THE FATE OF MINERAL OILS INJECTED INTO THE PERITONEAL CAVITY OF MICE

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The ordinary method of testing the carcinogenic activity of mineral oils is by means of frequent applications to the skin, usually the interscapular region (*J. Indust. Hygiene*, 1931, **13**, 204). This test was later supplemented by a consideration of certain physical characteristics of the oils, notably the refractivity (*J. Hygiene*, 1933, **33**, 464). Subsequently the injection test was instituted, the basis of the test being that when the injected oil is recovered from the animal there is found to be a reduction in the refractive index and density, the amount of this reduction depending more or less upon the degree of carcinogenic and dermatitic activity of the oil (*J. Hygiene*, 1935, **35**, 404). It is certain aspects of this test which we propose to discuss in the present paper.

In performing the test it was noted that the quantity of mineral oil remaining free in the peritoneal cavity after injection varied considerably, and it seemed to us more than possible that there was some relation between carcinogenicity and the quantity of oil recovered. Accordingly some experiments were undertaken to investigate this question, the oil recovered, following our standard technique (*J. Hygiene*, 1935, **35**, 125), being weighed, and the density subsequently determined. As we only inject 0·5 c.c. into a 20 g. animal the technical error is great, and in order to lessen this five animals have been used for each oil, the product obtained being massed. In this manner thirty oils were injected into 150 animals, the percentage recovered on the 7th day being calculated, and the oils then divided into three groups of ten samples. Correlation values (perfect correlation being unity) of the percentage of oil lost and carcinogenic potency, etc. were then determined for each group, the average values obtained for those cases in which we are immediately interested being:

Carcinogenic potency	0·368	Refractivity (1) $\frac{n-1}{d}$	0·601
Refractive index fall	0·580	,, (2) $n-\frac{d}{2}$	0·650
Refractive index	0·616	Kinematic viscosity	−0·001
Index fall and index	0·597	Refractivity and index fall	0·734

n = refractive index; d = density.

Here we have striking evidence of the relation of the animal reaction, illustrated by carcinogenic potency, refractive index fall and quantity of oil lost on injection, to the refractivity and the refractive index of the oils. It is also to be noted that the highest degree of correlation with percentage of oil lost is given by a combination of index fall and refractivity, while there is no correlation with viscosity. Further, refractivity as determined by the second formula provided somewhat better values than that given by the first formula.

The experiments with which we are at present concerned incidently confirmed the observation made some time ago that the toxicity of mineral oils on injection, as indicated by loss of body weight of the animal, is related to the carcinogenicity of such oils for the skin. The comparative correlations with percentage loss of body weight were:

Carcinogenic potency	0·484	Refractivity (1)	0·536
Refractive index fall	0·673	,, (2)	0·694
Refractive index	0·584	Kinematic viscosity	−0·033
Index fall and index	0·687	Refractivity and index fall	0·725
		Loss of oil	0·691

These values run more or less parallel with those given with percentage loss of oil after injection, but on the whole are a little higher. Neither set of values was materially improved by segregating the thirty oils into three viscosity groups, owing to a smaller deviation from the mean of the average data.

Although the standard time we adopt for allowing the animal to effect the refractive index of injected oils is 7 days, it was considered advisable to find out how the above correlation values would compare with those obtained when the oils were allowed to remain in the animals for a shorter time: that selected being 3 days. The percentage loss of oil for a period longer than 7 days has not been examined as it was felt that technical errors would be magnified owing to the smallness of the quantity of oil recoverable.

The average percentage loss of oil by the 3rd day gave the following correlation values:

Carcinogenic potency	0·286	Refractivity (1)	0·361
Refractive index fall	0·444	,, (2)	0·393
Refractive index	0·455	Viscosity	−0·060
Index fall and index	0·394	Refractivity and index fall	0·520

and those for loss of body weight:

Carcinogenic potency	0·659	Refractivity (1)	0·762
Refractive index fall	0·759	,, (2)	0·747
Refractive index	0·417	Viscosity	−0·274
Index fall and index	0·538	Refractivity and index fall	0·853
		Loss of oil	0·625

It will be noted that in none of the eight sets of data examined does the percentage loss of oil by the 3rd day correlate as well as the loss by the 7th day, the mean deviation being greater in the latter samples. The average loss by the 3rd day was 43·81 % while by the 7th day it was 51·65 %. Conversely, the loss of body weight by the 3rd day gave, on the whole, better correlation values than did loss by the 7th day, the deviation from the mean being smaller in the latter. The average loss of body weight by the 3rd day was 6·13 % while by the 7th day it was only 3·22 %. Thus, provided the dose of oil injected be not too large, the animal rapidly overcomes the temporary indisposition caused by the injection, and apparently the normal weight would soon be regained.

Now, although carcinogenic potency of mineral oils correlates to a high degree with their refractivity or their fall in index on injection, we have found that, on taking random samples, the highest degree of correlation is obtained by utilizing a combination of both the above data. It is significant that in each of the four sets of eight correlations now under discussion the highest value is again given by refractivity in combination with refractive index fall. Further, while correlation of refractive index fall on the 7th day with that on the 3rd day gave a value as high as 0·930, there was a significant difference in the values when carcinogenic potency was correlated with the two sets of figures, those of the 7th day giving 0·662 and those of the 3rd day 0·760. On the other hand, on substituting refractivity for carcinogenic potency the corresponding values obtained were 0·700 and 0·628 respectively.

The general conclusions we have arrived at as a result of these experiments are that percentage of oil lost is of more value on the 7th day than on the 3rd day, while percentage loss of body weight is of more value on the 3rd day. On the other hand decidedly better values are, on the whole, given by utilizing loss of body weight than by utilizing amount of oil recovered. On taking the mean of the values given on the 3rd and the 7th days and comparing loss of body weight with loss of oil, it was found that with the exception of the refractive index the higher values were in all instances obtained with the former. Thus:

	Loss of body weight	Loss of oil
Carcinogenic potency	0·571	0·327
Refractive index fall	0·716	0·512
Refractive index	0·500	0·535
Refractive index and refractive index fall	0·612	0·495
Refractivity (1)	0·649	0·481
,, (2)	0·720	0·521
Kinematic viscosity	−0·153	−0·030
Refractivity (2) and refractive index fall	0·789	0·627

It is obvious that there must be a considerable experimental error for the correlation values of loss of oil on the 7th day with that on the 3rd day was only 0·685, while that of loss of body weight on the 7th day with that on the 3rd day was but little better, viz. 0·716. There does not seem to be much difference in the value of the refractive index fall for estimating carcinogenic potency whether taken on the 3rd or the 7th day but further oils require to be tested before one can come to a definite decision as regards this point. Viscosity itself is found to play a negligible part in the animal reaction. At the most it would seem that there is possibly a tendency for the reaction to be a little sharper if the viscosity of the oils is lowered. In this as in other aspects, the above experiments confirm our previous findings.

It will perhaps be appreciated from these and our previously recorded experimental results how extraordinarily easy it is to segregate dangerous from relatively harmless mineral oils so as to avoid the risk of cancer of the skin and dermatitis. One can apply the oil to the skin, inject and determine fall in refractive index, inject and determine quantity of oil recoverable, inject and determine loss of weight of the animal, determine the refractivity of the oil, determine the refractive index and the viscosity.

We have here six different tests all of which intercorrelate one with another to a more than significant degree. While a combination of the data from several tests is a better guide than one test alone, the best single test, in our opinion, is that of injection and subsequent determination of the fall in the refractive index. The test, if possible, should be made on more than one animal, five being a very suitable number, and if performed in this manner we consider we have a more reliable result as to the activity of the oil in less than a fortnight than we should have when painting 100 animals for a period of 40–50 weeks.

It is important to remember when judging relative merits of our different procedures for estimating carcinogenic potency of mineral oils that there are several factors which have to be taken into consideration. One of the most important is that of the error introduced by the variable sensitivity of the animals. Whilst all the physical constant determinations are accurate, those for the oils recovered from the animal are of necessity somewhat less valuable as absolute criteria. Still less reliable are the determinations relating to the percentage of oil recovered from the peritoneal cavity, and the loss of body weight of the animal, and it is for this reason that, as already stated, five animals instead of one were utilized to obtain our figures. The least accurate test of all would appear to be the painting of the skin of animals, and to minimize the error at least 100 animals must be used for the examination of a single specimen of oil.

Bearing these points in mind, and in view of the fact that refractivity of the original oil and refractive index fall of the recovered oil both give a high degree of correlation with carcinogenic potency etc., one would have thought, at first sight, that the correlation value of refractivity and refractive index fall would have been very high indeed. But this is not so, the reason for the relatively poor value obtained being, of course, presumably due to the variable refractivity of the inert constituents which are intermixed with the active constituents in all the samples of active oils subjected by us to examination. In other words, two oils of quite different refractivity, although of similar viscosity may give an identical refractive index fall owing to one being of a paraffinic and the other of a naphthenic type, and it is in an attempt to clarify the position that we are considering the application to our data of the refractivity intercept, as advocated by Kurtz & Ward (*J. Franklin Inst.* 1936, **222**, 563). It is presumably for similar reasons that our results are not absolutely smooth when utilizing the refractive index in combination with viscosity for determining the toxicity of mineral oils. We have pointed out in previous communications that if our oils are segregated according to their toxicity, determined by the fall in the refractive index on injection into the peritoneal cavity of mice, that the correlation of refractive index and viscosity is very high, in the case of the first 100 random samples examined, as high as 0·850. If the index and viscosity are embodied in an empirical formula, involving a constant determined by us, an arbitrary figure is obtained which represents with a high degree of accuracy the toxicity of the oil in question.

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The arbitrary figures obtained in this way, like the refractivities are, of course, only measures of carcinogenicity when the oils lie within the carcinogenic viscosity range. On analysing the 100 random samples referred to above it was found that the arbitrary figures and carcinogenic potency only gave a correlation of 0·649 while the figures correlated with the refractive index fall gave the remarkably high value of 0·929.

SUMMARY

Mineral oil injected into the peritoneal cavity of mice tends gradually to disappear. The amount of oil recoverable after a given time depends upon its toxicity, as registered by the carcinogenicity for the skin, refractive index fall, refractivity etc. The 7th day after injection is apparently about the best time to collect the oil.

Loss of body weight of the animal is also a measure of the carcinogenicity for the skin, refractive index fall, refractivity etc. of the oil sample under test. The 3rd day after injection is apparently about the best time to record loss of body weight.

The best animal test for measuring the activity of mineral oils is the injection test, and subsequent determination of the refractive index fall.

The best physical test is to determine the refractive index and viscosity and to combine these in an empirical formula.

A combination of the two tests is the best of all.

The relative amounts of carcinogenic and dermatitic constituents responsible for the general activity can be gauged by a consideration of the viscosity.

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