ABSTRACT

WILLIAM HAMILTON LOWRY. Retrospectively Assigning Context of Observation for F-344 Rats Chronically Exposed to Formaldehyde. (Under the Direction of ALVIS G. TURNER)

One approach to estimating age-specific tumor incidence rates using scheduled sacrifice information involves determining context of observation for each tumor bearing animal. Context of observation is defined as determination of whether the tumor of interest contributed directly or indirectly to the cause of death, or alternatively, the tumor was an incidental finding at necropsy, in an animal dying of an unrelated cause. The present study was undertaken to assign retrospectively the context of observation for nasal squamous cell carcinoma in F-344 rats exposed to 15 ppm of formaldehyde for up to 24 months. Results indicate that greater than 90% of the tumors were classifiable as definitely incidental (2%) or definitely fatal (88%) with a high degree of confidence. This study demonstrates that context of observation can be assigned even long after the bioassay has been completed, provided the data have been properly archived.

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Table of Contents

Tables

Figures

Appendices

LITERATURE REVIEW

Regulatory History

In 1946, the American Conference of Governmental Industrial Hygenists (ACGIH) set the threshold limit value (TLV) for formaldehyde at 10 parts per million (ppm). Responding to reports documenting formaldehyde as an irritant, the ACGIH reduced the TLV to 5 ppm in 1948. In 1970, this TLV was adopted under the Construction Safety Act and incorporated in the Occupational Safety and Health Administration (OSHA) construction standards. However, complaints of irritation at concentrations far below this ceiling forced the ACGIH to further reduce the ceiling limit to 2 ppm (Federal Register, 1987).

The National Institute of Occupational Safety and Health (NIOSH) in 1976 recommended that OSHA reduce the permissible exposure limit (PEL) measured over a period of 30 minutes to 1 ppm. This recommendation was based on reports of eye, skin, and respiratory irritation experienced by a few workers at 0.3 ppm and widespread complaints at levels exceeding 1 ppm (Federal Register, 1987).

During the year of 1976, the Consumer Product Safety Commission (CPSC) received numerous reports from individuals complaining of adverse health effects from formaldehyde exposure. As a result, the National Research Council's Committee on Toxicology was asked to evaluate the literature and

determine safe concentrations from long-term continuous exposure in the home. Based on available data, the Committee concluded that there was no human population threshold for the irritant effects of formaldehyde, even at extremely low airborne concentrations (NAS 1980). Regarding formaldehyde carcinogenicity, the Committee prefaced their report by stating:

> "It must be recognized that the concern and deliberations that led to development of this document have to a certain extent been superseded by the recent preliminary report from the Chemical Industry Institute of Toxicology (CIIT), which indicated that formaldehyde exposure induced nasopharyngeal carcinomas in rats. It is strongly recommended that when the CIIT study has been reported in detail, and the results are available for evaluation, an appropriate peer group should review in detail and comment on the investigation..." (NAS 1980).

In October of 1979, CIIT responded to reporting requirements established by section 8(e) of the Toxic Substances Control Act (TSCA). The Environmental Protection Agency (EPA), who enforces TSCA, was sent the preliminary findings of the animal bioassy indicating squamous cell carcinoma in rats exposed to formaldehyde concentrations of 14.3 ppm at interim sacrifices. The study was completed in 1981 and results indicated nasal cancers in rats exposed to concentrations of 14.3 and 5.6 ppm and in mice exposed to 14.3 ppm. This evidence was corroborated by a study at New York University (NYU) that also found nasal cancers in rats at 14 ppm (Federal Register, 1987).

In 1980, the Federal Panel on Formaldehyde, consisting of scientists from eight federal agencies, evaluated the existing data and concluded that it was

"prudent to regard formaldehyde as posing a carcinogenic risk to humans" (Federal Register, 1987). Based on the CIIT and NYU studies along with the Federal panel's conclusion, NIOSH in 1981 classified formaldehyde as a potential occupational carcinogen.

In 1982, the CPSC used the CIIT data, coupled with consumer complaint data, to ban urea-formaldehyde foam insulation (UFFI) in homes and schools. However, this ruling was overturned in subsequent litigation (Gulf South Insulation v. CPSC, 701 F. 2d 1137 5th cir. 1983) because of unsubstantiated evidence. The court faulted CPSC for relying solely on the data of the CIIT study in establishing a risk estimate for consumers. Also, the Court determined that consumer complaint data were not an acceptable method for determining the risk of injury due to the effects of an acute irritant (701 F.2nd at 1148).

In 1983, the ACGIH added formaldehyde to the list of industrial substances suspected of causing cancer in humans and reduced the Threshold Limit Value (TLV) to 1 ppm, measured as an 8 hour time weighted average (TWA). ACGIH also set the short-term exposure limit (STEL) for formaldehyde at 2 ppm (Federal Register, 1987). Also in 1983, the National Center for Toxicological Research (NCTR) sponsored a workshop on formaldehyde to try to resolve the controversies surrounding formaldehyde's acute and chronic health effects. Scientific studies were reviewed by over 60 scientists from government, industry and universities. A risk estimation panel attempted to develop a risk assessment for humans exposed to formaldehyde. They concluded that carcinogenicity was the only end point that could be assessed quantitatively. The panel also

concluded that the modeling for carcinogenicity should be based on the CIIT study instead of human epidemiology studies that did not provide adequate evidence for carcinogenicity (Federal Register, 1987).

The Department of Housing and Urban Development (HUD) in February of 1985 designated standards for off-gassing of formaldehyde from pressed wood products used to produce manufactured and mobile homes. These regulations prohibit the emissions of formaldehyde from plywood and particleboard which would result in an air concentration in excess of 0.2 ppm and 0.3 ppm, respectively (Federal Register, 1987). One year later, the EPA announced it would relinquish its authority to regulate formaldehyde in occupational exposure settings because of OSHA's jurisdiction in that area. However, EPA would continue to investigate non occupational situations, such as those settings which used pressed wood products made with formaldehyde-based resins. In April of 1987, EPA published their assessment of health risks associated with formaldehyde exposure. Their position states that formaldehyde should be classified as a "group B1 probable human carcinogen," based on 1)"sufficient" evidence that formaldehyde is an animal carcinogen, 2) "limited" evidence from human studies, and 3) other information, including short-term tests, (ie. gene mutation, sister chromatid exchanges, and chromosome abberation), pharmacokinetic studies, (DNA-protein crosslinks in the nose of rats exposed to > 2 ppm) and comparative metabolism studies, (showing non-linear increases in covalent binding at higher concentrations) (Nelson et al., 1986). EPA's "limited" (a causal interpretation is credible) evidence from human studies is

based on nine epidemiologic studies, but because of possible exposure to other agents, the findings could have been confounded (Federal Register, 1987). In 1988, an Ad Hoc Panel on Health Aspects of Formaldehyde, composed of independent, international scientists, evaluated the existing literature and concluded that: 1) for no malignancy in man is there convincing evidence of a relationship with formaldehyde exposure and 2) if a relationship does exist, the excess risk, in absolute terms, must be small. The panel futher stated that the apparent lack of consistency in studies of site-specific cancer risk, and the uncertainty resulting from unresolved confounding by known risk factors, prevented the panel from making more definitive conclusions (Universities Associated for Research and Education in Pathology, Inc., 1988).

A working group from the International Agency for Research on Cancer (IARC) in 1987 reviewed recent epidemiological evidence on formaldehyde and upgraded their designation to category 2(A), a probable human carcinogen. OSHA promulgated the final rule for occupational exposure to formaldehyde published in the Federal Register dated December 4, 1987 in 29 CFR Parts 1910 and 1926. This standard reduced the permissible exposure level (PEL) from 3 ppm of formaldehyde in air to 1 ppm as an 8 hour TWA, and reduced the 15 minute short term exposure limit (STEL) from 5 ppm to 2 ppm. OSHA also at this time revoked the peak allowable exposure of 10 ppm and included an "action level" of 0.5 ppm measured as an 8-hour TWA to reduce the compliance burden for employers whose employees have minimal exposure to

formaldehyde. This standard took effect on February 2, 1988 (Federal Register, 1987).

Physical and Chemical Properties of Formaldehyde

Formaldehyde is defined as the chemical entity HCHO, and is identified by the Chemical Abstracts Service Registry No. 50-00-0. Formaldehyde is a colorless gas that boils at -19° C upon condensing and freezes at -118° C. Because of this chemical's ability to polymerize so readily, it is sold or transported in solution. Formalin is the most frequently encountered solution, containing 37-50 per cent formaldehyde and 6-15 per cent alcohol stabilizer, usually in the form of methanol (Nelson et al. 1986). Paraformaldehyde occurs as a solid polymer that vaporizes to its monomeric form and can contribute as a source of formaldehyde gas. Pure forms of formaldehyde gas are stable at temperatures between 80° and 100° C, however stability is dependent upon purity and even water will enhance the rate of polymerization (Federal Register, 1987).

One molecule of formaldehyde consists of a single carbonyl group with two atoms of hydrogen, $H_2C=O$. Most reactions (Nelson et al., 1986) fall into the following three reaction sequences:

OXIDATION-REDUCTION

Cannizarro Reaction

$$2HCHO \text{ ----- } HCOOH + CH_3OH$$

ADDITION OR CONDENSATION

Bisulfite Addition:

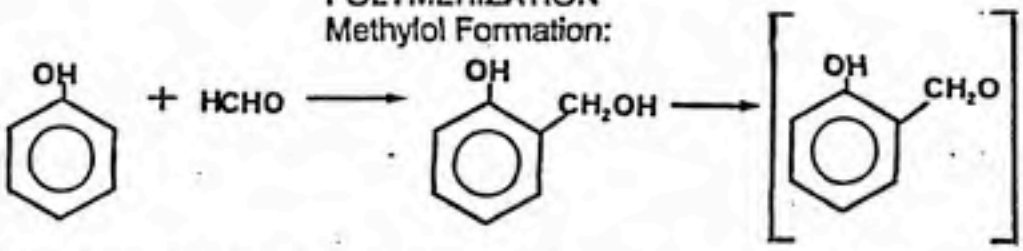
$$HCHO + NaHSO_3 \longrightarrow HOCH_2SO_3Na$$

Aldol Condensation:

$$HCO + R'(R'')CHC{=}OR \longrightarrow HOCHCR'(R'')C{=}OR$$

POLYMERIZATION

Methylol Formation:



Production and Use of Formaldehyde

Formaldehyde was first produced in the United States in 1901. It is now ranked 24th in production volume in the United States, with 5.7 billion pounds produced in 1985 (Federal Register, 1987). Formaldehyde is produced using two processes: the mixed oxide catalyst process or the silver oxide catalyst process. While both of these processes require methanol as the precursor, the processes differ in catalyst type, operating temperatures, and methanol/air ratios. Because of formaldehyde's high degree of chemical reactivity, it readily undergoes a wide variety of chemical reactions, as previously mentioned. Addition or condensation reactions are of great commercial value, for these reaction yield such products as pentaerythritol and hexamethylenetetramine, which are used in the wood product and apparell manufacturing industries respectively. However, methanol formation is the most important commercial reaction because the derivatives are used as the starting point in resin production (Federal Register, 1987).

The manufacture of resins such as urea-formaldehyde, phenol-formaldehyde, and melamine formaldehyde comprise 59% of the total consumption of formaldehyde. Approximately 33% is used in the synthesis of such chemicals as pentaerythritol, hexamethylenetetramine, and butanediol. Two percent is consumed in the treatment of textiles, while small amounts are used in consumer and industrial products such as cosmetics, shampoo, glue, and preservatives (Federal Register, 1987).

Urea-formaldehyde resins represent an entire class of resins formulated by polymerizing different ratios of urea and formaldehyde at different pH levels and combining such additives as thickeners, hardeners, plasticizers, and curing agents. Greater than half of the urea-formaldehyde resins produced are used as adhesives in the manufacture of particleboard and plywood. Urea-formaldehyde foam insulation (UFFI) was a resin made from urea, water, and formaldehyde that was mixed on site and pumped, using a propellant, into both commercial and residential building (Federal Register, 1987).

Phenol-formaldehyde resins are produced by the condensation reaction of methylol derivatives and are used primarily as binders for softwood plywoods. They are also used in the production of compounds of plastic molds, insulation, and abrasives. Polyacetal resins and melamine-formaldehyde resins are used in plastic molded compounds such as plates and cups, buttons, and decorative laminates used in furniture (Federal Register, 1987).

Nelson et al. (1986) report a number of other uses of formaldehyde including the manufacture of rubber, photographic film, leather, explosives,

dyes, cosmetics, corrosion inhibitors, and embalming fluids. Other reported uses include the production of vaccines.

Exposure

Because formaldehyde is released from so many sources, it is difficult to assess the amount released into the environment from both direct and indirect processes. Attempts have been made to estimate exposure levels (Preuss et al., 1985) from four major types of exposure; ambient air, indoor exposure, consumer exposure, and occupational exposure (tables I-IV respectively). These three categories can be condensed by combining ambient air, indoor air, and consumer exposure into one category, environmental exposure, which involves exposure outside the workplace. Those exposures occurring in the workplace will be termed occupational exposure. Tables I, II, and IV indicate the mean and maximum level in parts per billion (ppb), the number of observations and the type of exposure. Ambient mean levels range from 0.4 ppb (background rural) to 24.0 ppb in the Los Angeles Basin during unfavorable climatic conditions. Possible sources of environmental exposures to formaldehyde include motor vehicle exhaust, photochemical smog, and the emissions from burning of gas, oil, coal, and wood (Nelson et al., 1986).

Indoor exposure to formaldehyde concentrations as demonstrated in tables II and III indicate that off-gassing from both UFFI and pressed-wood products such as particle board and plywood account for significant routes of exposure.

Table II demonstrates that mean concentration levels rose substantially after using UFFI in both conventional and nonconventional buildings. In conventional buildings, the mean concentrations rose from 9-20 ppb, before using UFFI, to 130-280 ppb the first 90 days after using UFFI. The increase is even larger among nonconventional buildings, citing mean concentration levels of 34 and 46 ppb before installation and 380 and 700 ppb after installation of UFFI (Preuss et al., 1985).

Occupational exposure is summarized in table IV. There are a number of populations that appear to be exposed to formaldehyde. The embalming and funeral service industry has the highest number of people with 2,600,000 exposed at a mean level of 740 ppb. The second largest group of workers exposed is the textile and apparel manufacturing industry with 800,000 people exposed to concentrations of a mean level of 250 ppb. While there are only 4000 workers estimated in the particle board manufacturing business, the mean exposure level is the highest at 920 ppb. Other sources with high mean concentrations include UF foam manufacturing at 740 ppb, with only 50 workers exposed, and metalworking machine operations at 500 ppb with 55,000 workers exposed (Preuss et al., 1985).

Health Effects

There is a wide range of health effects from exposure to formaldehyde either through the inhalation route or from dermal contact (NAS 1980 Appendix I). In humans, airborne concentrations as low as 0.1 ppm have been shown to cause

irritation in the eyes, nose, and throat. Irritation increases as concentrations increase with severe lacrimation (tearing of the eyes) and pulmonary reactions such as pnuemonia, bronchial inflammation, and pulmonary edema at airborne concentrations of 50 ppm. Concentrations of 100 ppm for 30 minutes or longer are believed to be fatal (CFR 29). Skin irritation is a well documented effect from dermal contact (Glass 1961, Sneddon 1968). Repeated dermal exposure can lead people to become sensitized provoking allergic reactions, although even the most sensitized persons can tolerate up.to 30 ppm formaldehyde in products topically applied (NAS 1981). There are reports of various other types of health effects on the central nervous system, reproductive system, and blood, but the end point of primary interest from a regulatory point of view to date has been carcinogenicity.

The Role of Epidemiology

Epidemiology has played a key role in detecting exposure to carcinogens. Methods used for assessing risk to humans from exposure to potential carcinogens should be supported by human data (Clayson et al., 1985). However, these studies are of very limited value under certain circumstances. For substances that have been recently introduced into the commercial market, the use of epidemiologic studies to determine carcinogenicity would not be feasible because of the long latency period involved in developing the disease of interest (Armitage P. 1982). There are several other limitations (U.S. Interagency Staff Group on Carcinogens (USISGC) 1986) that are difficult to overcome.

Problems related to hazard evaluation include occupational exposure levels that are usually at higher levels than the exposure level of interest. Determining causal relationships at lower concentrations than exposure levels observed, using extrapolation techniques may produce spurious results. Another problem arises in determining causality. The USISGC (1986) state that in a strict sense it is never possible to prove causality. A hypothesis concerning the cause of any noted increased incidence following exposure may, however, be given evidential support, based on dose-response relationships, consistency and reproducibility of results, the strength and specificity of the association, its biological plausibility, and other considerations. A causal hypothesis can provide compelling evidence that can lead to preventative action as in the case of cigarette smoking and lung cancer.

While there are many limitations to using epidemiologic studies in the risk assessment process, there are many strengths as well. The major strengths include the studies' abilities to directly assess the risk from environmental exposure to carcinogens. This in turn may give some insight into the human carcinogenesis process which allows the extrapolation from exposure to similar chemicals that have yet to be tested. These studies compliment the data gathered in long-term animal studies to provide the decisionmaker with the necessary evidence to make well informed decisions pertaining to the protection of public health (US Interagency Staff Group on Carcinogens 1986).

Epidemiologic Evidence

There have been several epidemiologic studies performed on persons exposed to formaldehyde in occupational settings. A summary of these studies, as reported by Nelson et al. (1986), is shown in tables V and VI. Table V documents the mortality of industrial workers from the chemical and garment industry, while the mortality of professionals such as pathologists, anatomists and morticians is shown in table VI.

The studies in table V as reported by Nelson et al. (1986), indicate an increase in mortality for various types of cancer, but lack consistency. An increase in mortality from lung cancer was seen in two of the six chemical plants, and one of the three garment plants, while a decrease was observed among pathologists, anatomists, and morticians. Respiratory cancer was slightly increased in one of the four garment plants but was below the expected incidence among pathologists, anatomists, and morticians. There was an increase in buccal cavity and pharynx cancer in two of the industrial worker studies, however the authors point out that the industrial studies may be flawed because of their inability to detect unusual causes of death. Again among the professionals, there is a defference between the observed and expected values. An increase in cancer of the lymphopoietic system was reported among the professionals and garment industry workers, while the six studies of chemical workers were below those expected. There was a very slight increase in bladder cancer mortality among both industrial workers and professionals, with

relative risks of 1.07 and 1.08 respectively. Brain cancer was consistently high among the studies of professional workers exposed to formaldehyde, but not among industrial workers.

Overall, the epidemiologic evidence linking cancer with exposure to formaldehyde is "limited", meaning a causal interpretation is credible (Nelson et al., 1986). However, comparing different types of cancers among occupationally exposed workers reveals some inconsistency in the findings of these studies. Many workers in industrial settings are exposed to other compounds such as wood dust, a known carcinogen, which may bias the results. Also the exposure levels are difficult to determine. In the studies listed in tables V and VI, the author cites a range of mean exposures and the highest level reported. The mean exposures range from 0.17-3 ppm in the chemical industry studies, with the highest level reported as 5.4 ppm. The mean exposures range from 0.15 - 8.3 ppm among anatomists, with the highest mean level reported as 14.8 ppm.

A case-control study was performed by Vaughan et al. (1986) in an effort to determine if an association exists between occupational exposure to formaldehyde and cancer of the pharynx, or sinus and nasal cavity. Results from their study indicated that there was no significant association found between occupational exposure to formaldehyde and the types of cancers they were scoring. However, the authors admit to several limitations of the study which tended to conservatively bias the results.

In a study undertaken by the National Cancer Institute (NCI) in collaboration with the Formaldehyde Insitute (FI), Blair et al. (1987) reported the results of the

largest mortality study of industrial workers exposed to formaldehyde. However, selection of the cohort was criticized strongly because four of the ten plants used were studied previously, perhaps indicating that the author had prior knowledge of the plant population. The cohort consisted of over 25,000 workers who had been exposed for at least 14 years to formaldehyde. Standard Mortality Ratios (SMR) were evaluated in relation to dose (an 8 hour TWA > 0.1 ppm). Based on his analysis, the author concluded that these data provided little evidence that mortality from cancer was associated with exposure levels of formaldehyde experienced by the workers studied.

Laboratory Evidence

Long-term animal bioassays to determine the carcinogenicity of chemicals began in the 1920s. It was not the intent of these early researchers to use this procedure as a routine method for testing chemicals (Pito, 1981). By the 1960s, the National Cancer Institute (NCI) had begun large scale testing using standardized protocol (USISGC, 1986). Until this time, there were no uniform testing procedures. One conclusion drawn from these tests, with the exception of arsenic, was that known human carcinogens produce tumors in animals in properly carried out studies. The inverse of that statement, that substances causing tumors in animals will produce tumors in humans, may not be valid. Certain chemicals do not produce tumors in all species, but if a chemical has produced a carcinogenic effect in one species, "in the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for

which there is sufficient evidence of carcinogenicity in animals as if they presented a carcinogenic risk to humans" (IARC, 1984).

Currently, chronic bioassays are used to determine if exposure to a test substance alters the normal patterns of tumor development (Garth et al., 1986). Typical National Toxicology Program (NTP) design protocol requires randomized groups of animals exposed to various treatment levels by some route of administration. A group of non-exposed animals serve as a control for comparison. The animals are observed for the majority of their lifetime. Animals that die before the predetermined end point of the study, or animals that are killed at interim or terminal sacrifices, undergo necropsy histopathological examination of organ tissue from various sites. The basic data recorded for each animal includes the number of days on the study (time of death), necropsy (gross lesions), and histopathological evaluation, including diagnosis of those tissues that were examined, and any clinical observations noted during the course of the study. Statistical analysis is performed to determine the strength of the evidence of carcinogenicity for that test substance (Garth et al., 1986).

The biological event of interest is usually the occurrence of a tumor at a particular organ site. Garth et al. (1986) define a tumor as lesion within a well-defined class of neoplastic tissue, restricting the group of lesions to tumors of the same histological type and arising from the the same kind of tissue. While initially this appears to be a workable definition, many problems arise upon closer examination. Such outcomes as tumor multiplicity, acceleration of tumor development in exposed groups and unusual tumor morphology may also be

carcinogenic responses that require the judgement of an experienced pathologist.

Table VII, taken from a summary by Flamm et al. (1985) provides the results of numerous short-term and long-term animal studies. Many of the earlier studies reported no significant increase in tumor incidence, however, most of those studies used small numbers (ie. 6 rabbits, 10 rats) of animals in each group or used an exposure route (oral, drinking water) that did not produce a detectable increase in tumor incidence.

Regulatory agencies, such as OSHA, have stated that the CIIT chronic inhalation study clearly stands out as a superior data set from which to define a dose-response curve (29 CFR Part 1910). CIIT (1981) used two strains of animals, the Fischer 344 rat and the B6C3F mouse. There were 120 animals of each sex exposured to 0, 2.0, 5.6, and 14.3 ppm of formaldehyde. The animals were exposed for 6 hours per day, 5 days per week for up to 24 months. The scoring of squamous cell carcinoma (SCC) gave the following results when sexes were combined: for the mouse study, there were 0 tumors at 0, 2.0, and 5.6 ppm, and 2 tumors at 14.3 ppm. In the rat study there were 0 tumors at 0 and 2.0 ppm, 2 tumors (1 male, 1 female) at 5.6 ppm and 103 (51 males and 52 females) developing SCC at 14.3 ppm, yielding a dose-response relationship (non-linear) only in the rat population at the higher doses (see figure 1). The concentration dependence in this bioassay has permitted the use of quantitative risk assessment procedures by regulatory agencies to set exposure limits that will protect the public health of workers, consumers, and the public at large. The

CIIT study has been used in every formal risk assessment process by the regulatory agencies to set exposure limits for formaldehyde. A study reported by Albert et al. (1982), performed at New York University, corroborated CIIT's results using a different strain of rat, the Sprague-Dawley. Although only one concentration was used (14.2 ppm), and the length of study was slightly less than 2 years, a significant increase of nasal tumors was reported.

Results demonstrate a species sensitivity as well as a concentration dependence. The species difference (at 14.3 ppm there was a 50% tumor incidence in rats versus 3.3% tumor incidence in B6C3F1 mice), as proposed by Chang et al. (1982), involves the mouse's ability to reduce minute ventilation so as to have less formaldehyde available for deposition in the nasal cavity than the rat. Also, the difference in surface area in the nasal cavity between the mouse and rat (the mouse being much smaller) helps to explain the large difference in tumor incidence rates among the two species.

Both malignant and benign tumors were observed during experiments. Kerns et al. (1981) reported squamous cell carcinoma (SCC) in 103 rats at a exposure level of 14.3 ppm. In that same high dose group, three rats developed polypoid adenomas, a benign tumor. Two of these animals developed SCC. While the response of the polypoid adenomas was not dose related, there were some questions by the regulatory agencies as to whether the polypoid adenomas should be included in the risk assessment. Morgan et al., (1986) decided to look more precisely at the location of both the squamous cell carcinomas (SCC) and the polypoid adenomas. Morgan mapped the distribution

of the SCC and reported that they originated in the mucosa of the anterior nasal passage and developed from the surface epithelium and not the underlying glands. This point of origin differs from that of the polypoid adenomas which originated from poorly ciliated epithelium in the most anterior portion of the nasal cavity (fig. 2). Also the polypoid adenomas were confined to a small region of the nasal cavity whereas the SCC were invasive and found in multiple levels of the nasal cavity. Because of these differences and the fact that the squamous cell carcinomas are not considered to be the malignant counterpart of the polypoid adenomas, the data from the benign polypoid adenomas were not included in the formal risk assessments as proposed by OSHA. However, this controversy has not been resolved. The USEPA has proposed including the polypoid adenomas in their risk assessment.

Effects of Treatment

Determining effects on mortality due to exposure of a compound is of primary interest when analyzing chronic bioassay data (Garth et al., 1986). Comparing differences in mortality patterns among treatment groups can be performed using the Kaplan-Meier estimator of the survival function, a step function defined as follows:

$$S_i(t) = \prod_{k \epsilon R(t)} \left[1 - \frac{x_{ik}}{n_{ik}} \right]$$

Suppose that deaths are observed at K distinct times t_k, k=1,2,...K. Suppose that x_{ik} is the number of animals dying in group i at t_k, and n_{ik} is the number of animals at risk of dying in group i at t_k. For any t, let $R(t) = \{k: t_k \leq t\}$. Another way of stating it is that R(t) is the set of all k with death times occurring at or before t. A plot of the survival function $S_i(t)$ from beginning to the end of the experiment compares any effect of treatment on mortality with controls (Garth et al., 1986). See figure 3 as an example of this method of data treatment. It may be seen that there is very little effect on mortality at the low dose; however, there is a substantial effect at the high dose as demonstrated by many earlier deaths compared with the low dose or control group.

Classically, when using the Kaplan-Meier method for determining the effects of a compound on mortality, an assumption had to be made was that the tumor of interest was rapidly fatal. Therefore if an animal developed the tumor, it was assumed that he died from the tumor. Using context of observation information allows the investigator to determine whether the animal died from the tumor, or whether the tumor was an incidental finding at necropsy. Therefore the assumption that the tumor was rapidly fatal does not have to be made when context of observation information is available, demonstrating a more accurate and realistic effect of treatment on mortality.

Interpretation of data from chronic bioassays is used to determine whether the treatment also produced a carcinogenic effect. If biases due to randomization, animal husbandry, and pathological examination are eliminated, then the effects observed can be attributed to one of three events: 1) chance,

meaning that each treated individual has the same opportunity as untreated individuals for developing the outcome 2) differences in the length of time that each individual was at risk of developing the outcome, or 3) the real effects of treatment. The possibility of chance alone effecting the outcome can be determined using classical hypothesis testing. Longevity differences can be corrected so as to eliminate the bias due to intercurrent mortality, leaving only the effects due to treatment (Peto et al., 1980).

Peto et al. (1980) demonstrated the need to adjust for intercurrent mortality when analyzing chronic bioassay data. Intercurrent mortality refers to all interim deaths that are unrelated to the outcome of interest. Previously, investigators either reported crude proportions of tumor-bearing animals among treatment groups, or they partially corrected for intercurrent mortality by reporting the number of tumor-bearing animals as a percentage of the number of survivors when the tumor was first observed. This does not completely eliminate intercurrent mortality. If there are similar survival patterns among treatment groups, then expressing results in this manner may give a reasonably accurate picture of the effects of treatment. However, if the treatment reduces longevity, for example through acute toxicity, among different groups, animals may die at a younger age from this exposure, or other causes, then the treated animals do not survive a sufficiebt length of time to express the tumor of interest. Interpretation of the effects of treatment in these studies can have serious problems.

For example, consider a chronic bioassay comprised of 200 animals, 100 in the control group (unexposed) and 100 animals in the exposed group, (exposed to a chemical). Prior to 12 months, the control group shows 4/40 (10%) of the animals developing a tumor (where the numerator is the number of animals developing the tumor and the denominator is the number of animals that died) and the exposed group showed 18/90 or 20% of the animals developing a tumor. However, after 12 months, the control group demonstrated 21/60 (35%) and the exposed group demonstrated 7/10 (70%). Regardless of the fact that in both groups (prior to and after 12 months) the proportion of tumors amongst survivors was higher in the exposed group than the controls, the total fraction of animals developing tumors is 25/100 (25%) for both groups (see Table VIII). This suggest a need to take into consideration the differences in longevity between the exposed and control groups (Garth et al. 1986).

Table VIII

	Control	Exposed
Died prior to 12 months	4/40 (10%)	18/90 (20%)
Died after 12 months	21/60 (35%)	7/10 (70%)
Total for experiment	25/100 (25%)	25/100 (25%)

Determining Age-Specific Incidence Rates

Chronic bioassays for routine testing of chemicals has become an increasingly important tool used by regulatory agencies in setting standards for potential carcinogens. Until recently, however, analysis and interpretation of

chronic bioassay data have been focused primarily on frequency of tumor occurrence, neglecting such aspects as time to tumor onset and death, either from natural causes or as part of scheduled sacrifices. While advances have been made in the areas of experimental design and pathological evaluation of long-term animal studies (Gart et al., 1986), statistical methods for determining age-specific tumor incidence rates remain hampered by the lack of adequate data. This lack of adequate data is inherently problematic in the approach by which animal data are collected. Because tumor onset time is not an observable outcome for occult tumors, age at death with tumor must be substituted, and statisticians are required to make inferences based on assumptions (ie. the tumor is rapidly fatal) that may not always represent the real situation.

The use of age-specific incidence rates avoids biases due to the differences in mortality (Mc Knight 1987) by comparing tumor rates among only those animals surviving to each age. Determining context of observation for each individual animal will help to eliminate this bias due to intercurrent mortality. Context of observation identifies whether the tumor of interest contributed directly or indirectly to the death of the animal, or alternatively, whether the tumor was just an incidental finding at necropsy in an animal dying of an unrelated cause. Because of the unobservable nature of tumor onset time, a number of different types of time to tumor estimation procedures are presented in the literature. While a review of these procedures is beyond the scope of this paper, it is of interest to note that problems exist using each of these estimation procedures. A parametric approach by Borgan et al. (1984) for estimating tumor

incidence was of little value without data from frequent sacrifices. Other approaches by Turnbull et al. (1978) using sophisticated statistical software packages to fit log-linear and logistic models to data found it difficult to correlate the observed differences in the model fits in different groups to the differences in tumor incidences rates, except in special cases (McKnight and Crowley, 1984). More recently Portier (1986) and Portier and Dinse (1987) have proposed a semiparametric model which describes the tumor onset rate as parametric in form, but the death rate is adjusted in a nonparametric fashion and requires sacrifice information.

Using information from assigning context of observation is another approach to determining age-specific tumor incidence rates. Figure 4 is a schematic of the status of animals in a chronic bioassay study at any specific time. There are four different possibilities for each animal; 1) alive and not tumor (ANT), 2) dead and not tumor (DNT), 3) alive with tumor (AT), and 4) dead with tumor (DT). There are three different conditional death rates; 1) the rate at which tumor-free animals die without a tumor (λ D/NT), 2) the rate at which tumor-bearing animals die from the tumor (λ DFT/T), and 3) the rate at which tumor-bearing animals die from causes other than the tumor (λ DOC/T). The tumor incidence rate λ^T is the rate at which tumor-free animals develop a tumor. Because tumor onset is unobservable in occult tumors (tumors that develop in places that can not be seen, ie. internal organs), it is necessary to include tumor prevalence information. Tumor prevalence (p)is defined as the proportion of those animals alive with tumors, ie. $p = AT/(AT+ANT)$. Tumor prevalence can

be estimated from scheduled sacrifices since at any given time in a large population the prevalence of tumors among the sacrificed animals should be the same as the prevalence of tumors in the population. Combining this information together with context of observation information, and making a few assumptions, allows the tumor incidence rate to become estimable (Starr 1985).

Tumor prevalence can change with time. Tumor-free animals can develop tumors or die tumor-free. Tumor-bearing animals can die from the tumor or from other causes. The rate of change of prevalence with respect to time, dp/dt, is dependent upon the tumor incidence rate and the natural death rates of both tumor-free and tumor-bearing animals and can be expressed as follows:

$$(dp/dt) = (1-p)\lambda^{T} - p(1-p)(\lambda^{DFT/T} + \lambda^{DOC/T} - \lambda^{D/NT})$$

This equation can be solved in terms of the tumor incidence rate by rearranging to become

$$\lambda^{T} = (dp/dt)/(1-p) + p(\lambda^{DFT/T} + \lambda^{DOC/T} - \lambda^{D/NT}).$$

Prevalence information is estimated from frequent scheduled sacrifices, assuming the prevalence of the tumor in the sacrificed animals is representative of the prevalence in the population. The three conditional death rates ($\lambda^{DFT/T}$, $\lambda^{DOC/T}$, $\lambda^{D/NT}$). When sacrifice information is not available to estimate prevalence, the assumption that the presence of a tumor has no effect on the

risk of dying from other causes, $\lambda^{D_{\bullet}C/T}$ becomes equal to $\lambda^{D/NT}$ and these two terms cancel. When this is true, it has been shown that the prevalence of tumor-bearing animals dying of natural (nontumor) causes is the same, on average, as the prevalence rate. That is to say the natural deaths from causes other than the tumor serve as unscheduled sacrifices and yield information about tumor prevalence and the rate of change with time (see appendix II for derivation of formula and complete explanation). Thus the tumor incidence rate for occult tumors can now be estimated from the context of observation information (Starr 1985).

METHODS

The data used for this study were obtained from the final report issued by the Chemical Industry Institute of Toxicology (CIIT 1981). The 24 month inhalation toxicity study in male and female mice and rats was initiated in June of 1978 using 120 rodents per sex at exposure concentrations of 0, 2, 6, and 15 ppm for 6 hours per day, 5 days a week for 24 months. There was a post exposure period of 6 months. Control animals were subjected to the same procedures as the treated group, except exposure was to air without formaldehyde. Body weight, clinical observation, and mortality were monitored for the length of the study. Because a significant tumor incidence rate (> 50%) was observed only at the 14.3 ppm level in rats (1% tumor incidence rate at 5.6 ppm in rats), the 14.3 ppm was the only dose group considered in this study.

A record was developed for each animal for the purpose of retrospectively assigning context of observation (see figure 5). This record contained the following information: animal number, pathology number, sex, death status (unscheduled death or sacrifice), and number of days on study. Also included in each record were clinical observations, necropsy data (citing gross abnormalities in organs, cavities, and glands), and histopathology data listing any abnormal organs,including a diagnosis and a severity rating for any

abnormal organs. Tumors, (squamous cell carcinomas) were listed as being present. Each record was reviewed by a pathologist, Dr. Thomas Monticello. For tumor bearing animals, an initial context (using a four-way classification system, see below) was assigned based on the information contained in the record. Histopathological examination of tissue was used in determining the final context of observation.

Materials used for the histopathology portion of this study were derived from the same tissue sections and paraffin blocks of the previously reported chronic inhalation study of formaldehyde in F-344 rats (CIIT, 1981; Kerns et al., 1983). Sections of nasal passages at four representative levels of the nose (Figure 6) were examined by light microscopy for all rats reported to have squamous cell carcinomas (Kerns et al., 1983). For each animal, the following criteria were evaluated as being present or not present (a score of one or zero, respectively):

i) 50% or more of the nasal cavity occupied by tumor or the associated keratin produced by the tumor, at any one level

ii) tumor present in more than one level of the nose

iii) tumor invasion

iv) tumor necrosis

v) moderate to severe tumor-associated inflammation

Based on the results of each case, a four-way classification system was used in assigning context of observation (ie. whether the cause of death was directly or indirectly due to the tumor), as described by Peto (1980):

Context 1 - Tumor found in animal certainly or almost certainly did not directly or indirectly cause the animal's death.

Context 2 - Tumor was probably non-fatal to animal (ie. probably context 1, but not sure).

Context 3 - Tumor was propably fatal to animal (ie. probably context 4, but not sure).

Context 4 - Tumor found in animal certainly or almost certainly was fatal to animal. This category includes both direct causes of death by the tumor and indirect causes due to some consequence of the tumor (ie. obstruction of airway, tumor-induced infection, paraneoplastic effects of tumor).

RESULTS

Table IX list data for each animal. These data include the pathology number, number of days on study (time of death), sex, context of observation, tumor status, and death status (sacrifice or unscheduled death).

Table X summarizes the tumor status for all 240 rats exposed to 15 ppm of formaldehyde. As shown in table X, 103 rats (52 female and 51 males) were found to be tumor bearing, 121 rats were found to be tumor free and 16 animals were classified as either missing or critical tissues were not examined. Two of the five animals listed in the extended unscheduled death category were not available for nasal turbinate examination, while the three animals that were examined in that category were all found to be tumor bearing.

Table XI lists the criteria used to determine context of observation for tumor bearing animals. Neither of the two animals classified in the definitely incidental (DI) context met any of the criteria. Only one of the two animals classified in the probably incidental (PI) context met one of the criteria. In contrast, six of the seven animals were scored for two of the criteria in the probably fatal (PF)

context, and 74 of the 92 animals in the definitely fatal (DF) context met every criterion. Eighty-eight of the 92 animals in this category had multi-level involvement and necrosis.

Table XII summarizes the context of observation for all tumor bearing animals by death times of both unscheduled deaths and sacrificed animals. The data suggest that squamous cell carcinoma (SCC) is a fatal tumor in rats. Greater than 97% of the tumors were classified as either definitely fatal (88%) or probably fatal (9%). As expected, the majority of animals that died unscheduled deaths from the tumor (definitely fatal) occurred between 12 and 24 months, with more than half the tumors occurring between 18 and 24 months.

Figure 7 illustrates the Kaplan-Meier estimate of survival to death from tumor, assuming the tumor is rapidly lethal. The first tumor bearing animal died at day 358, and the last tumor bearing animal died at day 819.

Table XIII lists the data necessary to construct the Kaplan-Meier survival curve. This includes the number of responses (both positive and negative) and the number of days on study listed in the order of death times.

DISCUSSION

While assigning context of observation proved to be a simple and efficient approach used in statistical analysis of bioassay data, pathologists have been reluctant to perform this task. Results from this study indicate that it is possible to assign context of observation retrospectively with a high degree of confidence, and incorporate this data into the analysis for determining age-specific tumor incidence rates.

The use of a four way classification system, as suggested by Peto et al., (1980) allows the pathologist some latitude in determining whether the tumor of interest was observed in an incidental or fatal context by using a "probably" category. This has advantages for both the pathologist and the statistician. If the pathologist is unsure if the tumor was definitely fatal or definitely incidental, the pathologist has the option of classifying the tumor as probably fatal or probably incidental allowing for some degree of uncertainty in cases of multiple tumors or systemic diseases such as leukemia. The statistician has the option of reversing the numbers in the probably incidental and probably fatal categories to determine the effects of misclassification on the outcome. If the numbers in the "probably" categories are relatively small, then misclassification will likely have little or no effect on the outcome. Peto et al., (1980) evaluated over 4500 Colworth rats for liver and esophageal tumors using this type of classification

system. Although Peto used a separate category especially for scheduled sacrifices (context 0), they reported 5.6% of their cases with an uncertain context. In this study almost 10% of the cases had an uncertain context when animals used in scheduled sacrifices were included, but less than 4% are of uncertain context when scheduled sacrifices were not included. The rationale for including the animals in scheduled sacrifices with the unscheduled deaths is based on the assumption that the tumor is rapidly lethal, and when a tumor-bearing animal is sacrificed, the animal actually dies from the sacrifice but would have died soon after because of the tumor.

These data suggest that nasal tumors can be classified using context of observation with a high degree of confidence. The validity of this assumption is illustrated in Table XI which indicates that there were no responses to any of the criteria in the context definitely incidental and over 90% response to every criteria in the definitely fatal context. Interestingly, both of the animals that were placed in the probably incidental context, and 5 of the 7 animals placed in the probably fatal context, were from scheduled sacrifices and account for almost 90% of those animals with an "uncertain" context. If the results from the scheduled sacrifices were not combined as Peto et al., (1980) suggest, then the degree of certainty of context would be over 98%.

Criteria used to determine context of observation (tumor size, invasiveness, multi-level involvement, necrosis, and inflammation) were used to reduce much of the subjectivity that may arise involving the determination of whether a tumor is incidental or fatal. Using distinct and well defined criteria for determining

context of observation allows pathologists the possibility of producing results of similar quality if data are available.

The Kaplan-Meier estimator of survival to death from fatal tumors is an appropriate way of visually demonstrating cumulative tumor mortality. The graph displays the proportion of animals surviving at any given time when all other causes of death except from the tumor of interest are eliminated. It is unappropriate to combine both fatal tumors and incidental tumors when using this graph; therefore context of observation becomes important in determining only those tumor which were fatal. Usually this curve is used to compare mortality among treatment groups; however in the case of formaldehyde, because there were only two tumors in the 5.6 ppm dose, and no tumors in the 2.0 ppm and control group, the results of graphing these doses would be a horizontal straight line at 1.0 (except for a slight downward dip to account for the two tumors found at terminal sacrifice at the 5.6 ppm treatment level). Graphing the low dose and control groups would show insignificant effects due to treatment.

Peto et al., (1980) used information from context of observation to analyze his data from 4500 Colworth rats (16 treatment groups) exposed to N-nitrosodimethylamine (NDMA). Using the same categories as previously described (Contexts 1-4), Peto reported 42% of the tumors were classified as either definitely incidental (39%) or probably incidental (3%), and 58% as either definitely fatal (55%) or probably fatal (3%). Because nearly half of the tumors found were classified as either definitely incidental or probably incidental, Peto

used two different methods to analyze his data to eliminate the bias due to intercurrent mortality. For those rats in context 1 and 2 (definitely/probably incidental), Peto used the 'prevalence' method. For those animals in context 3 and 4 (definitely/probably fatal), he used the 'death rate' method. He discovered that if the 'prevalence' method had been applied to both the incidental tumors and fatal tumors, misleading inferences would have resulted, demonstrating a protective effect of NDMA. Conversely, if he used the 'death rate' method for both fatal and incidental tumors, he found a highly significant carcinogenic effect. This clearly demonstrates the need to eliminate the bias due to intercurrent mortality. However, in the case of formaldehyde, because greater than 97% of the tumors (SCC) were classified as either definitely fatal or probably fatal, using the "death rate" method would be appropriate for analyzing those tumors classified as definitely or probably fatal, while the remaining 3% of the incidental tumors should be analyzed using the prevalence method to avoid introducing bias.

CONCLUSIONS

There is a high degree of confidence (greater than 90%) that animals with tumors can be readily classified with a context of observation.

Squamous Cell Carcinoma was probably/definitely fatal to rats exposed to 14.3 ppm formaldehyde in 97% of the cases, and therefore an analysis based on the assumption that the tumor was rapidly lethal would not be expected to produce much bias.

It is evident that this classification can be done in a retrospective mode, long after the bioassay has been completed, provided that the information has been properly archived.

Age-specific tumor incidence rates can be estimated using scheduled sacrifice and context of observation information.

Context of observation information provides a simple and efficient approach to making inferences concerning age-specific tumor incidence rates. This information helps to eliminate potential bias due to intercurrent mortality among treatment groups.

This type of data also adds a new dimension to risk characterization by focusing attention on the time to tumor onset in addition to the probability of developing a tumor.

RECOMMENDATIONS

Carefully selected criteria to determine context of observation should be used to reduce much of the subjectivity that might arise when pathologists attempt to classify the tumor as incidental or fatal.

Investigators should properly archive all the information so retrospective analysis can be performed without loss of accuracy over time.

It is apparent from this study and from Peto's work that both nasal tumors and internal organ tumors can be classified with a high degree of confidence. Pathologists should be encouraged to determine context of observation both retrospectively and as the study is on-going.

More emphasis should be placed on time-dependent aspects of carcinogenesis bioassays. Comparing age-specific tumor incidence rates is a much more effective methods for determining effects from treatment.

More work needs to be done in quantaitative modeling field to accommodate time-to-tumor data that will allow regulatory agencies to focus on more than just the cumulative lifetime probability of developing cancer.

Change the terminology of context of observation to something that is understandable to most people. Recommendations include cause of death assignment, tumor context, or tumor lethality.

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Table I. Ambient Formaldehyde Levels

Type of Exposure	Number of Observations[a]	Exposure Level (ppb) Mean	Max.
Rural areas (background)[b]			
Eniwetok Atoll, South Pacific	7	0.4	0.8
Urban areas[c]			
Los Angeles Basin (typical)	27	8.0	12.0
Los Angeles Basin (severe inversion)	65	24.0	48.0
Bayonne, N.J.	NR	6.1	20.0
Camden, N.J.	NR	3.8	14.0
Elizabeth, N.J.	NR	5.5	18.0
Newark, N.J.	NR	6.6	20.0

[a]NR indicates not reported.
[b]Estimated number exposed: 60,000,000.
[c]Estimated number exposed: 167,000,000.
Source: Adapted from Ref. Preuss et al., (1985)

Table II . Indoor Exposure to Formaldehyde

Source of Exposure	Sampling and Analytical Methods[a]	Number of Observations	Mean Concentration Levels (ppb)
Canadian UFFI Study			
Homes with health complaints	CA	100	139
Control (non-UFFI) homes	CA	378	34
UFFI homes (no complaints)	CA	654	40
UFFI homes (no complaints)	CA	1146	54
United Kingdom UFFI Study			
Control (non-UFFI) buildings	MBTH-CA	50	47
UFFI buildings	MBTH-CA	1143	93
Buildings before-after UFFI			
Conventional (double-wall masonry) before	MBTH-CA	7	20-9.0
Conventional (double-wall masonry) after	MBTH-CA	7	130-280[b]
Nonconventional (prefab concrete) before	MBTH-CA	2	46, 34[c]
Nonconventional (prefab concrete) after	MBTH-CA	2	380, 700[d]

[a]CA is chromotropic acid and MBTH is 3-methyl-2-benzothiazolone hydrazone.
[b]Averages over first 90 days postinstallation.
[c]Averages of two houses prior to installation.
[d]Averages of the two houses measured prior to installation over first 90 days postinstallation.
Source: Adapted from Refs Preuss et al., (1985)

Table IV Occupational Exposure to Formaldehyde

Exposure Source	Estimated Number Exposed	Number of Observations	Exposure Level (ppb) Mean	Max.
Direct mfr. of formaldehyde	1400	135	410	2200
Resin mfr. (UF, PF)	6000	8	240	490
Plywood mfr. (UF, PF)	27,000	91	350	1200
Particle board mfr.	4000	6	920	1400
Wood furniture mfr.	60,000	6	100	140
Mobile home mfr.	32,000	—	—	—
UF foam mfr.	50	4	740	1260
UFFI installation	1000	17	420	1300
Metal molds–castings mfr.	60,000	11	390	690
Plastic products mfr.	17,000	8	350	500
Paper and paperboard mfr.	1000	64	470	990
Textile and apparel mfr.	800,000	30	250	310
Building paper and building board mfr.	4000	—	—	—
Paints and coatings mfr.	2300	—	—	—
Abrasive products mfr.	7000	—	—	—
Asbestos products mfr.	—	—	—	—
Nonresin CHO derivatives mfr.	250	—	—	—
Nitrogenous fertilizers mfr.	3000	1	500	500
Use of CH_2O-containing sanitation products	—	2	380	470
Use in agricultural pesticide applications	—	12	320	650
Biology-medical laboratories	—	—	—	—
Embalming and funeral service industry	2,600,000	6	740	1390
Metalworking machine operations	55,000	9	500	1200

Note: — indicates data not available.
Source: Preuss et al., (1985)

Table V - Formaldehyde exposure: mortality of chemical and garment workers.

Cause of death	Observed/expected (O/E) deaths: Chemical industry[a] A1	A2	A3	A4	A5	A6	Total A (85)	B (98)	C (89)	D (90)	E (87)	Garment industry[b] F (88)	Total A–F	O/E[c]
All causes	77/93	88/107	49/45	845/953	104/149	446/485	1619/1862	115/—[d]	24/—[d]	146/197		256/—[d]	1765/2059	0.86
All cancers	19/23	32/27	15/11	251/246	21/38	114/123	455/468	20/22	10/6	37/37	42/27	87/73	651/633	1.03
Skin							2/—		0/—	1/0.9		2/1.1	3/2.0	—
Buccal cav. and phar.							5/4.6		2/0.2			3/1.3	10/6.1	1.64
Respiratory								6/7.5	3/2.3	12/12.4		11/12.2	32/34.4	0.93
Nose	0/0.05	0/0.06	0/0.03	0/0.56	0/0.09	0/0.28	0/1.07	0/—	0/—	0/—	0/—	0/—	0/1.1	—
Larynx							4/4.5			1/—		0/—	4/4.5	—
Lung	6/9.3	11/11.5	7/4.7	128/103.4	7/13.3	46/51.6	205/193.8			11/11.7	18/7.6	11/11.6	245/224.7	1.09
Digestive							118/117	8/6.3	4/1.5	5/9.5	14/9.0	22/17.5	171/160.8	1.06
Esophagus									0/—			1/0.9	1/0.9	—
Colon									4/0.6	3/3.0			7/3.6	1.94
Prostate									0/—	4/1.3			4/1.3	—
Kidney							7/8.3		0/—	1/1.0			8/9.3	0.86
Bladder							18/16.9		0/—	1/0.8			19/17.7	1.07
Brain							5/12.5		0/—	3/1.6		1/2.1	8/16.2	0.56
Lymphopoietic							20/26.3	2/2.3	1/0.5	6/4.4	5/2.5	10/6.1	44/42.1	1.05
Leukemia							9/11.4			2/1.7		4/2.4	15/15.5	0.97

[a] Range of mean exposures, 0.17–3 ppm; no. of samples > 142; highest level reported, 5.4 ppm.
[b] Range of mean exposures, 0.7–0.74 ppm; no. of samples, 85; highest level reported, 2.7 ppm.
[c] O/E given only when observed and/or expected deaths ≥ 5.
[d] Proportional mortality study.

Source: Nelson et al., (1986)

Table VI Formaldehyde exposure: mortality of pathologists, anatomists, and morticians (includes combined totals of observed and expected deaths for Tables 1 and 2).

Cause of death	Observed/expected (O/E) deaths: Pathologist[a] G1 (99,100)	G2 (99,100)	Anatomist[b] H (92)	Mortician[c] I1 (101,102)	I2 (101,102)	J (103)	K (101,105)	L (106)	Total G–L	O/E[d]	Total Tables 1 and 2 Total A–L	O/E[d]
All causes	146/244	110/195	737/1129	1132/—[e]	1007/—[e]	319/322	333/—[e]	31/—[e]	1312/1890	0.69	3077/3949	0.78
All cancers	38/62	32/52	120/188	243/219	205/170	68/67	69/60	17/13	772/831	0.93	1423/1464	0.97
Skin			2/3.5	8/3.6[f]	2/3.4	0/0.9	0/—	1/—	12/11.4	1.05	15/13.4	1.12
Buccal cav. and phar.			1/6.8	8/7.1	8/6.1	1/2.1	3/1.6	0/—	21/23.7	0.89	31/29.8	1.04
Respiratory			13/45.3	74/70.7	43/46.0	20/21.6	13/17.3	4/3.7	167/205.5	0.81	199/239.9	0.83
Nose		0/0.1	0/0.4	0/0.5	0/0.6	0/0.2		0/—	0/1.8	—	0/2.9	—
Larynx			1/2.8	2/3.4	2/2.6	0/1.0		1/—	6/9.5	0.61	10/14.3	0.70
Lung	10/27.4	9/22.0	12/43.0	78/66.8	41/42.8	19/20.2	12/14.0	3/3.4	178/239.6	0.74	423/464.3	0.91
Digestive	12/19.8	8/15.5	38/66.4	68/65.2	69/57.0	17/22.6	16/19.3	5/4.2	233/270.0	0.86	404/430.8	0.94
Esophagus			2/4.6	5/5.3	2/4.1	0/1.7	1/1.3	0/—	11/17.0	0.65	12/17.9	0.67
Colon			20/18.5	29/20.3	30/16.0‡		5/5.4	1/1.4	85/61.6†	1.38	92/65.2†	1.41
Prostate			20/18.7	15/16.4	23/13.1†	3/3.4	5/6.8	3/1.5	69/59.9	1.15	73/61.2	1.19
Kidney			1/4.0	8/5.4	4/4.6	1/1.7	2/1.4	0/—	16/16.5	0.97	24/25.8	0.93
Bladder	1/2.1	2/1.9	5/7.2	7/7.3	8/5.8		6/2.5	0/—	29/26.8	1.08	48/44.5	1.08
Brain		4/1.2*	10/3.7†	9/5.8	9/4.7	3/2.6	1/1.6	0/—	36/19.6†	1.84	45/35.8	1.26
Lymphopoietic	8/3.8*	2/3.0	18/14.4	25/20.6	19/15.6	8/6.5	10/5.6	1/1.1	91/70.6*	1.29	135/112.7*	1.20
Leukemia	1/1.5	1/1.1	10/6.7	12/8.5	12/6.9	4/2.5	8/2.6†		48/29.8†	1.61	63/45.3*	1.39

[a] Range of mean exposures, 0.16–4.8 ppm; no. of samples, 78; highest level reported, 13.57 ppm.
[b] Range of mean exposures, 0.15–8.3 ppm; no. of samples, 32; highest level reported, 14.8 ppm.
[c] Range of mean exposures, 0.74–2.7 ppm; no. of samples, 200; highest level reported, 5.26 ppm.
[d] O/E given only when observed and/or expected deaths ≥ 5.
[e] Proportional mortality study.
* Significant increase, $p < 0.05$.
† Significant increase, $p < 0.01$.
‡ Significant increase, $p < 0.001$.

Source: Nelson et al., (1986)

Table VI Studies of the carcinogenicity of formaldehyde in laboratory animals

Species	No. animals per group	Compound and dose	Route and frequency	Length of study	Tumour incidence
Rat	10	1 ml of 0.4% formalin	Subcutaneous once/week	15 months	2/10 local sarcomas 1/10 liver 1/10 peritoneal cavity
Rat	20	1 ml of 9–40% HMT*	Subcutaneous once/week	Until tumour formation	7/20 local sarcomas
Rat (BD)	15/sex	0, 0.4 g HMT (total dose)	Oral, drinking-water	333 days	Negative
Mouse (CTM, SWR, C3HF)	27–100/sex	0, 0.5, 1.0, 5.0% HMT	Oral, drinking-water	110–130 weeks	Negative
Rat (Wistar)	12–48/sex	0, 1.0, 5.0% HMT	Oral, drinking-water	156 weeks	Negative
Rat (Wistar)	12–24/sex	0, 1.0% HMT	Oral, drinking-water for 20–40 weeks	3 generations	Negative
Rat (Wistar)	16–48/sex	0, 2.0% HMT	Oral, drinking-water for 50 weeks	2 years	Negative
Rabbit	6	0, 3% formalin	Direct application to palate 5 times/week	10 months	1/6 'carcinoma *in situ*' of the palate
Mouse (C3H)	42–60/group	0, 41, 80, 163 ppm formaldehyde	Inhalation 1 h/day 3 days/week	35–70 weeks	Negative
Hamster (Syrian golden)	88–132 males	0, 10 ppm formaldehyde	Inhalation 5 h/day 3 days/week	Lifetime	Negative
Rat (Fischer 344)	120/sex	0, 2.0, 5.6, 14.3 ppm formaldehyde	Inhalation 6 h/day 5 days/week	2.5 years	Nasal tumours (combined sexes, 103/200 at 14.3 ppm, 2/214 at 5.6 ppm)
Mouse ($B6C3F_1$)	120/sex	0, 2.0, 5.6, 14.3 ppm formaldehyde	Inhalation 6 h/day 5 days/week	2.5 years	Nasal tumours (2/240 at 14.3 ppm)
Rat (Sprague-Dawley)	100 males	0, 14.7 ppm formaldehyde + 10.6 ppm HCl	Inhalation 6 h/day 5 days/week	Lifetime	Nasal tumours (25/99)
Rat (Sprague-Dawley)	100 males	0, 14.3 ppm formaldehyde + 10.0 ppm HCl	Inhalation 6 h/day 5 days/week	588 days*	Nasal tumours (12/100)
Rat (Sprague-Dawley)	100 males	0, 14.1 ppm formaldehyde + 9.5 ppm HCl	Inhalation 6 h/day 5 days/week	588 days*	Nasal tumours (6/100)
Rat (Sprague-Dawley)	100 males	0, 14.2 ppm formaldehyde	Inhalation 6 h/day 5 days/week	588 days*	Nasal tumours (10/100)

*HMT, Hexamethylenetetramine, forms formaldehyde upon decomposition
*Interim results

Table IX

TIME TO TUMOR STATISTICS

	PATH #	DAYS	SEX	COB	TUMOR	DEATH
1	801581	5	M	1	0	UD
2	801587	181	M	1	0	6 MO SAC
3	801651	181	M	1	0	6 MO SAC
4	801569	181	M	1	0	6 MO SAC
5	801641	181	M	1	0	6 MO SAC
6	801628	181	M	1	0	6 MO SAC
7	801583	181	M	1	0	6 MO SAC
8	801650	181	M	1	0	6 MO SAC
9	801595	181	M	1	0	6 MO SAC
10	801636	181	M	1	0	6 MO SAC
11	801653	181	M	1	0	6 MO SAC
12	801797	181	F	1	0	6 MO SAC
13	801761	181	F	1	0	6 MO SAC
14	801701	181	F	1	0	6 MO SAC
15	801728	181	F	1	0	6 MO SAC
16	801776	181	F	1	0	6 MO SAC
17	801700	181	F	1	0	6 MO SAC
18	801690	181	F	1	0	6 MO SAC
19	801717	181	F	1	0	6 MO SAC
20	801693	181	F	1	0	6 MO SAC
21	801748	181	F	1	0	6 MO SAC
22	801762	252	F	1	0	UD
23	801656	274	M	1	0	UD
24	801719	319	F	1	0	UD
25	801627	353	M	1	0	UD
26	801767	353	F	1	0	UD
27	801754	358	F	4	1	UD
28	801763	361	F	1	0	UD
29	801711	364	F	4	1	UD
30	801639	364	M	1	0	12 MO SAC
31	801646	364	M	1	0	12 MO SAC
32	801585	364	M	1	0	12 MO SAC
33	801676	364	M	1	0	12 MO SAC
34	801643	364	M	1	0	12 MO SAC
35	801601	364	M	1	0	12 MO SAC
36	801596	364	M	1	0	12 MO SAC
37	801602	364	M	1	0	12 MO SAC
38	801619	364	M	1	0	12 MO SAC
39	801664	364	M	1	0	12 MO SAC
40	801766	364	M	1	0	12 MO SAC

PATH # = PATHOLOGY NUMBER
DAYS = DAYS ON STUDY
COB = CONTEXT OF OBSERVATION
TUMOR = 1=PRESENT 0=ABSENT
DEATH = UD=UNSCHEDULED DEATH
MO SAC=MONTH OF INTERIM SACRIFICE

TIME TO TUMOR STATISTICS (CONT)

	PATH #	DAYS	SEX	COB	TUMOR	DEATH
41	801791	364	F	1	0	12 MO SAC
42	801777	364	F	1	0	12 MO SAC
43	801751	364	F	1	0	12 MO SAC
44	801774	364	F	1	0	12 MO SAC
45	801720	364	F	1	0	12 MO SAC
46	801746	364	F	1	0	12 MO SAC
47	801684	364	F	1	0	12 MO SAC
48	801787	364	F	1	0	12 MO SAC
49	801765	364	F	1	0	12 MO SAC
50	801617	390	M	1	0	UD
51	801710	420	F	4	1	UD
52	801640	432	M	4	1	UD
53	801659	432	M	4	1	UD
54	801771	433	F	1	0	UD
55	801745	438	F	4	1	UD
56	801610	474	M	4	1	UD
57	801796	477	F	1	0	UD
58	801633	479	M	4	1	UD
59	801741	479	F	4	1	UD
60	801740	488	F	4	1	UD
61	801638	491	M	4	1	UD
62	801604	495	M	1	0	UD
63	801655	504	M	4	1	UD
64	801715	504	F	2	0	UD
65	801725	508	F	4	1	UD
66	801785	509	F	1	0	UD
67	801642	511	M	4	1	UD
68	801792	512	F	4	1	UD
69	801736	520	F	4	1	UD
70	801567	521	M	1	0	UD
71	801772	525	F	4	1	UD
72	801677	530	M	1	0	UD
73	801574	531	M	4	1	UD
74	801729	531	F	1	0	UD
75	801775	533	F	4	1	UD
76	801708	533	F	4	1	UD
77	801697	535	F	4	1	UD
78	801652	537	M	1	0	UD
79	801618	539	M	4	1	UD
80	801742	543	F	4	1	UD

PATH # = PATHOLOGY NUMBER
DAYS = DAYS ON STUDY
COB = CONTEXT OF OBSERVATION
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MO SAC = MONTH OF INTERIM SACRIFICE

TIME TO TUMOR STATISTICS (CONT)

	PATH #	DAYS	SEX	COB	TUMOR	DEATH
81	801575	545	M	4	1	UD
82	801566	545	M	4	1	UD
83	801626	545	M	4	0	UD
84	801733	546	F	1	0	UD
85	801594	547	M	3	1	18 MO SAC
86	801649	547	M	4	1	18 MO SAC
87	801605	547	M	1	0	18 MO SAC
88	801565	547	M	1	0	18 MO SAC
89	801680	547	M	1	0	18 MO SAC
90	801789	547	F	1	0	18 MO SAC
91	801798	547	F	1	0	18 MO SAC
92	801681	547	F	1	0	18 MO SAC
93	801750	547	F	3	1	18 MO SAC
94	801623	550	M	3	1	18 MO SAC
95	801624	550	M	1	0	18 MO SAC
96	801675	550	M	1	0	18 MO SAC
97	801589	550	M	1	0	18 MO SAC
98	801630	550	M	1	0	18 MO SAC
99	801786	550	F	1	0	18 MO SAC
100	801764	550	F	1	0	18 MO SAC
101	801696	550	F	1	0	18 MO SAC
102	801716	550	F	1	0	18 MO SAC
103	801747	550	F	1	0	18 MO SAC
104	801673	551	M	3	1	18 MO SAC
105	801663	551	M	1	0	18 MO SAC
106	801620	551	M	1	0	18 MO SAC
107	801616	551	M	1	0	18 MO SAC
108	801600	551	M	1	0	18 MO SAC
109	801571	551	M	1	0	18 MO SAC
110	801590	551	M	1	0	18 MO SAC
111	801573	551	M	1	0	18 MO SAC
112	801615	551	M	1	0	18 MO SAC
113	801730	551	F	1	0	18 MO SAC
114	801752	551	F	1	0	18 MO SAC
115	801686	551	F	1	0	18 MO SAC
116	801770	551	F	1	0	18 MO SAC
117	801682	551	F	3	1	18 MO SAC
118	801779	551	F	4	1	18 MO SAC
119	801683	552	F	4	1	UD
120	801598	552	M	1	0	18 MO SAC

PATH # = PATHOLOGY NUMBER
DAYS = DAYS ON STUDY
COB = CONTEXT OF OBSERVATION
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DEATH = UD=UNSCHEDULED DEATH
MO SAC=MONTH OF INTERIM SACRIFICE

TIME TO TUMOR STATISTICS (CONT)

	PATH #	DAYS	SEX	COB	TUMOR	DEATH
121	801734	552	F	1	0	18 MO SAC
122	801794	552	F	1	0	18 MO SAC
123	801780	552	F	1	0	18 MO SAC
124	801783	552	F	4	1	18 MO SAC
125	801577	555	M	1	0	UD
126	801667	560	M	4	1	UD
127	801749	567	F	4	1	UD
128	801558	569	M	3	1	UD
129	801706	571	F	1	0	UD
130	801688	572	F	4	1	UD
131	801609	574	M	4	1	UD
132	801621	574	F	4	1	UD
133	801670	574	M	1	0	UD
134	801722	577	M	1	0	UD
135	801658	577	M	1	0	UD
136	801795	583	F	1	0	UD
137	801756	586	F	1	0	UD
138	801564	588	M	4	1	UD
139	801699	588	F	4	1	UD
140	801707	590	F	1	0	UD
141	801698	595	F	4	1	UD
142	801723	596	F	4	1	UD
143	801685	596	F	4	1	UD
144	801799	603	F	4	1	UD
145	801597	606	M	4	1	UD
146	801579	607	M	4	1	UD
147	801582	609	M	4	1	UD
148	801629	614	M	4	1	UD
149	801760	615	F	4	1	UD
150	801592	619	M	1	0	UD
151	801599	619	M	1	0	UD
152	801714	620	F	4	1	UD
153	801625	621	M	4	1	UD
154	801709	624	F	4	1	UD
155	801757	627	F	4	1	UD
156	801781	629	F	1	0	UD
157	801622	630	M	4	1	UD
158	801663	631	M	1	1	UD
159	801666	631	M	4	1	UD
160	801687	631	F	1	0	UD

PATH # = PATHOLOGY NUMBER
DAYS = DAYS ON STUDY
COB = CONTEXT OF OBSERVATION
TUMOR = 1=PRESENT 0=ABSENT
DEATH = UD=UNSCHEDULED DEATH
MO SAC=MONTH OF INTERIM SAC

TIME TO TUMOR STATISTICS (CONT)

	PATH#	DAYS	SEX	COB	TUMOR	DEATH
161	801737	633	F	1	0	UD
162	801744	641	F	1	0	UD
163	801671	642	M	4	1	UD
164	801731	643	F	4	1	UD
165	801678	644	M	1	0	UD
166	801790	652	F	4	1	UD
167	801738	652	F	1	0	UD
168	801561	654	M	4	1	UD
169	801778	663	F	4	1	UD
170	801593	665	M	3	1	UD
171	800727	672	F	4	1	UD
172	801679	673	M	4	1	UD
173	801782	673	F	4	1	UD
174	801769	675	F	1	0	UD
175	801724	679	F	1	0	UD
176	801726	683	F	4	1	UD
177	801611	686	M	4	1	UD
178	801702	686	F	4	1	UD
179	801580	686	M	4	0	UD
180	801784	687	F	1	0	UD
181	801645	688	M	4	1	UD
182	801755	689	F	4	1	UD
183	801768	689	F	1	0	UD
184	801607	693	M	4	1	UD
185	801635	694	M	4	1	UD
186	801759	696	F	4	1	UD
187	801692	697	F	4	1	UD
188	801703	699	F	4	1	UD
189	801659	705	M	4	1	UD
190	801712	705	F	4	1	UD
191	801753	706	F	1	0	UD
192	801788	706	F	1	0	UD
193	801603	711	M	4	1	UD
194	801713	712	F	4	1	UD
195	801576	715	M	4	1	UD
196	801562	718	M	4	1	UD
197	801586	721	M	4	1	UD
198	801606	721	M	1	0	UD
199	801578	727	M	4	1	UD
200	801591	727	M	4	1	UD

PATH # = PATHOLOGY NUMBER
DAYS = DAYS ON STUDY
COB =CONTEXT OF OBSERVATION
TUMOR = 1=PRESENT 0=ABSENT
DEATH = UD=UNSCHEDULED DEATH
MO SAC= MONTH OF INTERIM SACRIFICE

TIME TO TUMOR STATISTICS (CONT)

	PATH#	DAYS	SEX	COB	TUMOR	DEATH
201	801718	727	F	4	1	UD
202	801613	728	M	4	1	UD
203	801739	728	F	1	0	UD
204	801691	728	F	2	1	24 MO SAC
205	801743	728	F	4	1	24 MO SAC
206	801721	728	F	4	1	24 MO SAC
207	801800	728	F	4	1	24 MO SAC
208	801735	728	F	1	0	24 MO SAC
209	801694	728	F	1	0	24 MO SAC
210	801732	728	F	1	0	24 MO SAC
211	801608	728	M	4	1	24 MO SAC
212	807327	728	M	4	1	24 MO SAC
213	801657	728	M	1	0	24 MO SAC
214	801632	728	M	1	0	24 MO SAC
215	801612	728	M	1	0	24 MO SAC
216	801665	728	M	1	0	24 MO SAC
217	801570	728	M	1	0	24 MO SAC
218	801704	729	F	3	1	24 MO SAC
219	801695	729	F	4	1	24 MO SAC
220	801793	729	F	4	1	24 MO SAC
221	801705	729	F	1	0	24 MO SAC
222	801773	729	F	1	0	24 MO SAC
223	801689	729	F	1	0	24 MO SAC
224	801758	729	F	1	0	24 MO SAC
225	801672	729	M	2	1	24 MO SAC
226	801584	729	M	1	0	24 MO SAC
227	801614	729	M	1	0	24 MO SAC
228	801631	729	M	1	0	24 MO SAC
229	801654	729	M	1	0	24 MO SAC
230	801660	729	M	1	0	24 MO SAC
231	801668	740	M	1	0	EXT UD
232	801637	753	M	4	1	EXT UD
233	801634	767	M	4	1	EXT UD
234	801644	808	M	1	0	EXT UD
235	801568	812	M	4	1	EXT UD
236	801674	819	M	1	1	27 MO SAC
237	801648	819	M	3	1	27 MO SAC
238	801662	819	M	1	0	27 MO SAC
239	801572	819	M	1	0	27 MO SAC
240	801661	819	M	1	0	27 MO SAC

PATH # = PATHOLOGY NUMBER
DAYS = DAYS ON STUDY
COB = CONTEXT OF OBSERVATION
TUMOR = 1=PRESENT 0=ABSENT
DEATH = UD=UNSCHEDULED DEATH
MO SAC=MONTH OF INTERIM SACRIFICE

TABLE X

CHARACTERIZATION OF RATS EXPOSED TO FORMALDEHYDE

AT 15 PPM

Time to death in months	TUMOR BEARING			TUMOR FREE			NASAL TURBINATES NOT EXAMINED			ANIMALS MISSING			
	M	F	TOTAL	M	F	TOTAL	M	F	TOTAL	M	F	TOTAL	TOTAL
6	0	0	0	10	10	20	0	0	0	0	0	0	20
12	0	0	0	10	10	20	0	0	0	0	0	0	20
18	4	4	8	15	15	30	1	0	1	0	0	0	39
24	3	7	10	10	7	17	0	0	0	0	0	0	27
27	2	0	2	3	0	3	0	0	0	0	0	0	5
U.D.*	39	41	80	13	18	31	1	4	5	4	4	8	124
Ext. U.D.*	3	0	3	0	0	0	1	0	1	1	0	1	5
TOTAL	51	52	103	61	60	121	3	4	7	5	4	9	240

UD = unscheduled deaths.
Ext. UD = extended unscheduled deaths.

TABLE XI

CONTEXT OF OBSERVATION IN TUMOR-BEARING ANIMALS

Context	Total Number with Context	Number of Animals with each Criterion*				
		Tumor Size ≥ 50%	Involvement of more than one level	Invasion	Necrosis	Inflammation
Definitely Incidental (DI)	2	0	0	0	0	0
Probably Incidental (PI)	1	0	0	0	0	1
Probably Fatal (PF)	9	0	4	0	5	6
Definitely Fatal (DF)	91	88	90	81	90	84
Total # of Tumor-Bearing Animals	103					

*Histopathological criteria as described in text.

TABLE XII

CONTEXT OF OBSERVATION OF TUMOR-BEARING ANIMALS BY TIME OF DEATH (MONTHS) FOR UNSCHEDULED DEATHS AND SCHEDULED SACRIFICES

CONTEXT	6	12	18	24	27	Subtotal	Total
Definitely Incidental							2
unscheduled deaths	0	0	0	1	0	1	
sacrificed	0	0	0	0	1	1	
Probably Incidental							1
unscheduled deaths	0	0	0	0	0	0	
sacrificed	0	0	0	1	0	1	
Probably fatal							9
unscheduled deaths	0	0	0	2	0	2	
sacrificed	0	0	5	1	1	7	
Definitely fatal							91
unscheduled deaths	0	2	23	52	3	80	
sacrificed	0	0	4	7	0	11	
Total	0	2	32	64	5		103

TABLE XIII

KAPLAN-MEIER TUMOR FREE SURVIVAL CURVE

	RP	RN	RT
1	0	1	5
2	0	1	181
3	0	1	252
4	0	1	274
5	0	1	319
6	0	2	353
7	1	0	358
8	0	1	361
9	1	20	364
10	0	1	390
11	1	0	420
12	2	0	432
13	0	1	433
14	1	0	438
15	1	0	474
16	0	1	477
17	2	0	479
18	1	0	488
19	1	0	491
20	0	1	495
21	1	1	504
22	1	0	508
23	0	1	509
24	1	0	511
25	1	1	512
26	1	0	520
27	0	1	521
28	1	0	525
29	0	1	530
30	1	1	531
31	2	0	533
32	1	0	535
33	0	1	537
34	1	0	539
35	1	0	543
36	2	1	545

RP = RESPONSE POSITIVE
RN = RESPONSE NEGATIVE
RT = RESPONSE TIME IN DAYS

KAPLAN-MEIER SURVIVAL CURVE (CONT)

	RP	RN	RT
37	0	1	546
38	3	6	547
39	1	9	550
40	3	12	551
41	2	4	552
42	0	1	555
43	1	0	560
44	1	0	567
45	1	0	569
46	0	1	571
47	1	0	572
48	2	1	574
49	0	2	577
50	0	1	583
51	0	1	586
52	2	0	588
53	0	1	590
54	1	0	595
55	2	0	596
56	1	0	603
57	1	0	606
58	1	0	607
59	1	0	609
60	1	0	614
61	1	0	615
62	0	2	619
63	1	0	620
64	1	0	621
65	1	0	624
66	1	0	627
67	0	1	629
68	1	0	630
69	2	1	631
70	0	1	633
71	0	1	641
72	1	0	642

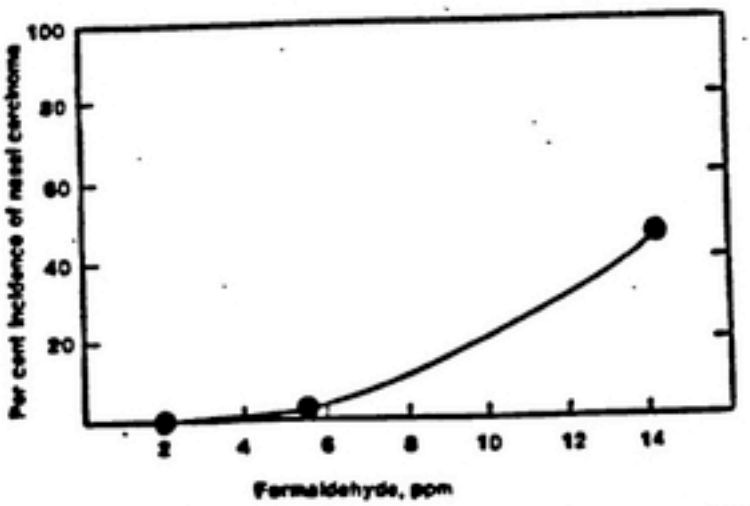
RP = RESPONSE POSITIVE
RN = RESPONSE NEGATIVE
RT = RESPONSE TIME IN DAYS

KAPLAN-MEIER TUMOR FREE SURVIVAL CURVE (CONT)

	RP	RN	RT
73	1	0	643
74	0	1	644
75	1	1	652
76	1	0	654
77	1	0	663
78	1	0	665
79	1	0	672
80	2	0	673
81	0	1	675
82	0	1	679
83	1	0	683
84	2	1	686
85	0	1	687
86	1	0	688
87	1	1	689
88	1	0	693
89	1	0	694
90	1	0	697
91	1	0	697
92	1	0	699
93	2	0	705
94	0	2	705
95	1	0	711
96	1	0	712
97	1	0	715
98	1	0	718
99	1	1	721
100	3	0	727
101	7	9	728
102	4	9	729
103	0	1	740
104	1	0	753
105	1	0	767
106	0	1	808
107	1	0	812
108	2	3	819

RP = RESPONSE POSITIVE
RN = RESPONSE NEGATIVE
RT = RESPONSE TIME IN DAYS

FIG. 1



Dose response of formaldehyde in Fischer 344 rats. Data from Kamm *et al.* (1983).

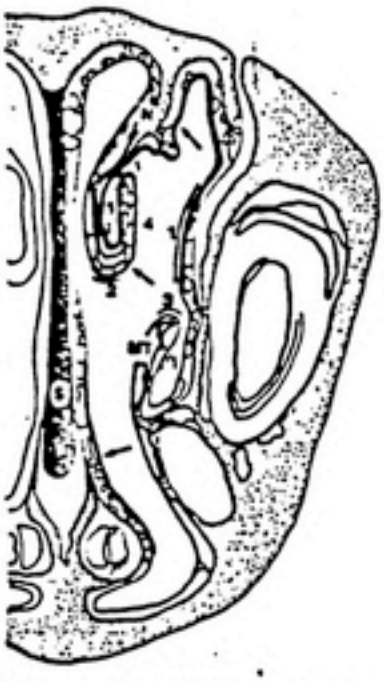
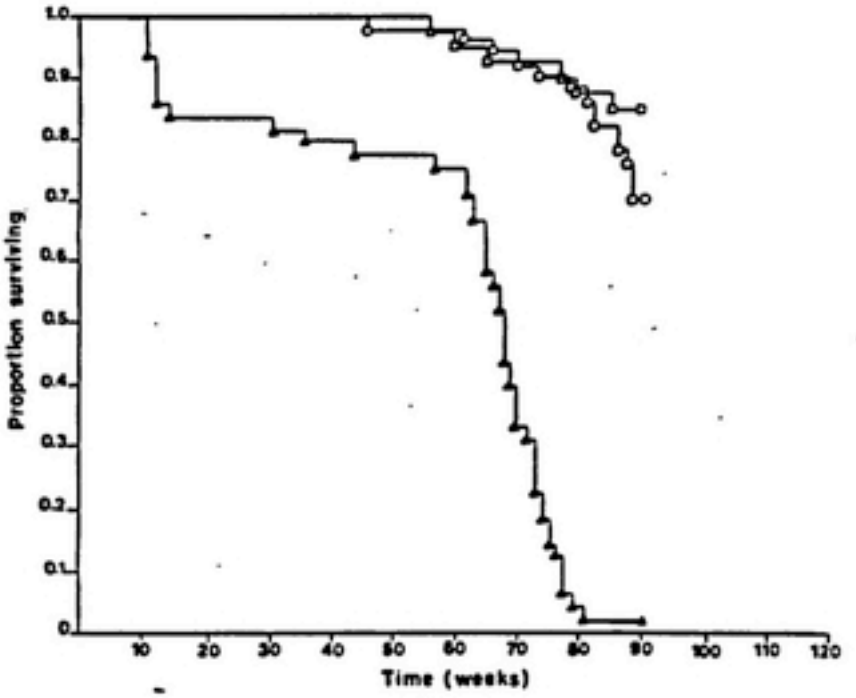


FIG. 2 Diagram of Level II of the rat nose showing the sites of attachment (fine lines) of polypoid adenomas and the number of rats with a polypoid adenoma at each site. The bold lines, indicated by the arrows, show the principal sites of origin of the majority of squamous cell carcinomas (see Table 1 and Figs 2 and 3). N = nasoturbinate; MT = maxilloturbinate; S = nasal septum.

Source: Morgan et al., (1986)

Fig. 3 Kaplan–Meier estimates of survival curves for three groups of female mice (□, control; ○, low dose; ▲, high dose) treated with 1,2-dichloroethane



Source: Garth et al., (1986)

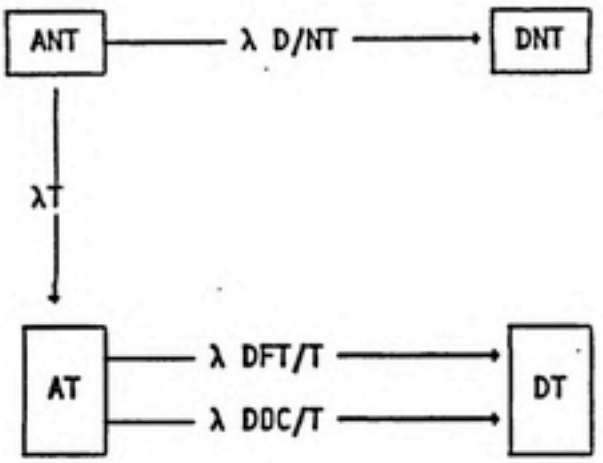


Fig. 4

Schematic of bioassay animal status at any specific time. Animals are either alive and tumor-free (ANT), dead without tumor (DNT), alive and tumor present (AT), or dead with tumor present (DT). As time passes, tumor free (ANT) animals can develop tumors at a rate of λ^T (tumor incidence rate) or die tumor free at a rate λ D/NT. Tumor-bearing (AT) animals can die from tumor or from other causes at rates λ DFT/T or λ DOC/T respectively.

Source: Starr, (1985)

Fig.5

TYPICAL RECORD

Pathology Number: 801757
Animal Number: 2303

Sex: F
Status: UD

Days on Study: 627
# of Slides: 16
# of Blocks: 4

Histopathology

Organ	Diagnosis
Colon A	Nematodiasis P
Adrenal Gland A	Zonal Fasciculata Lipicosis Bilateral 2
Pituitary Gland A	Pars Distalis Adenoma P
Bone Marow-Femur A	Hyperplasia 3
Lymph-node Mandibular A	Lymphocytic and plasmacytic hyperplasia diffuse 3
Spleen A	Lymphoid Depletion 2
Nasal Turbinate A	Epithelial Dysplasia Multifocal 2
	Hyperkeratosis focal 3
	Olafactory epithelium squamous metaplasia multifocal 2
	Rhinitis seropurulent 2
	Squamous cell carcinoma P
	Squamous metaplasia multifocal 2

Necropsy

Nasal Cavity	mass
Pituitary	enlarged and/or cystic focal or diffuse

Clinical Observations

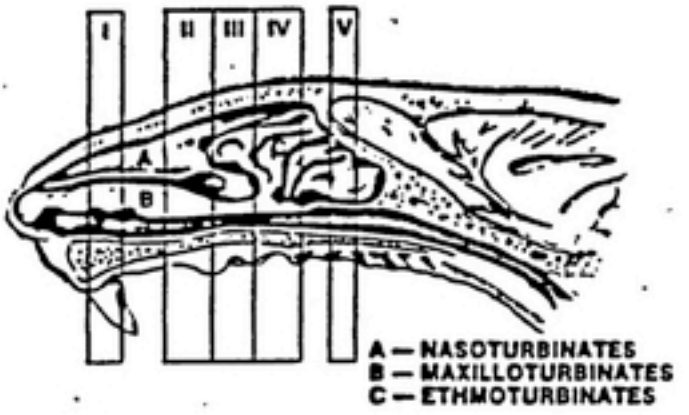
From Date	To Date	Abnormal Observations
800212	800229	Nasal/Occular Discharge

800302	800303	Nasal/Occular Discharge
800302	800303	Red Eyelids

Comments

Context: 4

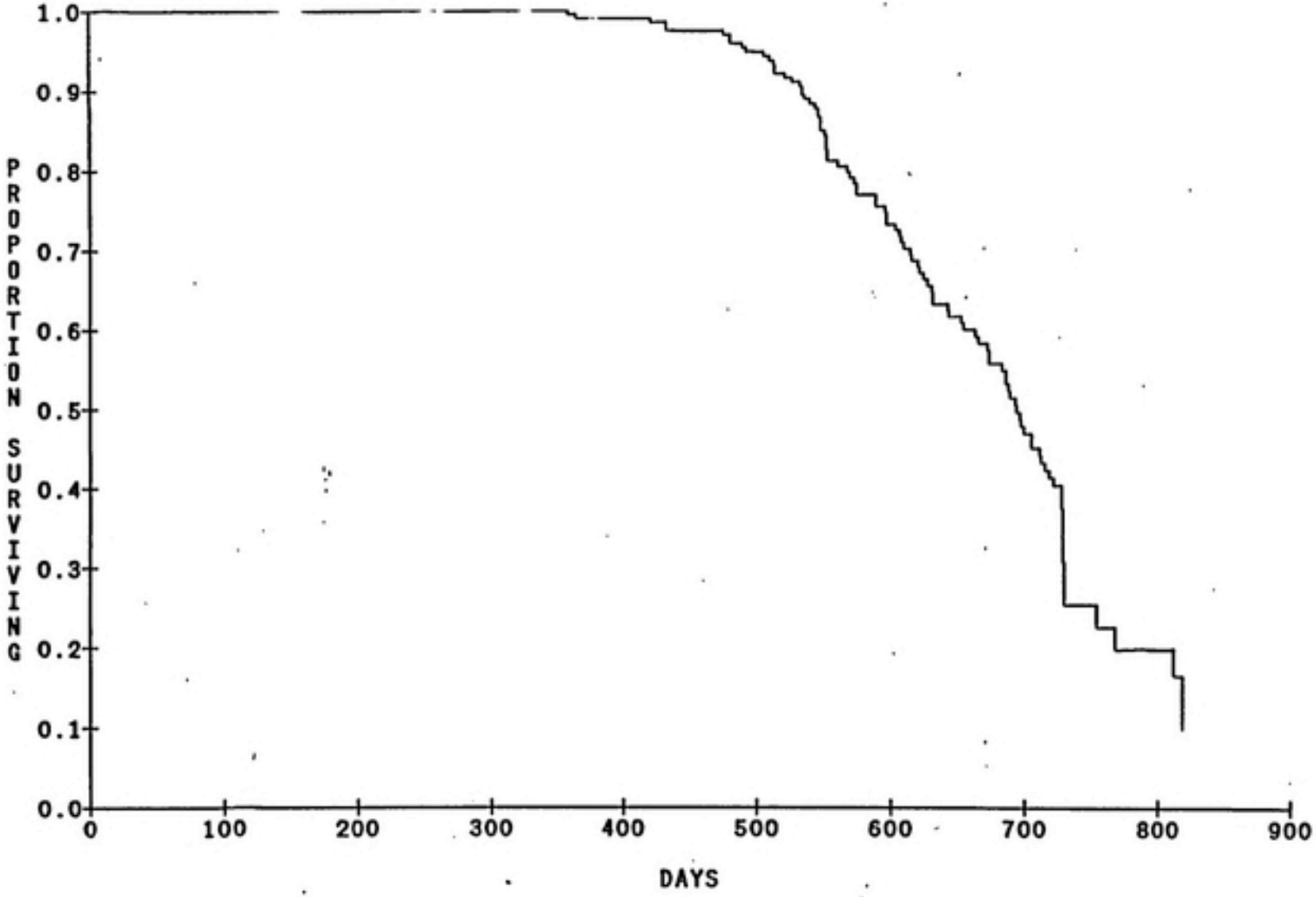
FIG. 6



Midsagittal section of a rat head that demonstrates the turbinates that are included in each level (I to V) for microscopic evaluation.

Source: Kerns et al., (1983)

igure 7 Kaplan-Meier Estimates of Tumor FreeSurvival



APPENDIX I

Table 1. Summary of Selected Animal Acute-Inhalation Data on Formaldehyde

Species	Concentration, ppm	Duration of Exposure	Effects	Reference
Rat	820	0.5 h	LC_{50} (over 3 wks)	Skog, 1950
	482	4 h	LC_{50} (approx.)	Nagornyi et al., 1979
	250	4 h	Death in 2-4 of 6	Carpenter et al.,1949
Cat	650-1,600	8 & 4 h	Death, pulmonary edema, emphysema	Iwanoff, 1911
	735	2 h	Death	Iwanoff, 1911
	650	4 h	Irritation, recovery in 6 d	Iwanoff, 1911
	300	3.5 h	Irritation, recovery in hours	Iwanoff, 1911
Guinea pig	0.3-50	1 h	Increased airway resistance, decreased lung compliance	Amdur, 1960
Mouse	414	4 h	LC_{50}	Nagornyi, et al., 1979
	3.1	10 min	RD_{50}	Kane and Alarie, 1977

Table 2. Summary of Effects of Prolonged Exposure to Formaldehyde in Animals

Concentration, ppm	Duration of Exposure	Species	Effects	Reference
41.5, 83	1 h/d, 3 d/wk x 35 wk	Mouse	Hyperplasia and metaplasia of the tracheal epithelium	Horton et al, 1963
50	5 h/d, 1 d/wk x 18 mo	Hamster	Squamous cell metaplasia	Nettesheim, 1976
15*	6 h/d, 5 d/wk lifetime	Rat	Squamous cell carcinomas in the naso-maxillary epithelium, epithelial dysplasia and squamous cell metaplasia of nasal turbinates	CIIT, 1979b; CIIT, 1980
15*	6 h/d, 5 d/wk lifetime	Mouse	None to date	CIIT, 1979b; CIIT, 1980
12.7	6 h/d, 5 d/wk x 13 wk	Mouse, rat	Nasal irritation, decreased body weight	Battelle Columbus Laboratories, 1977a
10	5 h/d, 5 d/wk x 18 mo	Hamster	Cell proliferation, hyperplasia	Nettesheim, 1976
8	60 d	Rat	Respiratory tract and eye irritation, decreased body weight, decreased number of alveolar macrophages	Dubreuil et al., 1976
6*	6 h/d, 5 d/wk lifetime	Rat	Squamous cell carcinoma of skin in 1 rat, epithelial dysplasia and squamous cell metaplasia of nasal turbinates	CIIT, 1979b; CIIT, 1980
6*	6 h/d, 5 d/wk lifetime	Mouse	None to date	CIIT, 1979b; CIIT, 1980
4.6	45 d	Rat	Yellowing of body hair, decreased body weight	Dubreuil et al., 1976
4	6 h/d, 5 d/wk x 13 wk	Mouse, rat	None observed	Battelle Columbus Laboratories, 1977a

Table 2. (continued)

Concentration, ppm	Duration of Exposure	Species	Effects	Reference
3.8	90 d	Rat, rabbit, dog, monkey, guinea pig	1 of 15 rats died, some inflammation of lungs in all species	Coon et al., 1970
2.4	90 d	Rat	Peribronchial and perivascular hyperemia	Fel'dman and Bonashevskaya, 1971
2*	6 h/d, 5 d/wk lifetime	Rat	Epithelial dysplasia and squamous cell metaplasia of the nasal turbinates	CIIT, 1979b, CIIT, 1980
2*	6 h/d, 5 d/wk lifetime	Mouse	None to date	CIIT, 1979b, CIIT, 1980
1.6	90 d	Rat	Yellowing of body hair	Dubreuil et al., 1976
0.82	90 d	Rat	Peribronchial and perivascular hyperemia	Fel'dman and Bonashevskaya, 1971
0.028	90 d	Rat	None observed	Fel'dman and Bonashevskaya, 1971
0.0098	90 d	Rat	None observed	Fel'dman and Bonashevskaya, 1971

*Study in progress; only interim findings have been reported.

Table 3. Summary of Human Inhalation Data on Formaldehyde

Concentration, ppm	Exposure	Effects	Reference
20	Chamber (< 1 min)	Discomfort, lacrimation	Barnes and Speicher, 1942
13.8	Chamber (30 min)	Eye and nose irritation	Sim and Pattle, 1957
0.5-10	Indoor residential air	Eye irritation, headaches, GI tract symptoms, skin problems, respiratory complaints	Sardinas et al., 1979
4-5	Occupational (10-30 min)	Irritation, discomfort, lacrimation	Fassett, 1963
0.67-4.82	Indoor residential air (Infants)	Vomiting, diarrhea, lacrimation	Wisconsin Division of Health, 1978
0.02-4.15	Indoor residential air	Eye and upper respiratory tract irritation, headache, tiredness, nausea, diarrhea	Wisconsin Division of Health, 1978
0.9-2.7	Occupational	Upper respiratory tract irritation, lacrimation	Blejer and Miller, 1966
0.3-2.7	Occupational	Annoying odor, lacrimation, irritation of respiratory tract, disturbed sleep	Shipkovitz, 1968
0.03-2.5	Indoor residential air	Drowsiness, nausea, headache, nose and respiratory tract irritation	Breysse, 1977

Table 3. (continued)

Concentration, ppm	Exposure	Effects	Reference
0.9-1.6	Occupational	Intense eye irritation and itching; dry, sore throat; increased thirst; disturbed sleep	Morrill, 1961
0.25-1.39	Occupational	Upper respiratory tract irritation, coughing, headaches	Kerfoot and Mooney, 1975
0.4-0.8	Occupational	Lowered $FEV_{1.0}/FVC$, upper respiratory tract irritation	Shoenberg and Mitchell, 1975
0.13-0.45	Occupational	Burning and stinging of eyes, nose, and throat; headache	Bourne and Seferian, 1959

Table 4 Summary of Clinical Studies with Formaldehyde

Concentration, ppm	Duration of Exposure	No. Subjects	% of Subjects Responding	Effects	Reference
0.03-3.2*	35 min	33	45	No significant change in eye blinking rate	b
			36	Doubling of eye blinking rate	
			19	Increases in eye blinking rate	
0.03-2.1*	20 min	33	33	Doubling of eye blinking rate	b
			20	"Desire to leave the room"	
			10	Medium eye irritation	
			7	Strong odor, strong eye irritation	
1.6	5 h/d x 4 d	16	94	"Slight discomfort," conjunctival irritation, dryness of nose and throat	a
0.83	5 h/d x 4 d	16	94	"Slight discomfort," conjunctival irritation, dryness of nose and throat	a
0.03-0.5*	5 min	33	11	Doubling of eye blinking rate	b
			3	"Desire to leave the room"	
			2	Medium eye irritation	
0.42	5 h/d x 4 d	16	31	"Slight discomfort," conjunctival irritation, dryness of nose and throat	a
0.25	5 h/d x 4 d	16	19	"Slight discomfort," conjunctival irritation, dryness of nose and throat	a

*Total exposure for 35 min at concentrations increasing from 0.03 to 3.2 ppm.

[a]Andersen, 1979

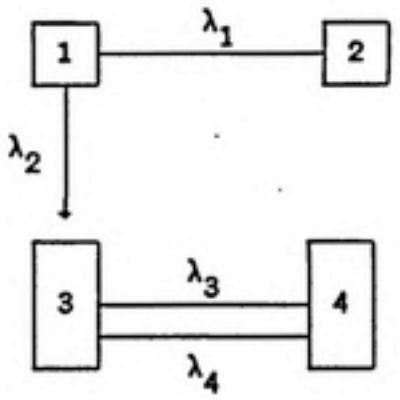
[b]Weber-Tschopp et al., 1977

Table 5. Summary of Irritation Thresholds[a] (33 Subjects Exposed to Formaldehyde at 0.3-3.2 ppm for 35 min)

Response	Threshold Concentration
Throat irritation	2.1 ppm
Eye blinking rate	1.7 ppm
Eye irritation	1.2 ppm
Nose irritation	1.2 ppm
"Desire to leave the room"	1.2 ppm

[a]Weber-Tschopp et al., 1977

Appendix II



1) $\frac{dN_1(t)}{dt} = -(\lambda_1 + \lambda_2)N_1(t)$

2) $\frac{dN_2(t)}{dt} = \lambda_1 N_1(t)$

3) $\frac{dN_3(t)}{dt} = \lambda_2 N_1(t) - (\lambda_3 + \lambda_4)N_3(t)$

4) $\frac{dN_4(t)}{dt} = (\lambda_3 + \lambda_4)N_3(t)$

SOLUTIONS:

1) $N_1(t) = N_1^o e^{-(\lambda_1 + \lambda_3)t}$

2) $\frac{dN_2(t)}{dt} = \lambda_1 N_1(t) = \lambda_1 N_1^o e^{-(\lambda_1 + \lambda_2)t}$

$N_2(t) = \int_o^t \lambda_1 N_1^o e^{-(\lambda_1 + \lambda_2)t}\, dt + N_2^o$

$= \frac{\lambda_1}{(\lambda_1 + \lambda_2)} \left(1 - e^{-(\lambda_1 + \lambda_2)t}\right)$

3) $$\frac{dN_3(t)}{dt} = \lambda_2 N_1(t) - (\lambda_3 + \lambda_4)N_3(t)$$

$$\frac{dN_3(t)}{dt} + (\lambda_3 + \lambda_4)N_3(t) = \lambda_2 N_1(t)$$

$$= \lambda_2 N_1^{\circ} e^{-(\lambda_1 + \lambda_2)t}$$

$$\therefore N_3(t)e^{(\lambda_3 + \lambda_4)t} = \int_o^t e^{(\lambda_3 + \lambda_4)t} \lambda_2 N_1 e^{-(\lambda_1 + \lambda_2)t} dt + N_3^{\circ}$$

$$\lambda_2 N_1^{\circ} \int_o^t e^{(\lambda_3 + \lambda_4 - \lambda_1 - \lambda - 2)t} dt$$

$$= \frac{\lambda_2 N_1^{\circ} (e^{(\lambda_3 + \lambda_4 - \lambda_2 - \lambda_2)t} - 1)}{(\lambda_3 + \lambda_4 - \lambda_1 - \lambda_2)}$$

$$\therefore N_3(t) = \frac{\lambda_2 N_1 (e^{-(\lambda_1 + \lambda_2)t} - e^{-(\lambda_3 + \lambda_4)t})}{(\lambda_3 + \lambda_4 - \lambda_1 - \lambda_2)}$$

4) $$\frac{dN_4(t)}{dt} = (\lambda_3 + \lambda_4)N_3(t)$$

$$= \frac{(\lambda_3 + \lambda_4)\ \lambda_2 N_1^{\circ} (e^{-(\lambda_1 + \lambda_2)} + - e - (\lambda_3 + \lambda_4)t)}{(\lambda_3 + \lambda_4 - \lambda_1 - \lambda_2)}$$

$$\therefore N_4(t) = \frac{(\lambda_3 + \lambda_4)\ \lambda_2 N_1^{\circ}}{(\lambda_3 + \lambda_4 - \lambda_1 - \lambda_2)} \int_o^t (e^{-(\lambda_1 + \lambda_2)t} - e - (\lambda_3 + \lambda_4)t) dt$$

$$= \frac{(\lambda_3 + \lambda_4)\lambda_2 N_1^{\circ}}{(\lambda_3 + \lambda_4 - \lambda_1 - \lambda_2)} \left[\frac{1 - e^{-(\lambda_1 + \lambda_2)t}}{(\lambda_1 + \lambda_2)} - \frac{(1 - e^{-(\lambda_3 + \lambda_4)t})}{(\lambda_3 + \lambda_4)} \right]$$

SUMMARY:

1) $N_1^{\circ}(t) = N_1 e^{-(\lambda_1 + \lambda_2)t}$

2) $N_2(t) = \frac{\lambda_1}{(\lambda_1 + \lambda_2)} (1 - e^{-(\lambda_1 + \lambda_2)})$

3) $N_3(t) = \frac{\lambda_2 N_1^{\circ} (e^{-(\lambda_1 + \lambda_2)t} - e^{-(\lambda_3 + \lambda_4)t})}{(\lambda_3 + \lambda_4 - \lambda_1 - \lambda_2}$

4) $N_4(t) = \frac{(\lambda_3 + \lambda_4)\lambda_2 N_1^{\circ}}{(\lambda_3 + \lambda_4 - \lambda_1 - \lambda_2)} \left[\frac{(1 - e^{-(\lambda_1 + \lambda_2)t})}{(\lambda_1 + \lambda_2)} - \frac{(1 - e^{-(\lambda_3 + \lambda_4)t})}{(\lambda_3 + \lambda_4)} \right]$

Some other definitions:

$P(t) = N_3(t)/[N_1(t) + N_3(t)]$

$$\frac{dP(t)}{dt} = \frac{\frac{dN_3(t)}{dt} \left[N_1(t) = N_3(t) \right]}{[N_1(t) + N_3(t)]^2}$$

$$- \frac{\left[\frac{dN_1(t)}{dt} + \frac{dN_3(t)}{dt} \right] N_3(t)}{[N_1(t) + N_3(t)]^2}$$

$$= \frac{\frac{dN_3(t)}{dt} [N_1(t)]}{[N_1(t) = N_3(t)]^2} - \frac{\frac{dN_1(t)}{dt} [N_3(t)]}{[N_1(t) + N_3(t)]^2}$$

$$= \frac{N_1(t)\ dN_3(t)/dt}{[N_1(t) + N_3(t)]^2} - \frac{P dN_1(t)/dt}{[N_1(t) + N_3(t)]}$$

$$\frac{N_1(t)}{[N_1(t) + N_3(t)]} = 1 - P$$

$$\therefore \frac{dP(t)}{dt} = \frac{(1-P)dN_3(t)/dt)}{[N_1(t) + N_3(t)]} - \frac{PdN_1(t)/dt}{[N_1(t) + N_3(t)]}$$

$$= \frac{(1-P)\ dN_3(t)/dt - PdN_1(t)/dt)}{[N_1(t) + N_3(t)]}$$

$$\frac{dP(t)}{dt} = \frac{(1 - P(t))\ (\lambda_2 N_1(t) - (\lambda_3 + \lambda_4)N_3(t))}{(N_1(t) + N_3(t))}$$

$$+ \frac{P(t)\ (\lambda_2 + \lambda_2)\ N_1(t)}{(N_1(t) + N_3(t))}$$

Dropping the explicit time reference.

$$\frac{dP}{dt} = \frac{(1 - P)\ (\lambda_2 N_1 - (\lambda_3 + \lambda_4)N_3)}{(N_1 + N_3)}$$

$$+ \frac{P\ (\lambda_1 + \lambda_2)\ N_1}{(N_1 + N_3)}$$

$$= \lambda_2(1 - P)\ (1 - P) - (1 - P)\ (\lambda_3 + \lambda_4)P$$

$$+ P\ (\lambda_1 + \lambda_2)\ (1 - P)$$

$$\frac{dP}{dt} = \lambda_2(1 - P)\ (1 - P) + P(1 - P)\ (\lambda_1 + \lambda_2 - \lambda_3 - \lambda_4).$$

solving for λ_2:

$$\lambda_2 = \frac{dP/dt}{(1 - P)} + P(\lambda_3 + \lambda_4 - \lambda_1)$$

but this requires that λ_1, λ_3 and λ_4 be estimable from the data. (P and dP/dt may be estimated from the sacrifice data).

By looking at the animals dying with a tumor, we may find the fraction dying in any interval from the tumor and the fraction dying from other causes. Let f_1 be the fraction of animals dying with a tumor who die from the tumor.

then $f_1 = \frac{\lambda_3}{(\lambda_3 + \lambda_4)}$

or $\lambda_3 f_1 + \lambda_4 f_1 = \lambda_3$

$$\lambda_4 f_1 = \lambda_3 - \lambda_3 f_1 = \lambda_3(1 - f_1)$$

$$\lambda_4 = \lambda_3(1 - f_1)/f_1$$

Also, we might assume that the presence of a tumor does not affect the rate of death (λ) from all other causes. In other words:

$$\lambda_1 \sim \lambda_4$$

$$\therefore \lambda_2 = \frac{dP/dt}{[1 - P]} + \lambda_3 P$$

But we know that the rate at which animals are dying free of a tumor is

$$R_1 = \lambda_1 N_1$$

The total rate of dying, R, is:

$$R = R_1 + (\lambda_3 + \lambda_4)N_3$$

but also, $\lambda_4 = \lambda_3(1 - f_1)/f_1$

$$\therefore R = R_1 + \lambda_3(1 + (1 - f_1)/f_1)N_3.$$

$$\text{or } \lambda_3 = \frac{(R - R_1)}{(1 + (1 - f_1)/f_1)N_3}$$

$$\therefore \lambda_2 = \frac{dP/dt}{[1 - P]} + \frac{(R - R_1)P}{\left[1 + \frac{(1 - f_1)}{f_1}\right] N_3}$$

$$= \frac{dP/dt}{[1 - P]} + \frac{(R - R_1 \quad N_3}{\left[1 + \frac{(1 - f_1)}{f_1}\right] N_3(N_1 + N_3)}$$

or

$$\lambda_2 = \frac{dP/dt}{[1 - P]} + \frac{(R - R_1)}{\left[1 + \frac{(1 - f_1)}{f_1}\right] (N_1 + N_3)}$$

To summarize, finding λ_2 requires:

P = fraction of <u>living animals</u> with a tumor in the "sacrifice" group.

$\frac{dP}{dt}$ = Rate of change of P during some small increment of time.

R = total rate at which the animals are dying in the "non-sacrafice" group.

R_1 = Rate at which animals are dying <u>free of a tumor</u>.

f_1 = fraction of animals <u>dying with a tumor</u> who are judged to have died <u>from</u> the tumor.

P and dP/dt come from the "sacrafice" group. R, R_1 and f_1 come from the "non-sacrafice" group.