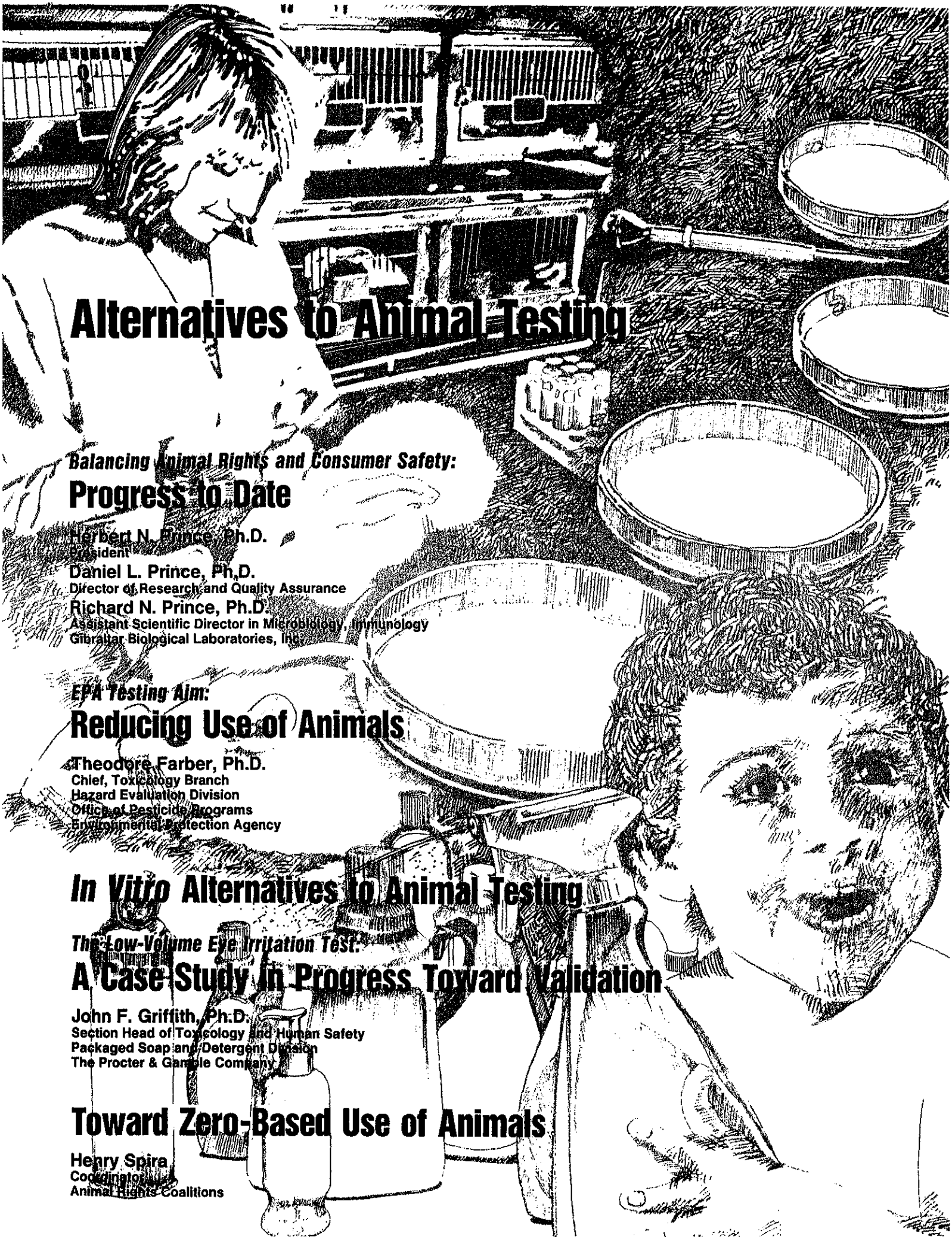

Alternatives to Animal Testing

Balancing Animal Rights and Consumer Safety: Progress to Date

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EPA Testing Aim: Reducing Use of Animals

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In Vitro Alternatives to Animal Testing

The Low-Volume Eye Irritation Test: A Case Study in Progress Toward Validation

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Toward Zero-Based Use of Animals

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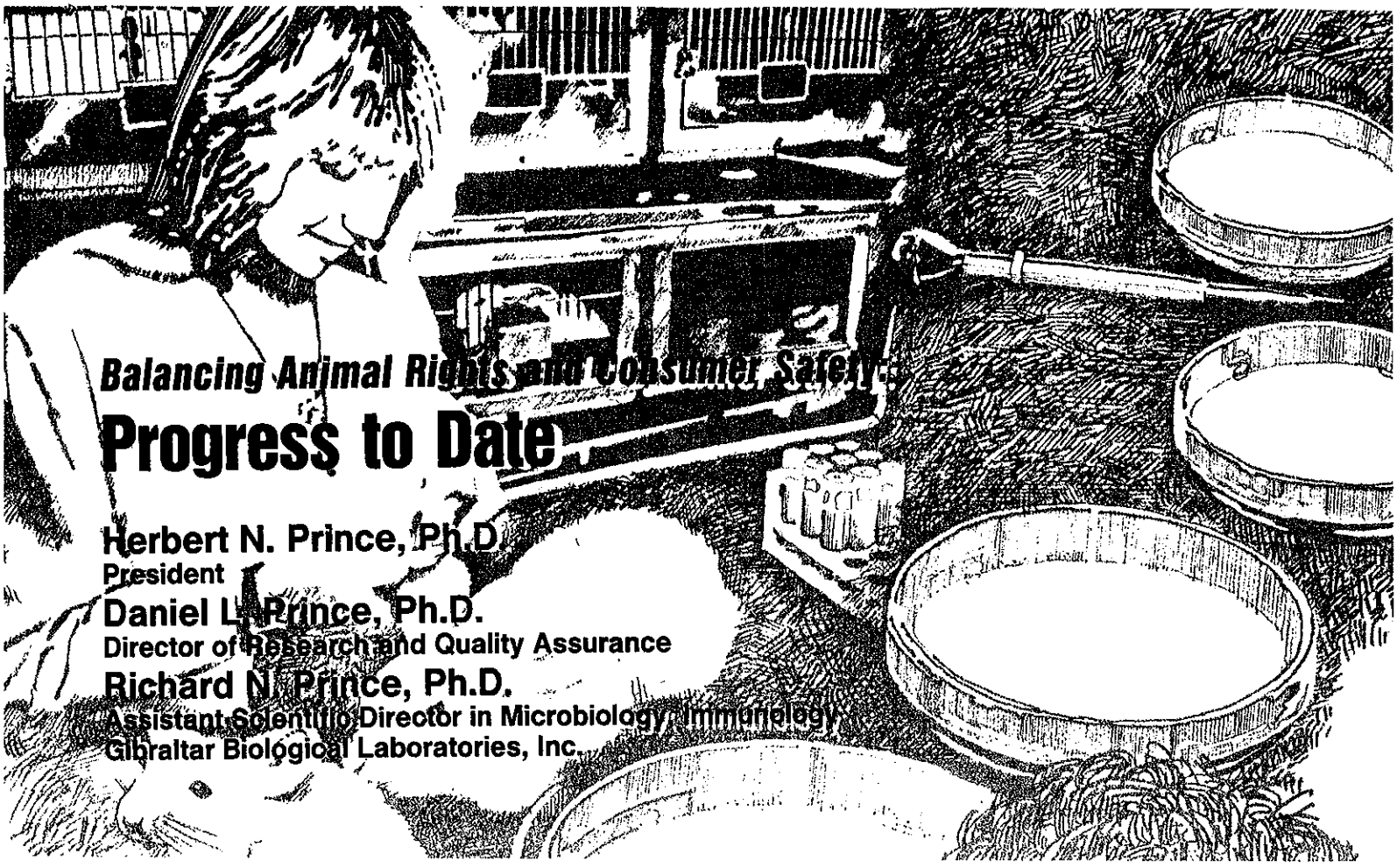

Balancing Animal Rights and Consumer Safety:

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Toxicology is the science of surrogate victims. Its sister science, pharmacology, is the science of surrogate patients. The toxicologist takes a healthy animal, exposes it to test compounds, and observes for signs of injury or sickness. The pharmacologist, on the other hand, works with injured or sick animals and attempts to make them healthy.

Manufacturers of specialty chemicals, disinfectants, and cosmetics are not responsible for curing sickness. Rather, these industries are interested in producing useful and safe products to be used in the household, workplace, or in the environment as pesticides. Accordingly, manufacturers, consumers, and government authorities all rely upon toxicologists for safety assessment. Because of the uncertainties associated with extrapolating from animals to man, toxicologists face a difficult task.

Is the toxicologist by education and training qualified to sacrifice animals so as to answer the question "Is my product safe?" Today, toxicologists are increasingly pensive about their duties and responsibilities.

Do they have the right to deliberately make an animal sick? Do they have the right to sacrifice animals? Are there viable alternative methods?

Who is more important, the animal or the consumer? Can the consumer be protected without sacrificing animals? Can the consumer be protected with fewer animals?

Can the toxicologist make these judgments without pressure from the outside? How powerful is the

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toxicologist? Where does the power come from? As of the moment, the toxicologist, a practicing biologist, has extraordinary power. It comes from a society that either makes demands on government and industry or gives up its right to sue. The latter is unlikely.

The balance between animal rights and consumer rights in a free and orderly society revolves around either the doubtful or unalienable right of the consumer to have safe products—be they products of need or products of convenience. Thus, the animal stands either at the altar of need or convenience. Is this fair? The operative word becomes, therefore, "sacrifice."

Whose rights do we sacrifice? The dictionary says that to sacrifice is ". . . to forego something of value for something that is perceived to be of greater value." An animal is something of value. But as a surrogate victim, whether breathing our air, drinking our water, using a product, or being injected with a drug or vaccine, it must defer to something of greater value, a noninjured human.

But we must not ask the animal to defer forever; not until we have exhausted all means at replacement refinement, or reduction. For almost 7 years we have seen a worldwide attempt.

"The balance between animal rights and consumer rights in a free and orderly society revolves around either the doubtful or unalienable right of the consumer to have safe products—be they products of need or products of convenience, thus the animal stands either at the altar of need or convenience."

What are the results? A composite of the types of systems that have been attempted is given in Table I. Progress has been slow and limited to screening for topical irritation.

TABLE I
Mechanism Chart of Alternative Toxicity Assays as the Animal or Microbial Cell Responds to Injury[a]

Cytological (biocidal)		
A. Cell culture cytopathology (CPE)	Eye irritation	Cosmetics, topical drugs, devices,
1. HTD	Skin irritation	dental products, detergents, germi-
2. MEM elution	Vaginal irritation	cides, consumer products
3. Direct inoculation	Buccal irritation	
4. Agar overlay		
B. Tissue		
5. Chick embryo CAM		
C. Inverterbrate metazoan		
6. *Artemia* (brine shrimp)		
Diffusion		
Inhibition of "uptake" or enhancement of "exiting" in a cell culture system	Eye irritation Vaginal irritation	Cosmetics, topical drugs, germicides, consumer products
1. NR_{90} (dye)		
2. UI_{50} (metabolite)		
3. DAc-fluorescein		
Physiological		
Chemotactic response	Eye irritation	Cosmetics, topical drugs
Histamine release	Vaginal irritation	
Prostaglandin release (submorphological signs of irritation)	Buccal irritation (systemic target organs mechanism studies)	
Exfoliative		
Washing of corneal epithelium after injury	Eye irritation	Cosmetics, topical drugs
Release		
Cell culture isotopic	Buccal irritation	Devices, dental products
Chromium membrane release	Systemic implants	
Submorphologic cell membrane		
Bacterial point mutation		
Ames test	Mutagens, carcinogens	Raw materials, chemicals, drugs
Antimicrobial (MIC or zone of inhibition)		
Yeast photoxicity	Skin irritation	Pesticides, intermediates, many products
GBL gram positive/negative screen		
Bacterial DNA damage		
E. coli—DNA repair	DNA-reactive screening	As above
Chromatid exchange, point mutation, DNA damage		
Friedlander *Saccharomyces* assay, cell cultures—fruit fly	Genotoxic and DNA-reactive screening	As above
Organ culture		
Rat hepatocytes	Carcinogens, irritants	As above

[a] Other assays are being developed within the United States and Europe. This is only a partial compilation.

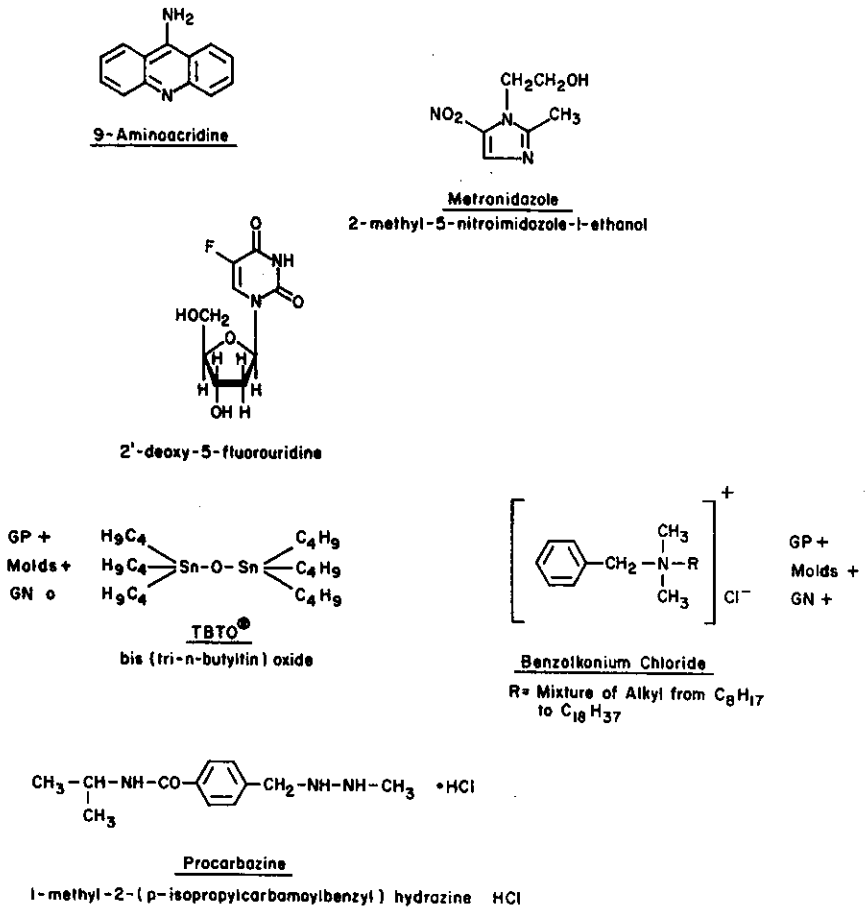

Figure 1 Compounds tested in the *Artemia* assay.

No animal models for public or consumer safety have been replaced yet as a result of this effort. That is how difficult the task appears to be. The medical community has not put a single drug or vaccine into clinical trial without animal testing.

No product liability cases have been set aside in favor of the manufacturer because *not performing* a safety test in animals has the same standing as performing the test. Probably no activist who has broken into a laboratory, destroying records and property, has also given up his rights to sue a manufacturer for injury from an "unsafe" product. No government agency has accepted an *in vitro* test to replace an animal.

But nonetheless, practically none of today's toxicologists, under pressure to be sure from responsible animal groups, have given up in this search for complete alternatives. The search continues, but it will remain difficult and slow. Patience is the key and timetables are not advisable. In the biological sciences, methods development followed by collaborative studies and peer review is a process that takes 10 or more years. Let's review the progress to date.

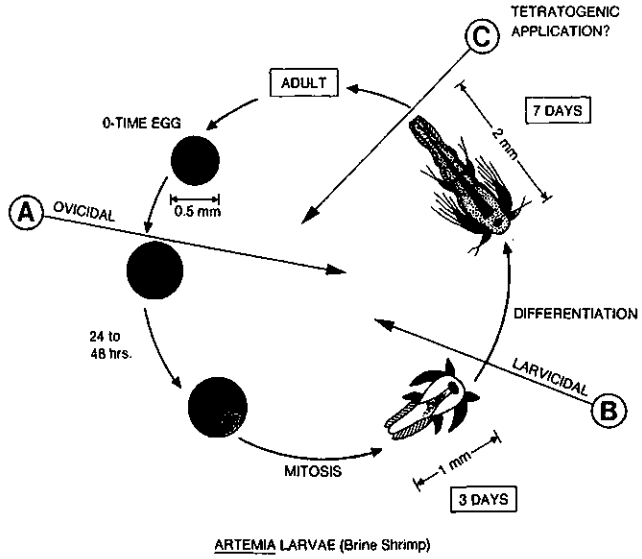

Figure 2 *Artemia* assay. In germination, the eggs are placed into artificial sea water. No feeding is necessary in the *Nauplius* and *Cypris* larval stages. To the adult stage and beyond, the cultures are fed with green algae, such as *Chlorella, Chalmydomonas, Scenedesmus*, etc. Introduction of the xenobiotic is made at stages A, B, or C. The xenobiotic is titrated for toxicity as in standard minimum bactericidal concentration assays.

Chronic Toxicity

No alternatives exist or have been attempted that will replace a 2-year study in a rat or dog. The question is: What will prolonged high, medium, or low exposure to any agent do to the central nervous system, functional organs, or hematopoietic system of humans? How can you predict where and what the results will be when the agent is given topically, orally, or parenterally? The same can be said for subchronic testing, where no alternative assay can predict the systemic effects of 90 days of feeding, inhalation, or dermal exposure. Progress in this area awaits the development of parallel *in vitro* tests that simulate the evolutionary complexity of metazoan forms, i.e., animals having many interrelating cells, tissues, and organs.

TABLE II
***Artemia* as a Screening Model for Biocidal Agents**

Substance	Minimum Larvicidal Concentration
	ppm
Metals	
TBTO	1.56
Copper	25.0
Antimicrobials	
Benzalkonium chloride	16.0
PVP-I_2	32.0
Phenol	64.0
Trichomonicides	
9-Aminoacridine	250–500
Metronidazole (Flagyl)	1000
Antitumor	
Procarbazine	250
Aminopterin	500
Fluorodeoxyuridine	1000

TABLE III
***In Vitro* Cytotoxicity Assay of Parenteral Drugs[a]**

Formulation	Minimal Toxic Concentration (MTC) of Active Ingredient		
	WI-38 Fibroblasts	MRC-5 Fibroblasts	HEP-2 Epithelioma
		μg/ml	
Thorazine injectable	8	16	4
Valium injectable	150	100	75
Verapamil	64	65	32
Hydrocortisone acetate	>2500	>2500	>2500

Method	Ranking of Toxicity		
	Most Toxic	Intermediate	Least Toxic
Human injection	Thorazine	Valium	Hydrocortisone acetate
Animal injection	Thorazine	Valium	Hydrocortisone acetate
Cytotoxicity Assay	Thorazine	Valium	Hydrocortisone acetate

[a] The drugs were dissolved in minimal essential medium (MEM) and fed in 0.1 ml amounts to tubes or microtiter plates containing confluent cell sheets, incubated at 35°C, and observed microscopically. Toxicity was assessed by morphological scoring of the cell sheet, cytotoxic effect (CTE), with and without staining. Staining with a vital dye or crystal violet increases the sensitivity of the assay and permits semiquantitative measurement. The morphological scoring criteria used here and with the direct contact assay (Table VI) is: cytotoxic effect scoring system: 0 = no toxicity, cell sheet indistinguishable from the control; 1 = cells are granular or are less refractile; no cells are lysed; 2 = at least 50% of the cell sheet is granular or there are lysed cells with sloughing; 3 = 75% of the cell sheet is destroyed; 4 = monolayer completely destroyed. Semiquantitative results are obtained by measuring the absorption of neutral red[10] or the integrity of the cell sheet by rapid staining with 0.04% Gram's crystal violet.

TABLE IV
***In Vitro* Antibacterial Assays of Thorazine Injectable and Valium Injectable: Gram-Positive Species[a]**

Formulation	Minimal Inhibitory Concentration of Active Ingredient		
	Staphylococcus aureus	*Bacillus subtilis*	*Streptococcus pyogenes*
Thorazine injectable	50	50	25
Valium injectable	320	320	320
Cortisone acetate	>1000	>1000	>1000

[a] The minimal inhibitory concentration (MIC) method is a classical technique in microbiology. The xenobiotic is dissolved in a solvent and serial dilutions are prepared in bacterial growth medium (trypticase soy broth, TSB, at 35°C). A standard number (100,000 organisms) of the microorganism is inoculated into each tube or well. In this experiment, aerobic incubation was at 35 ± 1°C for 48 hr. The MIC end point is the concentration of the xenobiotic in which there is no turbidity. Neutralization and solvent toxicity controls were run in parallel.

Teratology

No alternative assay exists or has been attempted that will simulate the extraordinarily complicated series of events that go from ingestion in the gastrointestinal tract of the woman to transport through the hepatic portal system to distribution to the right heart, the lungs, the left heart, the dorsal aorta, the uterine arteries, the endometrium, the placental barrier, the fetus, the one-, two-, three-, or four-chamber fetal heart to the differentiating epithelial, fibroblastic, ectodermal, entodermal, osseous, or cartilagenous tissue of the skull or spinal cord, palate, hind limbs, or forelimbs of the fetus. Some advances have been made in chromosome breakage studies in yeast and *Drosophila*, but the animal still must be used for confirmation.

"No product liability cases have been set aside in favor of the manufacturer because *not performing* a safety test in animals has the same standing as performing the test."

TABLE V
Activity of Sodium Lauryl Sulfate against Gram-Positive and Gram-Negative Bacteria Minimal Inhibitory Concentration End Point

Organisms	Sodium Lauryl Sulfate		Tween 20 (Polysorbate 20)		Benzalkonium Chloride	
E. coli	50,000	[a]	250,000		10	
Ps. aeruginosa	50,000		30,000		20	
Proteus mirabilis	50,000		30,000		40	
Sal. pullorum	50,000 (A)		500,000		20	
TA 100 Ames	50,000		250,000		0.63	
TA 1535 Ames	50,000		50,000		1.5	
E. coli 190	50,000		250,000		20	
E. coli 191	50,000		250,000		2.5	
Acinetobacter	50,000		500,000		5.0	
S. aureus (P.S.)	31	[a]	250,000	[c]	0.4	[b]
S. aureus (P.R.)	62	[a]	30,000	[c]	0.81	[b]
S. aureus (TSS)	62		30,000		0.62	
S. epidermidis	62		30,000		0.31	
S. pyogenes	8		125,000		0.31	
Strep. pneumoniae	8	[e]	2,000		0.31	
B. pumilus	31		500,000		0.25	
Lactobacillus casei	16		500,000		1.25	
Garderella vaginale	1.0		2,000		0.31	
Candida albicans	1.25		500,000		10.0	
Aspergillus niger	62		500,000		10.0	

[a] Gram-negatives were resistant to sodium lauryl sulfate (>50,000 ppm).
[b] Gram-positives and gram-negatives were both sensitive to benzalkonium chloride (average = 7 ppm).
[c] Gram-positives and gram-negatives were both resistant to Tween 20 (average = 200,000 ppm).
[d] P.S., penicillin-sensitive; P.R., penicillin-resistant.
[e] Gram-positives were sensitive to sodium lauryl sulfate (average value = 31 ppm).

TABLE VI
Direct Contact Assay Cytotoxicity of Sodium Lauryl Sulfate to MRC-5 Cells (Human Diploid Fibroblast)[a]

Concentration[b]	Microscopic Reading of Room Temperature Incubation at[c] 3–5 min	2 hr	6 hr	24 hr
ppm				
10,000	+	+	+	+
5,000	[+]	+	+	+
2,500	0	[+]	+	+
1,000	0	0	+	+
500	0	0	+	+
250	0	0	[+]	[+]
125	0	0	0	0
Cytotoxic Onset (minimum toxic concentration)	5000	2500	250	250

[a] Sodium lauryl sulfate was suspended in sterile water. Two-fold serial dilutions were performed and 0.1 ml pipeted/well. Four replicates were made per concentration. Incubation was at 35 ± 1°C with 6–8% CO_2. Scoring was by CTE as in Reference 7 and Table III.
[b] Dose was 0.1 ml/1.0 ml of MEM culture.
[c] + = cytotoxic (granulation, rounding, crenation, lysis); 0 = no effect.

TABLE VII
Effect of Sodium Lauryl Sulfate in the Chick Embryo Chorioallantoic Membrane Test (9 to 10 Days)[a]

Sodium Lauryl Sulfate	Result (No. Dead/ No. Treated)
%	
10	4/4[b]
5.0	4/4[c]
2.5	2/4[d]
1.0	All alive and normal

[a] The chorioallantoic membrane was dropped by induction of negative pressure. The shell above the shell membrane was carefully removed; 0.1 ml of formulation was placed on the surface of the chorioallantoic membrane. The shell opening was sealed with transparent tape. Incubation was at 35 ± 1°C for 3–5 days.
[b] Thrombosis and blanching.
[c] Thrombosis of sinus vessels.
[d] Thrombosis and necrosis.

Carcinogenicity

Very little progress has been made. Bacteria do not get cancer. Cell cultures die out or become transformed if they are continually propagated *in vitro*. Taking the cells from the intact animal to the test tube unleashes strange forces that put cells and animals worlds apart. Prediction becomes a risky business. Cancer can't be predicted but *in vitro* testing can give guidance. The intact animal is still required and will be for many years.

Mutagenicity

Excellent progress has been made, especially with the continued refinement of the Ames test[1] for spontaneous reversion in *Salmonella* and sister chromatid exchange in Chinese hamster cultures and fruit flies. The various EPA tiers of testing continue to offer hope that time, money, and animals will be saved as the result of genotoxicological research. The Ames test continues to offer excellent evidence for specific classes of compounds that carcinogens indeed exert their effect by mutation.

Topical and Local Irritation

Because of the work done at Rockefeller University,[2,3] grants from Johns Hopkins University,[4] and

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some of the work done in our own laboratory[5–7] and elsewhere, cell culture and certain bacterial assays have given us the ability to accurately predict what substances or formulations will cause eye irritation and intramuscular irritation. Pharmacological successes have also been reported[4] as the mechanisms of liver function, prostaglandin irritation, and protein synthesis are probed in cell and organ cultures. Continued success is likely in this area. Less success has been obtained in the area of skin irritation. Good results have been achieved in phototoxicity,[8] wherein a yeast culture can predict results in the guinea pig. An *Artemia* assay (brine shrimp)[9] did not predict antimitotic events but does offer some promise for local effects, and in our laboratory the use of a battery of gram-positive and gram-negative bacteria can predict the irritating potential of nonionic, anionic, and cationic detergents.

Results of Studies with Diverse Agents and Sodium Lauryl Sulfate

The following tables and figures describe results in our laboratory with alternative assays that can be of aid in ranking the topical effects from the skin, eye, vaginal, and intramuscular administration of disinfectants, sporicides, insecticides, drugs, herbicides, and cleansers. The strategy, which is limited to topical effects, is to reduce the number of animals in the primary screen and then use the intact animal for the final demonstration of safety for labeling and regulatory guidance. An alternate strategy has been to test small numbers of agents in a battery of animal tests—not to reduce animal numbers, but to select the most sensitive animal.

Figure 1 shows a series of compounds tested in the *Artemia* brine shrimp model.

Figure 2 shows a life cycle of the model, which is designed to determine effects in rapidly dividing cells as a guide to wound healing, bone marrow depression, or biocidal effects. Table II depicts representative results. The greatest effect was seen with the tributyl tin compound (TBTO).

Thus, *Artemia* will successfully predict antifoulant activity and it has been used in our laboratory to predict activity against barnacles. The *Artemia* model shows that the greater the irritant potential the lower the inhibitory concentration. The

TABLE VIII
Draize Primary Irritation Score Obtained with Various Concentrations of Sodium Lauryl Sulfate[a]

SLS	Score	Remarks
%		
20	6.7	Eschar and corrosion persisting to 4 weeks; erythema and edema equal
15.0	3.5	Reversible erythema and edema
10.0	1.7	Transient erythema and edema
7.5	0.0	No effect
5.0	0.0	No effect

[a] 0.5 ml by patch to intact and abraded skin of six albino rabbits for 24 hr followed by observations for erythema and edema at 24 and 72 hr with calculation of the primary irritation score by the method of Draize (Consumer Product Safety Commission; FHSA). Similar results were obtained with the Subdivision F Assay, Series 81-4 (EPA-FIFRA).

TABLE IX
Guinea Pig Immersion Tests[a]

Sodium Lauryl Sulfate	Score	Results
%		
0.25	3.3[b]	Toxic
0.125	5.4[c]	Toxic
0.0625	8.1[d]	Borderline
0.037	9.6[e]	Negative

[a] Tests were for 4 hr in 37°C bath for 3 consecutive days read on day 6. Score of 9 or 10 is negative.
[b] With death, abdominal distention, and dermal lesions.
[c] Moderate effect; scurffing and fissuring (average 19 determinations = 5.4, range is 4.3 to 6.3, SD is 1.15).
[d] Minimal irritation (slight scaling and/or necrosis).
[e] Virtually no effect (range of 0 to 10).

TABLE X
Eye Irritation (Rabbit) Induced by 0.1 ml of Sodium Lauryl Sulfate per Eye[a]

	2 hr		CPSC[b] Fractional Score at				Corneal Opacity at			
Concentration (%)	Discharge	FS[c]	2 hr	Day 1	Day 3	Day 7	2 hr	Day 1	Day 3	Day 7
20.0	3/3	3/3	3/3	3/3	3/3	3/3	0/3	1/3	2/3	3/3
15.0	3/3	3/3	3/3	3/3	3/3	3/3	0/3	1/3	2/3	3/3
10.0	3/3	3/3 2/3	3/3	3/3	3/3	3/3	0/3	0/3	3/3	3/3
5.0	3/3	2/3	2/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
1.0	3/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
0.5	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
							0/3	0/3	0/3	0/3

[a] This table shows the extreme sensitivity of the Draize eye irritation test. Employing the presence of discharge at 2 hr, the test detected irritation at 1.0%; fluorescein staining at 2 hr at 5.0%, gross CPSC score at 5.0%, and corneal opacity at 10%. Thus, the eye irritation is not a single assay but rather is composed of a series of tissue and reaction components.
[b] CPSC, Consumer Product Safety Commission.
[c] FS, fluorescein staining under long wave ultraviolet light.

use of the model to replace rodents in parasitic and antitumor models is not indicated, since two potent trichomonicides and three antitumor agents were of little value. From the point of view of wound healing, however, compounds such as TBTO and benzalkonium chloride, which kill cells by direct contact, would be successfully detected in this model thus saving animals; but compounds that suppress healing by inhibition of cell division would be missed.

Table III shows the ability of cell culture assays to predict the relative scale of intramuscular irritation induced by four drugs; cortisone, Valium, verapamil, and thorazine. The correlation between *in vitro* toxicity, animal irritation, and human irritation is perfect. This assay should prove useful in reducing the number of rabbits and rats employed in the classical intramuscular irritation test employed to assess the safety of injectable drugs.[6] It should be noted that cell culture assays of this type have been used since 1965 to rule out irritancy effects in the device industry, with such tests as MEM elution and agar overlay.

The data in Table IV show that the use of gram-positive bacteria predicts irritation in rabbits and in man to the same extent as cell culture (Table III). Gram-negative bacteria were not predictive of intramuscular irritation.[6]

Table V gives additional insight into the ability of bacteria to predict topical irritation. Benzalkonium chloride was the most active (gram-positive and gram-negative) followed by sodium lauryl sulfate (gram-positive only) and Tween 20 (inactive). This ranking clearly mirrored the results in animals and man for known skin and eye irritants.[2,11] Additional studies seem warranted for specific classes of compounds and species of bacteria.

Additional studies were conducted with sodium lauryl sulfate employing cell cultures, chick embryos, rabbits, and guinea pigs. The purpose of these studies was to see which of these models could predict toxicity in animals and which of the animal models was the most sensitive. The data in Table VI show the rapidity of action of the cell culture assay.

TABLE XI
Minimum Toxic Doses (Phylogenetic Scale)[a]

Test	Sodium Lauryl Sulfate
	%
Corneal opacity	10.0
Skin irritation	10.0
CPSC eye irritation responses (3/6)	5.0
Draize eye irritation score (>15)	5.0
Gram-negative bacteria	5.0
Fluorescein eye at 2 hr[b]	5.0
Chick embryo	2.5
Eye discharge at 2 hr[c]	1.0
Guinea pig immersion	0.125
Cell culture	0.025
Strains of herpes and influenza viruses	0.0125 to 0.0500
Gram-positive bacteria	0.0020

[a] The increasing sensitivity of the assay is shown from top to bottom of table.
[b] Similar results at 24 hr.
[c] Eye discharge at 24 hr was less sensitive, detecting irritancy at 10% sodium lauryl sulfate, but not at lower concentrations.

Results could be detected within 5 min and the maximum cytotoxic effect of sodium lauryl sulfate, a known skin and eye irritant, was detected in 6 hr.[7] We have found that the equivalent results are obtained with a variety of cells (L929, MRC-5, WI38). This is likely due to the similarity of the lipid bilayers among mammalian cells grown in monolayers.

Results of a sodium lauryl sulfate assay in the chick embryo (Table VII) revealed that concentrations greater than 1.0% are required in this model, and that this experimental model may prove useful in reducing the number of rabbits used for eye irritation assays.

The results in Table VIII show the response of rabbit skin to sodium lauryl sulfate in the standard Draize model. Irritation was not noted until the application of patches bearing a 10% concentration of the agent.

By switching from the rabbit to the guinea pig skin model a more sensitive response was obtained (Table IX). Whereas the Draize test required a 10% concentration to detect irritancy, the guinea pig immersion test was sensitive down to a concentra-

TABLE XII
Cell Culture Predictions with Various Chemical Agents, Formulations, and Device Extracts[a]

Cell Culture	Irritation			Eye			Oral Toxicity	Leukopenia
	Intramuscular	Intracutaneous	Skin	Discharge	Conjunctivitis	Corneal Opacity		
+	+	+	±	+	+	±	−	−
−	−	−	±	±	±	±	±	±

[a] +, positive result for irritation; −, negative result for irritation; ±, the assay may or may not elicit irritation. A positive (cytotoxic) cell culture result is generally predictive of *in vivo* intramuscular, intracutaneous, ocular discharge, and conjunctivitis toxicity parameters. A negative (noncytotoxic) *in vitro* result is generally predictive of only *in vivo* intramuscular and intracutaneous results.

TABLE XIII
Cytotoxicity Data for Subdivision F Toxicity Tests and Cell Cultures (EPA 40 CFR Part 158)

Test	Alternative	Screening Leads	Possibility to Replace Animal
81-1. Oral rat	No	No	Remote
81-2. Dermal rabbit	No	No	Remote
81-3. Inhalation rat	No	No	Remote
81-4. Eye rabbit	No	Yes	Possible
81-5. Skin irritation rabbit	No	No	Remote
82. Series subchronic	No	No	Remote

tion of 0.0625%. Eye irritation results with sodium lauryl sulfate are shown in Table X. Corneal opacity was observed in the 10% treatment group. The minimum irritating concentration was 1%. More sensitive results were obtained at 2 hr post-treatment.

Table XI gives a phylogenetic scale and summarizes the toxicity tests performed with sodium lauryl sulfate employing three animal systems (Draize skin, Draize eye, guinea pig immersion), cell cultures, chick embryos, bacteria, and viruses. The most sensitive system was gram-positive bacteria followed by cell culture and the guinea pig immersion test. Various strains of ether-sensitive influenza and herpes viruses were rapidly inactivated. Least sensitive were the Draize skin irritation test and the corneal opacity component of the eye irritation test. Fluorescein staining of the corneal epithelium and mucous discharge were sensitive indicators of irritation in the Draize eye model and predict well for eye injury in man and occurred as early as 2 hr after instillation. Conjunctivitis was elicited at the same concentration at which inhibition of gram-negative rods occurred. Cell culture remains the most generally useful predictor of topical irritation in animals and man, especially eye irritation. However, it cannot distinguish close analogues within a chemical group[12] nor can it predict blindness. Numerous authors have shown the correlation between cytotoxicity and eye irritation,[2,7,13] and this correlation remains a major achievement of the alternative movement. However, the ability of cell cultures to discriminate among surface-active agents within one closely related group has been questioned.

Table XII gives an overview of the ability of cell cultures to predict (with neither false positives nor false negatives) in a series of *in vitro* assays. Table XIII presents the current status of cytotoxicity testing in replacing the intact animal studies mandated by FIFRA, EPA 40, CFR Part 158, Subdivision F, and Series 81 and 82. It is hoped that future studies will yield findings which will alter the composition of this table in favor of *in vitro* alternatives.

The data presented in this report send two clear messages:

- In the sole interest of consumer safety, toxicologists should pursue the phylogenetic approach described here and develop systems with increasing sensitivity among current animal models.
- In the interest of animal welfare, biologists should develop alternative models beyond the well established cell culture techniques so as to reduce the number of animals used.

These approaches are not mutually exclusive. Neither approach will be easy. How successful will we be? Let there be patience on both sides. The rallies in Central Park, the marches to the State houses, the press releases on torture and cruelty, the hurried enactment of legislation, the TV interviews—these techniques merely stampede government into legislating the currently impossible; however, they are disquieting to those of us who are professionally charged with the responsibility of assuring the delivery of safe products *now*. One cannot ban something that exists and ask that it be replaced by something that does not exist. The replacement comes first. Legislation comes second. Working together, with a clearer understanding of limitations and goals, industry, government, ethicists, and toxicologists may someday develop a larger number of accepted alternative tests than described here. □

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