pesticide during distillation, perhaps catalyzed by the metal surfaces of the still; and deposition of vaporized residues in the condenser coils. Rinses of the water-cooled condensers used in the laboratory experiments show that such deposition can occur although not in sufficient quantity to account for all of the missing residues.

Acknowledgment

Literature Cited

(1) Dickenson, W., *Trans. Faraday Soc.* **52**, 31 (1956).
(2) Downing, G., Norton, L. B., *Anal. Chem.* **23**, 1870 (1951).
(3) Gould, G. E., *J. Econ. Entomol.* **53**, 526 (1960).
(4) O'Donnell, A. E., Johnson, H. W., Jr., Weiss, F. T., *J. Agr. Food Chem.* **3**, 757 (1955).
(5) Pack, D. E., Ospenson, J. N., California Chemical Corp., Richmond, Calif., private communication, 1961.
(6) Schechter, M. S., Pogorelskin, M. A., Haller, H. L., *Anal. Chem.* **19**, 51 (1947).
(7) Shell Chemical Co., New York, Agricultural Chemicals Division, Technical Bulletin SC: 59-45 and 35.

SAFETY EVALUATION OF CHEMICALS

Relationship between Short- and Long-Term Feeding Studies in Designing an Effective Toxicity Test

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DURING more than 15 years, in the two laboratories involved, chemicals have been tested by mixing them in the diet and feeding these diets to rats. The usual procedure is first to determine an LD_{50}, or the amount of chemical expected to kill half of a group of small animals, usually rats, after a single dose. Second, if the chemical is a potential food ingredient or perhaps will become a residue on food crops, these acute oral toxicity data are used to plan dosage levels, and the material is then included in the diet of rats for a short-term experiment of 30- to 90-day duration. If the no-ill-effect dosage level of the material fed to the test rats is such as to sustain economic interest, a life-span or 2-year test of the chemical in the diet of rats will be started. Additional long-term study will also be undertaken using a nonrodent species, probably the dog.

A no ill-effect level determined as a result of these dietary feeding studies in laboratory animals is defined variously as one which shows no measurable effect, no ill effect, or no evidence of adverse effect attributable to the test material when judged by any of the toxicological or biochemical criteria employed. The term maximum no-effect level, used below, thus refers to the highest dietary concentration having no ill effect. Conversely, the minimum effect level is defined as the lowest dietary concentration at which any significant ill effect attributable to the test material was produced.

Table I. Relationship of Dosage Levels of Short-Term and 2-Year Feeding of Materials in the Diet of Rats

Material Number	Duration of Short-Term Test	Percentage of Material in Diet				Ratio: Short-Term/2-Years	
		Short-term		2-Years		2-Years	
		Minimum effect	Maximum no-effect	Minimum effect	Maximum no-effect	Minimum effect	Maximum no-effect
1	105	0.015	0.005	0.03	0.01	0.5	0.5
2	90	4.0	2.0	8.0	4.0	0.5	0.5
3	90	1.0	0.3	2.0	0.2	0.5	1.5
4	120	3.0	1.0	5.0	0.5	0.6	2.0
5	90	0.25	0.0625	0.256	0.064	1.0	1.0
6	90	0.01	0.003	0.01	0.003	1.0	1.0
7	97	0.1	0.03	0.1	0.03	1.0	1.0
8	90	8.0	4.0	8.0	4.0	1.0	1.0
9	130	1.0	0.3	1.0	0.2	1.0	1.5
10	30	0.05	0.012	0.04	0.01	1.2	1.2
11	30	25.0	10.0	20.0	5.0	1.2	2.0
12	90	0.75	0.375	0.40	0.13	1.9	2.9
13	90	10.0	3.0	5.0	1.0	2.0	3.0
14	90	0.03	0.01	0.0125	0.0062	2.4	1.6
15	130	3.0	1.0	1.0	0.2	3.0	5.0
16	50	0.3	0.1	0.1	0.03	3.0	3.3
17	98	0.01	0.003	0.003	0.001	3.3	3.0
18	90	16.0	8.0	4.0	2.0	4.0	4.0
19	29	0.25	0.06	0.06	0.02	4.2	3.0
20	210	0.25	0.05	0.05	0.01	5.0	5.0
21	90	0.225	0.15	0.04	0.02	5.6	7.5
22	130	0.1	0.03	0.005	0.0025	20.0	12.0
23	90	0.5	0.25	M[a]	0.5	...	0.5[b]
24	90	0.009	0.003	M[a]	0.004	...	0.8[b]
25	30	0.3	0.1	M[a]	0.1	...	1.0[b]
26	90	8.0	4.0	M[a]	2.0	...	2.0[b]
27	90	16.0	8.0	M[a]	4.0	...	2.0[b]
28	90	M[a]	3.0	3.0	1.0	...	3.0[c]
29	93	M[a]	5.0	5.0	1.0	...	5.0[c]
30	91	M[a]	0.18	0.06	0.02	...	9.0[c]
31	90	M[a]	1.0	M[a]	0.3	...	3.3[d]
32	90	M[a]	2.5	M[a]	0.5	...	5.0[d]
33	142	M[a]	25.0	M[a]	5.0	...	5.0[d]

[a] M = the maximum no-effect level was the highest dosage level fed.
[b] As the M level was on the 2-year test, the ratios are a maximum.
[c] As the M level was on the short-term test, the ratios are a minimum.
[d] As the M levels were on both the short-term and 2-year tests, the ratios are indicative only of which levels were used.

A compilation and comparison of ratios between results of short- and long-term feeding studies are presented. These data, on 33 materials fed for 90 days and 2 years, indicate that at least 50% of the time ratios of toxicity in rats for these periods were 2.0 or less. Therefore, one could transfer short-term test results with measured confidence into a prediction of the "no ill-effect" levels in 2-year studies. Only a few criteria of stress were effective in delineating the lowest dosage level of effect in such feeding tests. These are body weight gain, liver and kidney weight as percentages of body weight, and liver and kidney pathological study. With these criteria, except in special cases such as cholinesterase-inhibiting chemicals, one can perform a more reasonable, less expensive test. Furthermore, long-term dog studies were not, in 21 rat to dog long-term comparisons, more sensitive of effect than rats. Therefore, it is recommended that a short-term dog study is sufficient. If, in such a 3-month test, special effects on dogs should be discovered, additional long-term tests would be required. It is taken for granted that the experimental design will be subject to the judgment of an experienced toxicologist who will recognize the need for additional criteria in specific cases.
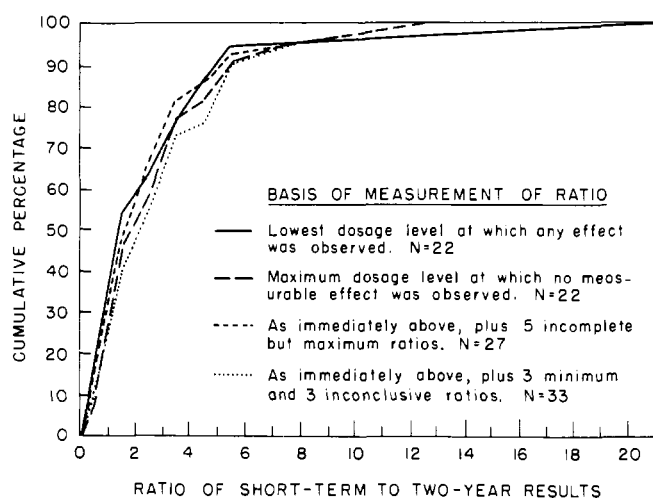

Figure 1. Cumulative percentage curves of short-term to 2-year ratios

One objective of the analysis of these dietary feeding data, is to determine whether one can predict, with a measured degree of confidence, the probable outcome of a 2-year chronic test in rats from short-term feeding tests. Safety could then be evaluated on the basis of 90-day tests in circumstances previously believed possible only with 2-year chronic feeding.

Methods

The data for these comparisons are a combination of separately derived results accumulated during more than 15 years by the two toxicological research laboratories. For a few materials, the 2-year chronic oral feeding portion was conducted by consulting laboratories. The uses and proposed uses for these materials include agricultural chemicals for various pesticide and veterinary chemical applications, direct additives to foods (such as thickeners, stabilizers, antimycotic agents), chemicals used in water treatment and flocculation, and

Table II. Identification of Materials

Material No.[a]	Chemical Name	Reference
1	O,O-Dimethyl-O-(2,4,5-trichlorophenyl) phosphorothioate	11
2	Polyethylene glycol (MW 4000)	17
3	o-Phenylphenol	9
4	Ethyl phthalyl ethyl glycolate	8
5	Butoxypolypropylene glycol (MW 800)	3
6	O-Methyl-O-(4-tert-butyl-2-chloro-phenyl)-methyl phosphoramidothioate	7
7	Sodium 2,2-dichloropropionate	12
8	Polyethylene glycol (MW 1540)	17
9	tert-Butylphenyl salicylate	7
10	2,4-Dimethyl-2-methylene-1,2,4-thiadiazolidine-5-thione	6
11	Hydroxypropyl methylcellulose	10
12	Di(2-ethylhexyl)phthalate	4
13	Polyacrylamide	7
14	3,5-Dinitro-o-toluamide	7
15	3(2-Biphenylyloxy)-1,2-epoxypropane	7
16	Bis(p-chlorophenoxy)methane	7
17	O-Methyl-O-(2,4-dichlorophenyl)-isopropyl phosphoramidothioate	7
18	Polyethylene glycol (MW 400)	17
19	2,4-Dichlorophenoxyethyl sulfate, sodium salt	5
20	4,4'-Isopropylidenebis(2-isopropyl-phenol)	7
21	1-Naphthyl-N-methyl carbamate	2
22	p-Chlorophenyl p-chlorobenzenesulfonate	7
23	Calcium disodium ethylenediamine tetracetate	7
24	Acrylamide	7
25	Dehydroacetic acid	19
26	Polyethylene glycol (MW 1500)	15
27	Polyethylene glycol (MW 200)	6
28	Sodium β-sulfopropionamide	7
29	Hydroxyethylcellulose	16
30	2,4,5-Trichlorophenoxyethyl sulfate, sodium salt	6
31	Methylpolysiloxane	13
32	Di-isobutyl adipate	7
33	Vinylidene chloride–vinyl chloride co-polymer	7

[a] Same as in Table I.

ingredients for food packaging materials.

To provide the most sound basis possible for decisions wherein the knowledge may best be utilized, both the qualitative and quantitative data were compared. Many criteria of effect are measured in short-term and 2-year

tests. These include mortality, growth, diet consumption, weight of certain organs, histopathological conditions of certain organs, and hematological and biochemical effects.

The two laboratories involved in this study have published two papers listing

Table III. Summary of Observations of Effects Detected in Short-Term and 2-Year Oral Studies

| | At Lowest Dosage Level in Which Any Effect Was Detected | | | | | | | | At Next Higher Dosage Level This Criterion Affected[b] | |
| | Short-term | | | | 2-Year | | | | | |
Criterion of Effect	No. of studies in which this criterion was followed	No. of pertinent[a] studies	No. where this criterion was affected	No. where it was the sole effect	No. of studies in which this criterion was followed	No. of pertinent[a] studies	No. where this criterion was affected	No. where it was the sole effect	Short-term	2-Year
Mortality	33	27	1	0	33	25	2[c]	0	0	0
Food intake	30	26	2	0	29	21	0	0	3	1
Body weight	33	27	10	6	33	25	15	7	9	2
Organ weights										
Liver	27	22	8	4	30	22	6	0	4	1
Kidney	27	22	7	3	30	22	5	3	2	1
Heart	17	13	0	0	17	13	0	0	0	0
Spleen	17	17	1	0	16	10	0	0	1	0
Testes	16	13	0	0	18	12	0	0	1	0
Lung	15	12	0	0	12	10	0	0	0	0
Brain	3	2	0	0	4	3	0	0	0	0
Thyroid	0	0	0	0	2	1	0	0	0	0
Stomach	1	0	0	0	1	0	0	0	0	0
Adrenal	0	0	0	0	3	0	0	0	0	0
Gross pathology	33	27	0	0	3	25	0	0	0	0
Micropathology										
Liver	30	24	3	1	33	25	9	1	3	3
Kidney	30	24	1	0	33	25	8	0	2	2
Heart	22	17	0	0	29	21	0	0	0	0
Spleen	21	16	0	0	23	16	1	0	0	0
Testes	21	17	1	1	33	25	0	0	0	0
Lung	20	16	0	0	32	25	0	0	0	0
Adrenal	16	12	0	0	27	20	0	0	0	0
Pancreas	16	12	0	0	23	17	0	0	0	0
Bone marrow	1	0	0	0	11	6	0	0	0	0
Voluntary muscle	1	1	0	0	6	3	0	0	0	0
Stomach	3	1	0	0	15	10	0	0	0	0
Intestine	3	1	0	0	21	14	0	0	0	0
Bladder	2	2	0	0	12	9	0	0	0	0
Brain	3	3	0	0	11	8	0	0	0	0
Sciatic nerve	2	2	0	0	0	0	0	0	0	0
Hematology	19	16	1	0	31	24	0	0	0	0
Blood urea nitrogen	10	7	0	0	6	3	0	0	0	0
Clinical urine analyses	1	1	0	0	7	5	0	0	0	0
Central nervous system	1	1	1	1	0	0	0	0	0	0
Neoplasm	33	27	0	0	33	25	0	0	0	0
Fertility	0	0	0	0	5	3	1	0	0	0
Cholinesterase	3	3	3	2	3	3	3	2	0	0

[a] Total number of studies in which this criterion was followed minus the studies in which none of the criteria were significantly altered at any dosage level.

[b] Times that effects were demonstrated by the criteria when they were not affected at the next lower dosage level.

[c] Highest level fed was the only one affected.

the efficiency of the criteria of stress in toxicological tests (14, 18). These indicated that while many and varied criteria are relied upon to demonstrate that a material has produced an effect, they differ widely in the efficiency with which they reveal effect. The criteria of body weight gain and of liver and kidney weights, calculated as percentages of total body weight, have proved quite sensitive. Therefore, another objective of this analysis is to compare these criteria on short- and long-term studies with the numerous other criteria that are widely accepted as necessary.

Results

Quantitative Comparison. Data on 33 materials were compared. The information, expressed as ratios between both the minimum effect and the maximum no-effect dietary percentage levels found after the short-term or 2-year chronic feeding, is presented in Table I.

The chemical names of these compounds are listed in Table II along with references to the earlier separate publication of the data.

In only five of the 33 short-term tests (Table I) were the durations less than 90 days. In 21, the duration was 89 to 98 days of doses. Therefore, the duration of the short-term used in the comparison was, a large majority of the time, approximately 90 days.

In 2-year tests, five of the materials, numbers 23 through 27, produced no adverse effect at the highest dosage level fed. Therefore, no minimum-effect level (one that differed significantly from the controls) was found. As the no-effect level for the 2-year test could be above the highest level actually fed, the ratio between the short-term and 2-year results is a maximum.

Similarly, in short-term tests, three materials produced no measurable difference from the controls at the highest

dosage level fed. Therefore, materials numbered 28, 29, and 30 resulted in ratios of short-term to 2-years that are minimal. Finally, three materials, 31 to 33 inclusive, had both the short-term and 2-year tests result in no effect at the highest levels fed. Here, the ratios are inconclusive. These ratios were, therefore, compared on several bases, shown in Figure 1.

When these ratios were tabulated, it was obvious that the usual statistics dependent on the *t*- or normal distribution could not apply. In approximately one half of the cases, the ratios were 2.0 or less. Therefore, nonparametric, percentile calculations were used. The midpoint, the 50th percentile or median, was close to 2 and similar for all five bases of measurement of the ratio. Therefore, 50% of the time the ratio between the short-term and 2-year tests was 2 or less.

The 2.5 and 97.5 percentiles were

Table IV. Frequency with which a Particular Effect Was Observed at the Lowest Level at which any Effect Was Observed

Criterion of Effect	Short-Term					2-Year				
	No of pertinent[a] studies	No. in which effect was found	Times found, %	No. of times effect was sole effect	Time sole effect, %	No. of pertinent[a] studies	No. in which effect was found	Times found, %	No. of times effect was sole effect	Times sole effect, %
Body weight	27	10	37	6	22	25	15	60	7	28
Liver weight	22	8	36	4	18	22	6	27	0	0
Kidney weight	22	7	32	3	14	22	5	23	3	14
Liver micropathology	24	3	12	1	4	25	9	36	1	4
Kidney micropathology	24	1	4	0	0	25	8	32	0	0
Food intake	26	2	8	0	0	21	0	0	0	0
Mortality	27	1	4	0	0	25	2	8	0	0
Spleen weight	17	1	6	0	0	10	0	0	0	0
Spleen micropathology	16	0	0	0	0	16	1	6	0	0
Testes micropathology	17	1	6	1	6	25	0	0	0	0
Hematology	16	1	6	0	0	24	0	0	0	0
Cholinesterase	3	3	100	2	67	3	3	100	2	67
Central nervous system	1	1	100	1	100	0	0	...	0	..
Fertility	0	0	...	0	...	3	1	33	0	0

[a] Total number of studies in which this criterion was followed minus the studies in which none of the criteria were significantly altered at any dosage level.

calculated to indicate the range that included 95% of the data; 32 of the 33 ratios were 9 or below. Material number 22 was unusual, with a ratio of 20 on the minimum-effect basis and 12 on the maximum no-effect basis. This material affected the 97.5 percentile so that, for all practical purposes, it was the upper limit. For the 33 cases, or for any of the other four groups of summaries, 95% of the cases were included in this 12 to 20 ratio limit.

The frequencies with which the different ratios occurred were accumulated and are presented in Figure 1. Here, the cumulative percentage distributions of all five bases are similar. Values can be taken from these curves to denote the ratios that include, for example, 90% of the cases. This ranges from about five to six.

Efficiency of Criteria. At least 36 criteria of effect have been examined in oral studies in these two laboratories. These are enumerated in Table III along with the frequency with which they have been used in short-term and 2-year tests. Efficiency is measured by the number of times that a criteria showed effect at the lowest dosage level where any effect was recognized. At higher dosage levels in each graded series, other of these criteria were often affected. All of the studies included in this survey followed a pattern aimed at defining the no ill-effect level of repeated oral intake for a chemical. Therefore, it is at this lowest effect level that a criterion will be proved as sensitive or insensitive. Also included in Table III are listings of the incidence with which these criteria were significantly altered at the next higher dosage level when they were insensitive at the lowest-effect level.

The concept of pertinent studies

must be considered. While 33 short-term and 2-year studies are compared herein, several resulted in no measurable effect at any dosage level whatever. In these, as no criteria could be assessed as efficient, the pertinent studies are those in which each criterion was followed minus those in which none of the criteria were significantly altered at any dosage level.

Table III shows that in short-term tests only 12 criteria were affected at these lowest effect levels; only seven of these were the sole effects at these levels. Similarly, in the 2-year tests only nine criteria were ever affected, and only four of these were sole effects. Many criteria examined in these reported studies were not efficient at any time—i.e., never resulted in effect at these lowest detectable effect levels. These include the weight of the heart, testes, and lung. Other criteria insensitive in all of these studies from both laboratories have been the cellular changes observed by microscopic examination (hereafter termed micropathology) of the heart, lung, adrenal, pancreas, stomach, intestine, and brain.

Examination of Tables III and IV reveals that several other criteria were affected a very few times but were never the sole effects. This group includes micropathology of the kidney and spleen, food intake, mortality, spleen weight, and hematology. Table IV summarizes the incidence and percentage of the pertinent studies in which significant effects were seen at these lowest-effect levels. Certain criteria examined only a few times appear quite efficient—i.e., cholinesterase and central nervous system effects. These criteria were followed only when the particular chemicals being tested were expected to influence them under some experi-

mental conditions. For example, in the case of organic phosphate compounds, inhibition of cholinesterase activity may be produced at dosage levels well below those where any other effect can be measured.

In approximately one third of the pertinent short-term studies the criteria of body weight, liver weight, and kidney weight were affected. While this is also true for these organ weights in the 2-year tests on the same materials, body weight is a much more sensitive criteria in the 2-year than in the short-term test. In 60%, or in 15 of the 25 studies, body weight was affected at the lowest effect level. Furthermore, in 22% of the short-term and 28% of the chronic studies, body weight decrease was the sole effect—i.e., the only criteria affected at this lowest dosage level.

Liver and kidney micropathology, similarly, were more sensitive in 2-year, than in short-term studies. In only 12 and 4% of the short-term tests were these criteria affected as compared to 36 and 32%, respectively, in these chronic studies. Only once was liver pathology the sole effect, and micropathology of the kidney was never the sole effect.

The criteria affected at the lowest effect level, hence judged to be most sensitive, and those at the next highest dosage level in both types of studies are contrasted in Table V. The material numbers in this table are the same as in Table I. Sixteen times in the 22 studies in which at least one dosage level was affected in both the short-term and chronic tests, the same criteria were affected in the tests of both durations. This does not mean that the criteria affected in the short-term were the only criteria affected in the 2-year tests. It does indicate, however, as for

Table V. Relationship of Ratio between Short-Term and 2-Year Oral Studies and the Sensitive Criteria of Effect Noted

Material No.[a]	Ratio: Short-Term/2-Years Minimum effect	Maximum no-effect	Short-Term Most sensitive criteria at lowest effect level	Short-Term Other criteria affected at next higher level	2-Year Most sensitive criteria at lowest effect level	2-Year Other criteria affected at next higher level
1	0.5	0.5	C	K,L	C	K,L
2	0.5	0.5	L	B	B	XX
3	0.5	1.5	K,L	B	B,K	XX
4	0.6	2.0	B	XX	B,D,K	XX
5	1.0	1.0	L	none	B,L,K	XX
6	1.0	1.0	C,K	L	C,L	XX
7	1.0	1.0	K	B,L,K,L	K	XX
8	1.0	1.0	B	none	B,L	XX
9	1.0	1.5	B	A	B	XX
10	1.2	1.2	K,L	A,B	K,L	A,B
11	1.2	2.0	B,D	XX	B	XX
12	1.9	2.9	B	none	B,F,K,L	XX
13	2.0	3.0	B	XX	B	XX
14	2.4	1.6	T	B,L,S,T	L,L	XX
15	3.0	5.0	L	XX	B	XX
16	3.0	3.3	L,L	K	B,L,K,L,S	K
17	3.3	3.0	C	none	C	L
18	4.0	4.0	B,K	L	B	none
19	4.2	3.0	K	A,B	B,D,K,L,K,L	XX
20	5.0	5.0	L	XX	L	L
21	5.6	7.5	K	B,L	B,K,L	XX
22	20.0	12.0	A,K,L,S,L	B,K	L,K,L	none
23	...	0.5	B,H	none	...	...
24	...	0.8	CNS	XX	...	...
25	...	1.0	A,B	XX	...	...
26	...	2.0	B	none	...	...
27	...	2.0	L	B,K	...	...
28	...	3.0	...	...	B	XX
29	...	5.0	...	...	K	XX
30	...	9.0	...	...	K	K,L
31	...	3.3	...	...	...	...
32	...	5.0	...	...	...	...
33	...	5.0	...	...	...	...

[a] Same as in Table I.

Abbreviations and symbols: A = food intake, B = body weight, C = cholinesterase, CNS = central nervous system, D = mortality, F = fertility, H = hematology, K = kidney micropathology, K = kidney weight, L = liver micropathology, L = liver weight, S = spleen micropathology, S = spleen weight, T = testes micropathology, T = testes weight, XX = no level fed higher than lowest effect level.

Table VI. Percentage of Studies in which One or More Effects of a Combination Were Observed at the Lowest Dosage Level at which any Effect Was Detected

Criterion of Effect	Frequency that at Least this or these Criteria were Affected at the Lowest Dosage Level where any Effect Was Detected Short-term Cumulative frequency	Short-term Cumulative percentage[a]	2-Year Cumulative frequency	2-Year Cumulative percentage[a]
Body weight	10	42	15	65
Body weight, Liver weight	18	75	17	74
Body weight, Liver weight, Kidney weight	21	88	20	87
Body weight, Liver weight, Kidney weight, Liver pathology	22	92	23	100
Body weight, Liver weight, Kidney weight, Liver pathology, Kidney pathology	23	96		
Body weight, Liver weight, Kidney weight, Liver pathology, Kidney pathology, Testis pathology	24	100		

[a] In two cases in both the short-term and 2-year studies, cholinesterase change was the sole effect. In one additional case, in short-term only, central nervous system effect was noted. These being special, unusual chemicals they are omitted from this table.

material number 8, that body weight was affected in both the short- and long-term tests. In six of these studies, different criteria were affected in the tests of different duration. For example, while the micropathology of the testes was the sole effect in the subacute study of material 14, this organ was not affected in the chronic study at either the lowest effect level or at the next higher dosage level. Here, in the 2-year study, liver weight and liver micropathology were the efficient criteria.

Discussion

From the results of these comparisons, it is obvious that at least one half of the time the ratio of short-term to 2-year results was 2.0 or less. Furthermore, a maximum ratio between the no-effect levels of these tests of different duration was 12. Therefore, it is concluded that one can transfer short-term results with confidence into a likely prediction of what the no ill-effect, two-year levels will be. The experienced toxicologist, having confidence in his prediction of the final no-effect level, has the basis for greater utilization of the short-term results in his scientific decisions.

The Miller Amendment to the Federal Food, Drug and Cosmetic Act of 1938 provides for the issuance of temporary tolerances for pesticide residues when such materials are being applied to food crops during field trials. With proper consideration of the influence of the other factors involved in the safety evaluation, the results of 90-day feeding studies can be a sufficient scientific basis for establishing a temporary tolerance under the Miller Amendment.

The subacute or 90-day dietary feeding study is carried out for $1/8$ of the lifetime of the rat; and in view of ratios defined herein, it is believed that the 100-fold margin of safety customarily considered in relation to the maximum no-effect dosage level at the end of 2-year tests should be ample when applied likewise to the conclusions from a 90-day study.

It should not be assumed that the authors are advocating the results of 90-day studies per se be considered as merely less time-consuming and, therefore, a substitute for the 2-year chronic oral study in rats. An experienced toxicologist knows that one must judge other factors in the evaluation of safety, including an understanding of the metabolism and excretion of the substance, the nature of the damage produced. and the activity of compounds of related chemical structure. The authors strongly believe, however, that the toxicological requirements for safety evaluation should be flexible, the final judgment being contingent upon the specific circumstances involved, and that in certain instances the 2-year study may be eliminated.

Design of Long-Term Tests

One advantage of recognizing sensitive criteria would be to utilize them in the design of an efficient 2-year study. If a battery of these sensitive criteria can delineate the lowest effect level efficiently, then these alone could be used. As more and more chemicals are now being tested as to their capacity to produce chronic toxicity, such tests must be stripped of unnecessary encumbrances

and modernized. As was suggested in previous papers (14, 18), certain combinations of criteria are sufficient for accuracy. These are listed in Table VI along with the cumulative frequencies and percentages of the studies in which they, at least, were affected at the lowest dosage level in which any effect was detected. For short-term studies, 92% of the time the four criteria—body weight, liver weight, kidney weight, and liver pathology—were sufficient to delineate this lowest-effect level. These same four were effective in 100% of the pertinent 2-year tests. For reasons discussed before, the two studies on chemicals having specific cholinesterase effect were omitted from consideration as was one short-term test that produced a central nervous system effect. To reach 95% efficiency for short-term tests, kidney pathology had to be added to the four criteria previously mentioned, and for 100% efficiency, the histopathology of the testes must be studied also.

In projecting these data, it should not be implied that these criteria actually will be 100% effective in defining the lowest effect level. Special cases, such as the cholinesterase-depressant materials, will occur. However, these special tests that should be added to the group of proved efficient criteria will be apparent to competent toxicologists. On the basis of the results of this compilation of data, it is suggested that reliable, efficient short-term and 2-year feeding tests can be conducted examining only the following criteria: body weight gain, liver and kidney weight (as percentage of body weight), and liver and kidney micropathology.

Following the above design would allow more materials to be screened. The major bottle-neck in these toxicological studies is the microscopic examination of the numerous tissues from organs that were either never affected or that were never the sensitive criteria at the lowest effect level—namely, the experimental dosage level needed to determine the acceptable amount of a chemical in the diet of humans.

It is taken for granted that other criteria will be followed in any well-organized long-term experiment. The above listing of the sensitive criteria includes all that need be examined routinely in rats. Information on gross effects and the incidence of mortality and of neoplasms are always recorded, although in none of the experiments were these among the required, sensitive criteria at the lowest effect level. A proper experiment must be supervised by an experienced toxicologist who must note unusual effects when they occur, generally finding these in single dose, tolerance, or short-term studies. The qualified toxicologist will use additional measures of effect when required as no one pattern can possibly cover all situations. The plan suggested is the minimum basic design.

Another problem considered in the efficient design of studies of this nature is the value of information derived from feeding dogs for 1 or 2 years. The logical question presented is whether rat feeding studies are sufficient. Is any additional useful toxicological information obtained by feeding these chemicals to dogs?

In 21 of the reported 2-year rat feeding studies, the same chemicals were included in the diets of dogs for periods of at least 1 year. Nineteen of the 21 studies were pertinent as some effect was produced in rats in at least one level fed. In none of the 21 were the dog studies more sensitive, in 14 they were equivalent, and in 7 they were less sensitive in delineating a lower use level than were the rat 2-year studies. In the authors' experience, the rat is definitely a more sensitive indicator of the no ill-effect level than the dog. By using the rat, a lower "safe" level will be established. It is concluded that the feeding of a chemical to dogs for 90 days should be an ample period to show whether this species would be more sensitive than the rat. If this proved to be the case, then long-term tests should be performed using dogs. If not, this step should be eliminated.

Many times, long-term feeding of essentially nontoxic additives are undertaken for "administrative expediency" (7). In such instances, it is proper to streamline the test as much as possible, for public health is not at risk. On the other hand, certain proposed additives will be found to be truly toxic in properly conducted preliminary studies. For these, proof of safety may not be streamlined. Here, mechanism of injury should be determined by which human symptoms of very early effects may be predicted.

Literature Cited

(1) Barnes, J. M., Denz, F. A., *Pharmacol. Rev.* **6,** 191 (1954).
(2) Carpenter, C. P., Weil, C. S., Palm, P. E., Woodside, M. D., Nair, J. H., III, Smyth, H. F., Jr., J. AGR. FOOD CHEM., **9,** 30 (1961).
(3) Carpenter, C. P., Weil, C. S., Palm, P. E., Woodside, M. D., Smyth, H. F., Jr., *Ibid.,* **7,** 763 (1959).
(4) Carpenter, C. P., Weil, C. S., Smyth, H. F., Jr., *Arch. Ind. Hyg. Occup. Med.* **8,** 219 (1953).
(5) Carpenter, C. P., Weil, C. S., Smyth, H. F., Jr., J. AGR. FOOD CHEM. **9,** 382 (1961).
(6) Chemical Hygiene Fellowship of Union Carbide Chemicals Co., Mellon Institute, Pittsburgh, Pa., unpublished data.
(7) Dow Chemical Co., Biochemical Research Laboratory, Midland, Mich., unpublished data.
(8) Hodge, H. C., Maynard, E. A., Blanchet, H. J., Jr., Hyatt, R. E., Rowe, V. K., Spencer, H. C., *Arch. Ind. Hyg. Occup. Med.* **8,** 289 (1953).
(9) Hodge, H. C., Maynard, E. A., Blanchet, H. J., Jr., Spencer, H. C., Rowe, V. K., *J. Pharmacol. Exptl. Therap.* **104,** 202 (1952).
(10) Hodge, H. C., Maynard, E. A., Wilt, W. G., Blanchet, H. J., Jr., Hyatt, R. E., *Ibid.,* **99,** 112 (1950).
(11) McCollister, D. D., Oyen, F., Rowe, V. K., J. AGR. FOOD CHEM. **7,** 689 (1959).
(12) Paynter, O. E., Tusing, T. W., McCollister, D. D., Rowe, V. K., *Ibid.,* **8,** 47 (1960).
(13) Rowe, V. K., Spencer, H. C., Bass, S. L., *Arch. Ind. Hyg. Occup. Med.* **1,** 539 (1950).
(14) Rowe, V. K., Wolf, M. A., Weil, C. S., Smyth, H. F., Jr., *Am. Ind. Hyg. Assoc. J.* **20,** 346 (1959).
(15) Smyth, H. F., Jr., Carpenter, C. P., Shaffer, C. B., *J. Am. Pharm. Assoc., Sci. Ed.* **36,** 157 (1947).
(16) Smyth, H. F., Jr., Carpenter, C. P., Weil, C. S., *Ibid.,* **36,** 335 (1947).
(17) *Ibid.,* **44,** 27 (1955).
(18) Smyth, H. F., Jr., Weil, C. S., Adams, E. M., Hollingsworth, R. L., *Arch. Ind. Hyg. Occup. Med.* **6,** 32 (1952).
(19) Spencer, H. C., Rowe, V. K., McCollister, D. D., *J. Pharmacol. Exptl. Therap.* **99,** 57 (1950).