13544

047466

Chemical:

PC Code:
HED File Code
Memo Date: **02/06/80**
File ID: **00000000**
Accession Number: **000-01-0050**

HED Records Reference Center
07/22/2002

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

168501
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DATE: February 6, 1980

SUBJECT: Risk Analysis for Carcinogenicity of Nitroso-Prowl.

Caswell #454BB

FROM: Chief, Toxicology Branch/HED (TS-769)

TO: Peter E. McGrath, Director
Hazard Evaluation Division (TS-769)

The following is a risk analysis for nitroso-Prowl alternative to that made by Mrs. Barton in her November 28th memorandum addressed to you, a copy of which I obtained from the Environmental Fate Branch late last week.

As requested by you, I have set down in some detail here the basic assumptions underlying this analysis; also presented here is a discussion on how such assumptions differ from those made by Mrs. Barton and by the CAG, whose calculations she had used to a large extent. Unfortunately, this comparison is not complete since, as I indicated to you previously, some of the assumptions or justifications for the CAG analysis are unknown to me while others are of questionable value.

ASSUMPTIONS:

A. Exposure Information for nitroso-Prowl (source:- EFB memorandum of 12/12/79)

1. Applicators

a) Inhalation - 4.3 ngm/cu.m./hr x 1.8 cu.m./hr x 10 hrs for a working day = 77.4 ngm/day; for a 60 kgm person, this is 77.4/60 = 1.29 ngm/kgm/day;

b) Dermal - 18 ngm/hr x 10% estim. absorption rate x 10 hrs for a working day = 18 ngms/day; for a 60 kgm. person, this is 18/60 = 0.30 ngm/kgm/day;

total - sum of (a) and (b) above 1.29 + 0.30 ngm/kgm/day = 1.59 ngm/kgm/day = 0.000,001,59 mgm/kgm/day.

EFB estimate (based on an 8 hr. working day, on only 10 working days per year, on a 70 kgm body-weight, and on a working life of 30 years out of a lifespan of 70 years) is 0.01 ngm/kgm/day for the "average lifetime exposure"; this is 159 times less than the estimate given above. It is this EFB estimate which was used by Mrs. Barton.

2. Incorporators

a) Inhalation - 1.4 ngm/cu.m./hr x 1.8 cu.m./hr x 10 hrs for a working day = 25.2 ngm/day; for a 60 kgm person, this is 25.2/60 = 0.42 ngm/kgm/day;

b) Dermal - 110 ngm/hr. x 10% estim. absorption rate x 10 hrs for a working day = 110 ngm/day; for a 60 kgm. person, this is 110/60 = 1.83 ngm/kgm/day;

total - sum of (a) and (b) above - 0.42 + 1.83 ngm/kgm/day = 2.25 ngm/kgm/day = 0.000,002,25 mgm/kgm/day.

EFB estimate (based on the same assumptions as given for applicators above) is 0.02 ngm/kgm/day; this is more than 110 times less than the estimate given above. It is this EFB estimate which was used by Mrs. Barton.

NOTE: It is questionable whether the so-called "average lifetime exposure" estimates are the proper ones to estimate "safe" levels of nitrosamines. It is known that, at least in experimental animals, only a relatively few and, even single exposures of DEN may induce cancer; in view of this it seems to me that the pertinent estimate to use is the daily rate of exposure rather than some average or cumulation of it based on merely 10 working days per year and on merely a fraction of one's lifetime.

B. The "surface area correction"

The Barton memorandum devotes a full page to this topic which ends in its "bottom line" with a value of 5.85 as the ratio for the surface area of a 70 kgm human and a 350 gm rat. Despite all the abstruse mathematics on which this calculation is based, such ratio is palpably incorrect as even a non-mathematician knows intuitively:- the surface area of an adult human is vastly greater than 5.85 times the surface of a rat. Rather than the one-third power of the ratio of their weights, the surface area ratio is the two-thirds power of the ratio of weights. Thus, the two-thirds power of 70/0.35 is 34.20 which is considerably more satisfying as the ratio of the surface areas of a 70 kgm. human and a 350 gm rat, than the one-third power of the same ratio which is only 5.85.

I have no objection to using the surface area correction even though I am not persuaded that it provides a "truer" estimate of the difference between rats and humans; however, my lack of such objection is based principally on the fact that whether we consider a 70 kgm. human to be "worth" 200 rats of 350 gms. each on a "weight" basis or only 34.20 such rats on a "surface area" basis, does not seem to me to make a great deal of difference justifying a fuss over it.

C. The Correction for Relative Molecular Weights

This correction is even more minor than the previous one; it is based on an EFB estimate of 102/310 as the ratio of the molecular weights of DEN and nitroso-Prowl respectively. This factor is only 0.3290 or less than one-third and it indicates that in a given weight there are one-third as many molecules of nitroso-Prowl as there are molecules of DEN.

D. The Animal Experiment

The Barton risk assessment relies on the work of the CAG who have assumed as proper an experiment where no control (unexposed animals) results are given, only thirty-three subjects (hamsters) were exposed, the exposure was by "spray atomizer" (?) and there was only one exposure level rather loosely defined as "1 to 2" mgms of DEN; also there is no report on the concentration of the test agent in the air breathed by the experimental animals, or the length of their confinement in an inhalation chamber (if indeed such chamber was at all used in this "study") or on the malignancy status of the tumors observed. Again, I wonder whether there was any histopathologic examination of such tumors which are merely reported as "lung and/or tracheal tumors". No information on the weight of the experimental animals is given.

Precisely why the CAG chose from amongst the dozens of experiments on the carcinogenicity of DEN this particular experiment - where they estimate a rate of exposure of 2.857 mgm/kgm/day to be associated with a response rate of 18/33 or 54.54% tumor-bearing animals - is not known to me. It is also not known to me just why Mrs. Barton selected this particular experiment for her calculations if we consider that the CAG also describe another experiment in hamsters exposed to DEN by gavage where the slope estimated by the CAG is more than twice as large as in the hamster inhalation experiment.

The rat experiment that I had originally selected for the risk analysis indicated a higher response rate (60% animals with malignant tumors) at a rate more than 19 times smaller (0.15 mgm/kgm/day) than the exposure rate estimated by the CAG for the hamster inhalation effort. In the rat experiment chosen by me there were at least 5 exposure levels, at least 225 animals exposed, the tumors were reported as having been malignant, and the long-term (historical) control rate of malignant tumor-bearing animals is only 1%.

To compare the relative "potency" of DEN as estimated by these two independent experiments, the following is informative with respect to the estimate of the slope parameter for the one-hit procedure:-

Hamster Inhalation Experiment:-

1-2 mgm DEN associated with 18/33 = 54.54% tumor-bearing animals and assuming a control incidence of zero; $P_t = 1 - \exp.(-\lambda x)$ where $P_t = 0.5454$ and x is estimated by the CAG to be 2.857 mgm/kgm/day; $1 - P_t = \exp(-\lambda x)$; $\ln(1 - P_t) = -\lambda x$; $\lambda = [-\ln(1 - P_t)]/x =$ $= -\ln(0.4546)/2.857 = 0.788337/2.857 = 0.2759$.

Since lung or tracheal tumors in the hamster are very rarely of the malignant variety and since only a small (if any) proportion of the tumors observed were likely malignant, the estimated slope probably represents a vast exaggeration of the slope referrable to malignant tumors; I shall, however, give it the benefit of such doubt.

Rat Feeding Experiment:-

0.15 mgm/kgm/day associated with 27/45 = 60% malignant tumor-bearing animals and assuming a control incidence of 1% animals with such tumors, i.e., a difference of 59% attributable to exposure; proceeding as above, $\lambda = [-\ln(0.41)]/0.15 = 0.891598/0.15 = 5.9440$, i.e., a factor of over 21.5 fold and, due to the malignancy status mentioned above, probably much larger than this.

We can conclude, therefore, that the "potency" of DEN as assessed in the hamster inhalation study selected by Mrs. Barton is only a small fraction of that manifested in the rat feeding study which I had selected as the basis for the risk analysis.

E. The Extrapolation Procedure

In her memorandum to which I referred earlier here, Mrs. Barton states that the one-hit extrapolation procedure "is the preferred one", presumably over other similar such procedures.

I would challenge such assertion - there is no basis whatsoever to decide which method is the preferred one since the mechanism of carcinogenesis for chemicals such as DEN (or others, for that matter) is not known. The one-hit model is predicated on assuming a specific such mechanism, but the evidence that precisely this mechanism is operative in areas other than radiation carcinogenesis is non-existent.

In my previous memorandum on this subject, I have given estimates resulting from both the one-hit and the log-probit (Mantel-Bryan) procedures; if I were asked to select one over the other of these two extrapolation procedures, I would select the latter since:-

a) it need not be based on any specific assumption with respect to the mechanism of carcinogenic action of the test agent;

b) data from several experiments can be utilized jointly in the sense that the data are not combined but, rather, the information resulting from a number of independent trials can be pooled; this advantage is not shared by the one-hit procedure.

c) the one-hit procedure (described by Hoel et.al. in J. of Toxic. and Envir. Health, 1, 133-151, 1975) is indicated by these authors to be only an "interim" procedure which should be subject to review as further research becomes available and at least every two years. We are now some five years down the pike since the date of that publication, and, as far as I know, no such updating has been carried out by the CAG or by anyone else on the one-hit method of extrapolation. Yet Hoel et.al., indicate in a footnote precisely what it was they had in mind:- where some knowledge on the carcinogenesis process is available, other models than the linear one might be appropriate; an example indicated is that of the bladder tumors resulting from renal concretions.

It may well be true that certain specific situations would seem to fit the one-hit model - the effect of a single unit of radiation on a single susceptible cell, or the effect of a single crystal of asbestos on a single cell. Yet there are other examples where the one-hit theory almost certainly does not apply:- bladder tumors occasioned by nitrilotriacetate (NTA) come about not as a direct effect of the agent but rather from the crstalluria occasioned by the high levels of NTA and the damage to the urinary tract when high concentrations of NTA are required to be excreted. Clearly, the NTA would not be acting as a direct carcinogen; if crystal formation is necessary for tumors to occur, then relatively large amounts of NTA would be required. Sitll another pertinent example here would be saccharin where dietary levels of 50,000,000 ppb were required to elicit moderate frequencies of bladder tumors in male rats, contrasting to levels of only 50 ppb or less at which diethylstilbestrol elicits high frequencies of tumors. Clearly something different from a one-hit model must be operative in the case of NTA and saccharin which seem to require overwhelming doses for carcinogenic action.

Still another example here, one that is closer to our field of pesticides, would be chlordane and heptachlor. For these hepatocarcinogens it seems likely that a condition of general hepatotoxicity need be fulfilled before cancer of the liver becomes manifest; this, again, would seem to be a clear case where the one-hit theory would almost certainly not be applicable.

F. The CAG Computations for the Estimate of the Slope Parameter of the One-Hit Procedure

As indicated to you repeatedly in the past, the assumptions underlying the CAG's computations are completely unknown to me.

For example, on page 11 of their report, their computations involve a factor of the one-third power of 70/0.15 - is this the relative surface areas of humans and hamsters? is so, is this the appropriate power for such ratio? The same computations for the slope estimate involve another factor of the third power of 5/18 whose logic escapes me completely. I suspect it may be related to the fact that the hamster tumors appeared rather early, but then how would the CAG justify precisely the third power of such ratio at precisely that site of the formula for computing the slope? At least these kinds of things are mysteries to me.

The value for the slope estimated by the CAG is 99.87. Mrs. Barton then took this estimate (which she properly rounded off to 100) and multiplied it by 0.33×10^{-8} mgm/kgm/day (the EFB's estimate of the average lifetime exposure for applicators corrected for the molecular weight difference between nitroso-Prowl and DEN) to arrive at a risk of 3.3×10^{-7} for these; similarly she obtained a risk twice as large, 6.6×10^{-7} for incorporators.

As mentioned earlier here, these estimated risks may be too low for the following principal reasons:-

1. An average lifetime exposure was assumed rather than the higher daily exposure rate; the justification for this assumption is not given;

2. for their estimation of the slope parameter, the CAG had used an experiment which not only is of questionable value, but which also tends to markedly understate the carcinogenic propensity of DEN. The reason for the CAG's selection of this particular experiment is not reported;

3. the rationale for the CAG's actual estimation procedure for the slope parameter escapes me;

4. the reason for the CAG's selecting only the one-hit extrapolation procedure is unknown to me;

5. the reason for the CAG's selecting the observed incidence rate (rather than some high upper confidence limit on it as would have been proper) also eludes me.

To give an illustration of the possible impact of this last point, consider the following example of hypothetical data:-

suppose in a certain study where animals were exposed at a level x = 1,000 ppm, say, of an agent on test, 30% of the control animals were observed to manifest a certain type of tumor which was also observed amongst the exposed animals but at a 70% rate. Suppose, further, that ten animals were tested in each of these two groups. The way the CAG would proceed with this particlar situation would be to carry out a test of statistical significance of the observed difference here. Since the result of this would be a clear lack of any such significance at some high probability level, they would stop right there without carrying out any further risk analysis.

However, the actual risk from a carcinogenesis point of view will not be affected by whether the CAG do or do not decide whether to assess it. Suppose, alternatively, that the same conditions are present in another situation where the same agent was tested at the same level of exposure and an identical response rate was noted in the control and exposed groups:- 30% and 70% respectively of the animals manifesting precisely the same type of tumor; where this situation differs from the previous one, however, is only in the number of animals tested; say, here 1,000 animals in each group were tested rather than merely 10. A test for statistical significance for the difference in response rates would now yield highly significant results and the CAG may well decide we are dealing now with a "carcinogen". Note, however, that absolutely nothing has changed except that the size of the two groups being compared is much larger in the second case. The response rate has not changed, neither has the level of both the artificial and the field exposure, and certainly the risk of humans exposed under field conditions to the agent on test has not changed. The CAG would now typically proceed with a formal risk analysis; in their computations they would have reference to the actually observed rate of response in the two groups without any consideration being given to the number of unexposed and exposed animals in each group. In other words, their methodology would not allow them to distinguish between these two markedly dissimilar situations, except that in the first case they would not carry out a formal risk assessment whatsoever. Were they nevertheless to carry out such a formal risk assessment in each of these two cases, their estimates of the human risk would be indistinguishable.

If one does take into account the number of animals tested in each situation and one utilizes this information by having reference to lower and upper limits of some wide confidence interval (say the 95% interval) one would say that at some specified upper limit on the risk such as, say, 1/1,000,000 there would correspond a "virtually safe" level of the agent on test such as 0.000,379 ppm for the first case, and one of 0.001,015 ppm in the second case; both of these estimates are given through use of the one-hit extrapolation method. Why do we view a higher level of the agent as "virtually safe" in the second case as opposed to the first one? Simply due to the fact that there

is considerable more information on the carcinogenicity of that agent when 1,000 animals per group were tested than when only 10 such animals per group were tested and this increment in information has resulted in a lessening of the uncertainties involved here. The equivalent estimate that the CAG would make in this case (which would be based on an assumption that they have "perfect" information here, i.e., as if an infinity of animals would have been tested in each group) would be something of the order of 0.001,180 ppm. The way they would phrase this result, however, would be to say that a field exposure level of 0.001,180 ppm would entail a human risk of 1/1,000,000. By the same token, they would regard the estimate of 0.000379 ppm (resulting from the first situation given here) and the estimate of 0.001,015 ppm (resulting from the second situation given here) as also causing exactly the same human risk. This would lead one to the conclusion that if one merely alters the size of some animal experiment, the risk to humans posed by a given fixed level of the agent on test can be increased or decreased. The logic of such conclusion is self-evident.

An alternative risk analysis for nitroso-Prowl

Assumptions

1) Take exposure information for nitroso-Prowl as developed by EFB, but utilize daily rates of exposure as opposed to "average lifetime" rates; these would be 1.59×10^{-6} mgm/kgm/day for applicators, and a slightly higher rate of 2.25×10^{-6} mgm/kgm/day for incorporators;

2) Apply the correction factor of 102/310 = 0.329 for the relative molecular weights of nitroso-Prowl and DEN; this would convert the exposure rates given above to 5.2316×10^{-7} mgm/kgm/day for applicators and 7.4032 mgm/kgm/day for incorporators;

3) Apply an additional factor of 5.85 for the surface area correction as suggested by Mrs. Barton; this would yield corrected rates of 3.06×10^{-6} and 4.33×10^{-6} mgm/kgm/day for applicators and incorporators respectively;

4) Compute estimates from the Druckrey rat feeding experiment whose results were:-

mgm/kgm/day	rate of animals bearing malignant tumors:-
0.15	27/45 = 60.00%
0.30	67/80 = 83.75%
0.60	49/60 = 81.67%
1.20	36/40 = 90.00%
2.40 and more	virtually 100.00%

additional assumptions are:-

a) a 1% historical incidence rate of control (unexposed) animals with malignant tumors as reported by Prof. Druckrey;

b) an upper 95% confidence limit on the observed incidence of exposed animals;

c) selection of the response noted at 0.15 mgm/kgm/day which provided the maximal estimates of the "virtually safe" level of DEN;

d) the one-hit method of extrapolation;

e) the log-probit method of extrapolation;

f) a variety of upper limits on the risk;

g) the basic premise (this is more than an assumption, it is a fact) that point estimates of the risk to humans naturally exposed to a carcinogen by virtue of occupation cannot be made from considerations arising out of an animal experiment where the subjects were artificially exposed; at most, what can be estimated are "virtually safe" levels of the chemical on test for a variety of upper limits on the risk of the population of animals from which the experimental subjects were drawn; if one could estimate the risk for a large population of humans from a finite and small sample of animals, this would lead to the unacceptable conclusions which I had discussed earlier there:- that the risk to the human population would increase or decrease depending on the size of the sample of animals used in the test;

h) identical susceptibility for humans and for the experimental animals.

Subject to the conditions listed above, the following estimates are made:-

Log-probit model

Upper 95% confidence limit on response noted at 0.15 mgm/kgm/day, 27/45 = 60.00%, is 74.30%; correction by Abbott's formula for control incidence yields a difference of 74.04% for the increment in response due to exposure; to this difference there corresponds a normal deviate of 0.644,256.

Upper limit on risk	Virtually safe level of DEN (mgm/kgm/day)
1/100,000,000	0.000,000,083
5/100,000,000	0.000,000,160
1/ 10,000,000	0.000,000,215
5/ 10,000,000	0.000,000,437
1/ 1,000,000	0.000,000,601
5/ 1,000,000	0.000,001,30
1/ 100,000	0.000,001,85
5/ 100,000	0.000,004,38

The upper limit on the risk corresponding to exposure rates $3.06 - 4.33 \times 10^{-6}$ mgm/kgm/day of nitroso-Prowl would be in the interval 1 - 5/100,000; more exactly;-

for 3.06×10^{-6} mgm/kgm/day for applicators there would correspond the following upper limit on the risk:-

0.000,003,06/0.15 = 0.000,020,400; 1/0.000,020,400 = 49019.6078; log 49019.6078 = 4.69036983; 4.69036983 - 0.644256,282 = = 4.046113550; normal area corresponding to this deviate is 2.6/100,000;

for 4.33×10^{-6} mgm/kgm/day for incorporators there would correspond the following upper limit on the risk:-

0.000,004,33/0.15 = 0.000,028,866; 1/0.000,028,866 = 34642.0324; log 34642.0324 = 4.539603363; 4.539603363 - 0.644,256,282 = = 3.89534708; normal area corresponding to this deviate is 4.9/100,000.

One-hit model

To go from an observed incidence (P_1) to an upper limit on the risk (P_2), the choice of the base of the logarithms is imaterial; thus one would use the ratio log $(1-P_2)$/log $(1 - P_1)$; here P_1 = 74.04% (see log-probit calculations), $1 - P_1$ = 0.2596 and log $(1 - P_1)$ = - 0.58569531.

Upper limit on risk	Virtually safe levels of DEN (mgm/kgm/day)
1/100,000,000	0.000,000,007,42
5/100,000,000	0.000,000,037,1
1/ 10,000,000	0.000,000,074,2
5/ 10,000,000	0.000,000,371
1/ 1,000,000	0.000,000,742
5/ 1,000,000	0.000,003,71
1/ 100,000	0.000,007,42
5/ 100,000	0.000,037,1

Comparison of this table with the previous similar one reveals once more what is the usual case:- at very low upper limits on the risk, the one-hit estimates are considerably smaller than the corresponding log-probit ones; thus at an upper limit on the risk of 10^{-8}, they are <u>smaller</u> by a factor of approximately 11.2. This relationship reverses at the higher upper limits on the risk with the one-hit estimates becoming the larger ones:- at an upper limit on the risk of 5×10^{-5}, they are <u>larger</u> by a factor of approximately 8.5. The two types of estimates are of comparable magnitude at an upper limit on the risk in the neighborhood of $5 - 10 \times 10^{-7}$.

Thus, depending on the actual upper limit on the risk, a single choice of the extraplating model can either underestimate or overestimate the "virtually safe" level of an agent by comparison with another such extrapolating model.

To find the upper limits on the risk associable with the estimated exposure rates for nitroso-Prowl, we use $P(x) = 1 - \exp(-\lambda x)$ where x is the level of exposure (3.06 or 4.33×10^{-6} mgm/kgm/day for applicators and incorporators, respectively) and λ or the slope parameter is estimated, as before, from the experimental data;-

$\lambda = -\ln[1 - P(x)]/x = -\ln(1 - 0.7404)/0.15 = -\ln(0.2596)/0.15 = 1.3486/0.15 = 8.9908.$

Note that this particular value of λ is more than 10 times smaller than the equivalent value estimated by the CAG from the hamster inhalation study.

Multiplying this by the exposure rate, we obtain the upper limits on the risk $8.9908 \times 3.06 \times 10^{-6} = 2.751 \times 10^{-5}$ for applicators and $8.9908 \times 4.44 \times 10^{-6} = 3.893 \times 10^{-5}$ for incorporators, which are rather close to the estimates given by the log-probit model.

Summary

Subject to the assumptions listed above, the upper limits on the risk associable with exposure to nitroso-Prowl are estimable as:

$2.6 - 2.8 \times 10^{-5}$ for applicators and $3.9 - 4.9 \times 10^{-5}$ for incorporators.

These estimates are approximately two orders of magnitude higher than those generated by Mrs. Barton who relied in part on other estimates for DEN originating with the CAG.

M. Adrian Gross