on the plate. The assay can be quantitated with respect to dose (added amount) of mutagen and modified to include "on-the-plate" treatment with the liver homogenate required to metabolically activate many compounds.

Fractions and/or control compounds to be tested were suspended in dimethyl sulfoxide (supplied sterile, spectrophotometric grade from Schwarz/Mann) to concentrations in the range of 10-20 mg/ml solids. The potential mutagen was in some cases assayed for general toxicity (bacterial survival) with strain TA1537. Normally, the fraction was tested with the plate assay over at least a 1000-fold concentration range with the two tester strains TA98 and TA100. Revertant colonies were counted after 48 h incubation. Data were recorded and plotted versus added concentration, and the slope of the induction curve was determined. It is assumed that the slope of the linear dose-response range reflects the mutagenic activity. Positive or questionable results were retested with a narrower range of concentrations. All studies were carried out with parallel series of plates plus and minus the rat liver enzyme preparation (7) for metabolic activation. Routine controls demonstrating the sterility of samples, enzyme or rat liver

S-9 preparations, and reagents were included. Positive controls with known mutagens were also included in order to recheck strain response and enzyme preparations. All solvents used were non-mutagenic in the bacterial test system.

SAMPLES

Samples that have been tested and their sources are listed below: (1) coal-liquefaction product from a process under development, courtesy of the Pittsburgh Energy Research Center (Synfuel A), or Coal A from ORNL repository; (2) coal-liquefaction product from the COED Pyrolysis Process, courtesy of FMC (Synfuel B), or Coal B from ORNL repository; (3) Louisiana-Mississippi sweet crude oil, courtesy of Dr. J. A. Carter of the Analytical Chemistry Division, Oak Ridge National Laboratory; (4) composite crude oil sample from materials obtained through the courtesy of Dr. Dee Latham of the Laramie Energy Research Center; (5) a crude shale-oil sample (B) from the above-ground simulated in situ oil-shale retorting process; (6) the aqueous product water consisting of the centrifuged water of combustion from the same process (both samples 5 and 6 courtesy of the Laramie Energy Research Center); (7) a coal-gasification aqueous condensate from a process under development, courtesy of Pittsburgh Energy Research Center; and (8) a separator liquor from a coal-liquefaction process, courtesy of FMC. The authors

recognize the possibility that these samples may bear no relationship to the process as it may exist in the future, nor should it be construed that these materials are representative of all natural crudes, synthetic or shale-oil processes. They are used here simply as appropriate and available materials for the research.

BIOASSAY RESULTS

Class Fractionation

Oil Samples. The bulk of the samples listed above were subjected to the fractionation scheme described by Swain et al. (11), as modified by Bell et al. (12). The scheme is described in detail as applied to oils in Rubin et al. (13) and in Part I of this presentation. As an example, a summary of the results from a sample of Synfuel A-2 (9) is given in Table 1. Subfractionation results are shown with the neutral fraction chromatographed on a Florisil column. The column was eluted with the solvents shown and, with this sample, collected in one fraction. The data includes the analytical weight analysis of the sample (column 1) along with the specific mutagenic activity (slope of dose-response curve) of each fraction (column 2). The product of these (column 3) represents a weighted value of each fraction relative to the contribution to the starting test material. Mutagenic activity is seen in both the acidic and basic fractions along with the neutral subfractions. However,

Table 1

Distribution of Mutagenic Activity of Synthetic Oil[a] (Synfuel A-2)

Fraction[b]	Relative weight (% of total)	Specific activity[c] (rev/mg)	Weighted activity[d] (rev/mg)
1. $NaOH_I$	20.9	1700	356
2. WA_I	2.2	180	4
3. WA_E	4.9	1260	62
4. SA_I	<0.1	30	0
5. SA_E	0.4	130	1
6. SA_W	0.4	120	1
7. B_{Ia}	6.8	38700	2633
8. B_{Ib}	0.1	1270	1
9. B_E	2.0	36200	725
10. B_W	0.6	570	3
Neutral	69.2	583 (570)[e]	403
TOTAL	107.6		4189
Neutral Subfractions			
Hexane	72.7	340	244
Hexane/benzene	5.0	710	35
Benzene/ether	19.8	1360	270
Methanol	2.3	1460	34
Subtotal	99.8		583
Initial sample, g Chromatographed, g	26.166 10.664		

[a]All assays carried out in the presence of crude liver S-9 from rats induced with Aroclor 1254.

[b]I = insoluble (fractions a and b), E = ether soluble, W = water soluble, WA = weak acid, SA = strong acid, B = base.

[c]rev/mg = revertants/mg, the number of histidine revertants from Salmonella strain TA98 by use of the plate assay with 2×10^8 bacteria per plate. Values are derived from the slope of the induction curve extrapolated to a milligram value. NT = not tested.

[d]Weighted activity of each fraction relative to the starting material is the product of columns one and two. The sum of these products is given as a measure of the total mutagenic potential of each material.

[e]Comparable to "specific activity," but based on the activity of the total neutral fraction rather than the summation of the individual fraction.

the major contributors to the mutagenicity appear to occur in the basic fractions, with activities also consistently present in the neutral materials.

A comparison of these activities and the total mutagenic potential of the various oil and aqueous samples is given in Table 2. Reasonable reproducibility is seen in similar samples, e.g., Synfuel A-1 and A-2; Synfuel B-1 and B-2. Synfuel A-3 represents the same material without prior centrifugation of the solids. The consistency of activities seen in all oils considered is illustrated. On a relative scale, the synthetic fuels show more mutagenic activity than the natural crude "control" samples shown. Shale oil appears to be only slightly higher than the natural crudes. References are given to the complete published compilations on these samples. See Table 2.

Each determination represents the slope of the dose-response curve. All testing was carried out in the presence of the rat-liver microsomal activation system. Slight mutagenic activity without enzyme treatment was occasionally noted.

The routine screening utilized strains TA100 (missense) and TA98 (frameshift); however, complete strain-specificity tests were carried out with selected materials. Fractions giving a positive response with strain TA98 were, in general, also positive with the other frameshift strains, TA1537 and TA1538. Additionally, positive results were

Table 2

Summary of Mutagenicity Testing Results with Synthetic Oils and Aqueous Samples - Class Fractionation Scheme[a]

Sample	SA_W		B_E		Neutral		Total Wt. Act. (rev/mg)	Reference
	Rel. Wt. (%)	Spec. Act. (rev/mg)	Rel. Wt. (%)	Spec. Act. (rev/mg)	Rel. Wt. (%)	Spec. Act. (rev/mg)		
Composite Crude-1	0.1	400	0.2	150	84.2	277	241	(9)
Composite Crude-2	0.1	750	0.2	500	84.2	166	147	(10)
La-Miss Crude	0.1	240	0.2	180	80.7	90	76	(9)
Shale Oil	0.6	160	7.1	952	86.7	112	178	(10)
Synfuel A-1	0.3	240	2.0	28900	73.6	517	4032	(9)
Synfuel A-2	0.4	120	2.0	36200	69.2	583	4189	(9)
Synfuel A-3	0.3	1010	3.1	43300	56.4	1094	7308	(9)
Synfuel B-1	0.4	0	2.6	1500	82.3	560	516	(9)
Synfuel B-2	1.6	0	1.8	3800	89.3	465	484	(9)
Separator Liquor 1.3 w/v%	53.9	0	0.5	850	1.5	0	17	unpubl.
Gasifier Condensate 0.9 w/v%	30.8	0	0.5	4000	1.9	100	211	(14)
Process Water (shale Oil) 1.0 w/v%	65.0	0	2.7	1575	2.4	52	68	(10)

[a]Strain TA98 with metabolic activation with Aroclor-induced preparation.

routinely noted with the sensitive missense strain TA100; however, reversion of the missense strain TA1535 was rare. TA98 appeared to be the best general indicator of mutagenic activity of these materials. Furthermore, liver preparations from rats induced with Aroclor 1254 (a gift of Monsanto) showed the best general applicability. However, individual differences in effectiveness do occur; for example, variously induced preparations show obvious differences between basic fractions and e.g., the neutral/methanol fraction (9). An Aroclor-induced preparation reacts best with the neutral fraction (polynuclear aromatic hydrocarbons?), while a phenobarbital-induced preparation works more efficiently with the basic fraction (heterocyclic nitrogen compounds?).

Primary candidates for the mutagens (and carcinogens?) responsible for activity in the basic fractions include quinoline, substituted quinolines, alkyl pyridines, acridine, naphthylamines, aza-arenes, benzacridines, and aromatic amines; in the neutral fractions potential threats may consist of benzanthracenes, dibenzanthracenes, substituted anthracenes, benzopyrenes, benzofluorenes, pyrene, substituted pyrenes, and chrysenes (see Ho et al., 15). Thus, work with these pure compounds is being carried out concurrently.

Reproducibility of results was shown by comparison of data from similar samples. Although discrepancies exist from fraction to

fraction, the general trend is apparent, and the sum of activities appears to be roughly reproducible. Again, when the major-component, neutral fraction is assayable, as with the Synfuel A, the summation of the subfraction values of the neutrals reflects the approximate additivity of the individual mutagenic determinations. For example, 570 revertants/mg with a direct assay of the neutrals from Synfuel A-2 compares with 583 revertants/mg based on the summation (Table 1).

An overview of the results points to a number of consistencies: (1) all crudes and Synfuels showed some mutagenic potential; (2) the neutral and basic fractions showed activities regardless of the source of the sample; and (3) the relative total mutagenic potentials varied over two orders of magnitude. Whether these results reflect a comparative biohazard of processes still under development is not the point in question here. The results simply show that biological testing - genetic reversion assays in this case - can be carried out with the newly developed tester systems, but only when coupled with the appropriate analytical separation schemes. Conceivably, the use of this approach could provide rapid information concerning health effects.

Aqueous Samples. Table 2 also lists sample results from a group of aqueous samples subjected to the class fractionation procedure ("Stedman procedure"). In general, greater activity is seen in the more

polar, more water soluble fractions rather than in the non-polar neutral materials. Caution has to be extended on work with any aqueous material because of the high potential for instability. Although we have used organic extraction here, techniques with resin concentration, e.g., XAD-2 may prove useful with aqueous samples (16, 17). Only in exceptional cases is the mutagenic activity directly observable in an unconcentrated sample.

Liquid Chromatographic Fractionation

In the initial studies with coal liquefaction products, the crude oils were fractionated using the scheme originally developed for cigarette smoke condensates ("Stedman procedure"). The scheme yields class separations based on the relative acid-base properties of the components. The samples are partitioned between ethyl ether and 1 N NaOH in a single-stage, continuous procedure to yield an aqueous acid fraction and organic phase base and neutral fractions. The organic fraction is extracted with 1 N HCl to yield an aqueous basic fraction and an organic basic fraction and an organic neutral fraction. The neutral material is subsequently subfractionated on a florisil column. These primary subfractions are then subjected to mutagenicity testing.

Realizing the potential for modification of the components within the procedure, we moved to consideration of a number of other methods. The fractionation procedure using Sephadex LH-20 can provide a gentle and

large-scale class separation for (initially) crude oils from shale oil and coal liquefaction processes. The procedure involves three steps using the gel in different modes: (1) lipophilic-hydrophilic partitioning; (2) molecular size separation; and (3) aliphatic-aromatic separation. The procedure (18) was designed by Jones, Guerin, and Clark of the Analytical Chemistry Division. Using fractions prepared as above, we have started a comparison of this procedure and the Stedman procedure for usefulness in preparation for bioassay. The preliminary mutagenicity studies confirm the suitability and utility of the method. Table 3 summarizes some of the results from shale oil. The method appears to be generally applicable to complex organic mixtures and achieves the goal of presenting a gentle and rapid separation scheme, useful with large-scale samples.

Subfractionation of Neutral Components. The polycyclic aromatic hydrocarbons (PAH) presumably occurring in the neutral fractions of the various schemes noted have been listed as major contributors to mutagenicity of the test materials. With natural crudes, these components appear to account for the bulk of the activity. With synthetic crudes, the contributions of both the basic and neutral fractions, must be considered. Further work is needed to define (isolate and identify) the mutagenic components of these important classes.

Table 3

Sephadex LH-20 Fractionation of Shale Oil Coupled With Mutagenicity Testing

Test Material-Fraction	% of Total	Specific[a] Activity (rev/mg)
Crude Oil	100	233
Hydrophilic	6	1300
Lipophilic	93	196
- Polymer	5	54
- Hydrogen-Bonding	5	1040
- Sieved	84	100
- Polymer	1	0
- Aliphatics	60	180
Aromatics		
- 1 and 2 Ring	14	24
- 3 and 4 Ring	5	132
- Polynuclear	4	1220

[a]Slope of dose-response curve with *Salmonella* strain TA98 plus rat-liver preparation induced with Aroclor 1254.

We have carried out a preliminary study with synfuel PAHs subfractionated and detected by the short-term mutagenicity assay. As shown in Table 4, shale oil (Sample B) can be separated into lipophilic and hydrophilic fractions with the Sephadex LH-20 partition chromatography described previously (18, 19). Further separation of the lipophilic fraction is achieved by neutral alumina and LH-20 using various solvents. The various subfractions can then be assayed for mutagenicity with the Salmonella histidine reversion system. As seen in the Table 4, activity seems to peak in the 4- and 5-ring subfractions, those containing known carcinogens/mutagens as benzo(a)pyrene, benzo(c)phenanthrene, and 3-methylcholanthrene.

Subfraction of Basic Components. Again, considering the results with class fractionation procedures, we developed a procedure (20) specifically designed for subfractionation of the basic materials, now realized to be a major contributor to mutagenic activity. An elution sequence using alumina and Sephadex LH-20 gel with a combination of solvents isolates 90% of the mutagenic activity from basic compounds into 0.5 wt% fraction of crude oil.

A basic alumina column eluted first with benzene and then ethanol isolates the mutagenic components of the ether soluble base fractions (ESB) of synthetic crude oils into a fraction of about 25 wt% of the ESB.

Table 4

Subfractionation of Neutral Components From Shale Oil: Distribution of Polycyclic Aromatic Hydrocarbons and Mutagenic Activity

Subfraction	Weight %	Specific activity[a] rev/mg of fraction	
		Without S-9	With S-9
Aromatic Fraction	100	60	170
I (polymeric)	5.7	0	0
II (1-ring)	47.0	0	0
III (2-ring)	33.7	0	0
IV (3-ring)	8.0	0	1000
V (4-ring)	2.7	1600	4000
VI (5-ring)	0.6	2600	3800
VII (>5-ring)	0.5	600	1500
TOTAL	98.2	62	214

[a]Number of histidine revertants from Salmonella strain TA98 by use of plate assay with 2×10^8 bacteria per plate. Values derived from slope of the induction curve. "With S-9" indicates test carried out in presence of crude enzyme preparations from rats induced with Aroclor 1254.

A further separation is achieved by eluting the ethanol isolate through a Sephadex LH-20 gel column with isopropanol followed by acetone. About 90% of the basic mutagenic activity is recovered in the acetone subfraction which comprises ∿0.5 wt% of the crude oil. Development of this separation scheme was made possible using the Ames microbial mutagenesis assay as the detector during exploratory liquid chromatographic separations. Table 5 lists some of the preliminary data from these studies.

COMPARATIVE MUTAGENESIS

In order to validate and compare the results accumulated in the Ames system with complex test materials from synthetic fuel technologies, we selected specific fractions or subfractions on the basis of their activity in the histidine reversion assay for further testing in the various other tests designed to detect mutagenicity. Preliminary results have been published in the Proceedings of the Second International Conference on Environmental Mutagens, Edinburgh, 1977 (21). For the purposes of a qualitative comparison, the results are given in Table 6. The selected fractions or subfractions utilized were basic and neutral isolates from synthetic crude oils from coal liquefaction processes [Synfuel A and B as described in Epler, et al. (9)]. With *Drosophila* (22) and in the mammalian cell gene mutation assay (23), the detection has been a function of newly developed

Table 5

Subfractionation of Basic Fraction:

Distribution by Weight and Mutagenic Activity

	Shale oil		Synfuel A-3	
	wt%	rev/mg[a]	wt%	rev/mg[a]
Basic fraction (A)	100	2,500	100	30,000
Alumina				
Benzene (B)	78	600	76	0
Ethanol (C)	---	---	---	---
LH-20				
Isopropanol (D)	12	0	12	0
Acetone (E)	10	20,000	12	222,000

[a]Assayed with Strain TA98 with Aroclor-induced preparation.

Table 6

Comparative Mutagenesis of Fractions From Synthetic Crude Oils[a]

Test system	Assay	Basic fraction	Neutral fraction	Crude[b] synfuel
Salmonella	$his^- \rightarrow his^+$	+	+	+
E. coli	$arg^- \rightarrow arg^+$ $gal^- \rightarrow gal^+$	+ +	+ +	NT NT
Yeast	$his^- \rightarrow his^+$ $CAN^s \rightarrow can^r$	+ +	+ +	NT NT
Drosophila	SLRL	+	-	NT
CHO cells	6-thioguanine resistance	+	NT	NT
Human leukocytes	chromatid aberrations	P	+?	NT
Mouse	dominant lethals	-	P	+
Carcinogenesis	skin painting[c]	P	P	P

[a]For references to published work or work in progress see text. The fractions utilized were generally those from Synfuel A-3, or Synfuel B-2. + = mutagenic; - = nonmutagenic; NT = not tested; and P = in progress.

[b]Crude synfuels are generally too toxic to test in most systems.

[c]Work of J. M. Holland, Oak Ridge National Laboratory, in progress.

fractionation schemes (e.g., the use of LH_{20}) (39, 40), that result in higher specific activity (more highly purified) mutagenic subfractions. In general, the results validate the initial screening carried out in the Salmonella assay but these other systems have not as yet been used to exhaustively test materials that are negative in the Ames system. Note, also, however, that the preliminary results of Generoso (personal communication) show that the crude synthetic fuel does induce dominant lethals in mice although the basic fraction alone appears to be negative.

For the comparative studies with microbial systems given here, we selected four synfuel fractions. The results with the frameshift strain TA98 with metabolic activation were considered. Fractions 6 [strong acid, water soluble (SA_W)]; 7 [base insoluble, fraction A (B_{Ia})]; 9 [base, ether soluble (B_E)]; and 14 (neutrals/methanol) were selected on the basis of their ability to revert the Ames strains. In order to validate the mutagenicity results obtained from the Salmonella histidine-reversion system, we extended the treatment with the selected test fractions to the E. coli 343/113 system of Mohn (24). The results obtained in the forward (gal^+) and reverse-mutation (arg^+) assays with E. coli support the results obtained with Salmonella. Both the basic fraction (#9) and the neutral subfraction (#14) are mutagenic upon metabolic activation with Aroclor-induced rat-liver homogenate (S-9).

Further validation of the bacterial results was obtained by assaying for both forward mutation and reverse mutation in the yeast system (21, 25). The Synfuel A fractions tested were weakly mutagenic and were effective without metabolic activation. Some antagonistic effects were encountered when metabolic activation was incorporated. The most active fraction, the ether soluble bases (B_E), also reverted the putative frameshift marker, hom3-10. This fraction may contain acridines and other nitrogen heterocyclics. Unpublished results from our group have pointed to similar effectiveness without activation in the Salmonella system when suspension tests rather than plate assays are used with crude mixtures.

To ascertain the comparative effectiveness in the human leukocyte chromatid aberration assay, we treated with selected test fractions from Synfuel B. The test materials used were the neutral subfractions and represent largely polycyclic aromatic hydrocarbons. The coal fractions were suspended in DMSO at a concentration of 20 mg/ml total solids. Two hours of control treatment with 5% DMSO and treatment with the four subfractions (Neutrals as Hexane, Hexane/benzene, Benzene/ether, and Methanol subfractions) over a concentration range of 0.1 - 1.0% (20 μg-200 μg) were ineffective in producing chromatid aberrations (1600 cells scored). However, metabolic activation was not included with

any exogenous enzyme source nor are the assumed constituents - PAH's - effective as chromosome breaking agents. Work with other fractions and the inclusion of metabolism is in progress.

Selected test fractions from Synfuel B were assayed in the Drosophila sex-linked recessive-lethal (SLRC). Fraction 13 (neutral/benzene/ether) is slightly effective as a mutagen for Drosophila at the higher concentrations fed.

Several other syncrude fractions which were scored as mutagenic in the Salmonella assays were tested in Drosophila. (All of the fractions require metabolic activation in the Salmonella assay.) Of the five fractions tested, only 12 and 13 gave any indication of an effect. Additionally, the highly active (in Salmonella) basic sub-fraction from the procedure previously described was tested. This basic material showed a significant dose-dependent response in the Drosophila SLRL assay. (see discussion by Nix and Brewen, these proceedings, reference 22.)

In conclusion, short-term tests with bacterial and fungal mutagenicity assays appear to detect effectively the mutagenic potential of complex environmental or industrial effluents; however, chemical fractionation is necessary to reduce toxicity and concentrate hazardous materials. Extension of the results to higher organisms, i.e., mammalian cells, Drosophila, and the mouse appears to be valid but needs more testing.

CONCLUSIONS

In these initial feasibility studies, the purpose has not been to reflect on whether a relative biohazard exists in comparison with other materials or processes. The results show that biological testing-within the limits of the specific system used-can be carried out with complex organic materials but perhaps only when coupled with the appropriate analytical separation schemes. An extrapolation to relative biohazard at this point would be, at least, premature. The primary use that such combined chemical and biological work may serve is to aid in isolating and identifying the specific classes or components involved. A number of precautions are listed below.

The detection or perhaps the generation of mutagenic activity may well be a function of the chemical fractionation scheme utilized. The inability to recover specific chemical classes or the formation of artifacts by the treatment could well corrupt the results obtained in addition to the possibility of an inability to detect the specific biological endpoint chosen. Along with the obvious bias that could accompany the choice of samples and their solubility or the time and method of storage, a number of biological discrepancies can also enter into the determinations. For example, concomitant bacterial toxicity can nullify any genetic damage assay that might be carried out; the

choice of inducer for the liver enzymes involved can be wrong for selected compounds; the choice of strain could be inappropriate for selected compounds; and additionally, the applicability of the generally used Salmonella test to other genetic endpoints and the validation of the apparent correlation between mutagenicity and carcinogenicity still remains a point of significant fundamental research. Furthermore, the short term assays chronically show negative results with, certain substances, e.g., heavy metals and certain classes of organics. Similarly, compounds involved in or requiring co-carcinogenic phenomena would presumably go undetected.

However, as a prescreen to aid the investigators in ordering their priorities, the short-term testing appears to be a valid testing approach with complex mixtures. Over-interpretation at this stage of research especially with respect to relative hazard or negative results should be avoided.

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