STATISTICAL ISSUES OF HAZARDOUS AGENTS

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Summary

Evaluation of hazards associated with exposure to chemicals, understanding of relationships between dose and adverse effect, extrapolation of effects from high experimental doses to low doses associated with actual exposures, and extrapolation from effects observed in animals to effects expected in humans are the main issues of statistical research in risk assessment of hazardous agents. We discuss statistical aspects of inhalation toxicology, proof of hazard, genetic toxicology and the role of oncogenes in carcinogenesis. Finally, we outline the need for more statistical research to identify hazardous agents by DNA and protein sequence analysis.

Hazard and risk

Adverse effects of chemicals on living organisms are studied by toxicologists. They examine the nature of these adverse or toxic effects and assess the probability of their occurrence. The probability that a chemical will produce harm under specified conditions is called risk.

The term ‘hazard’ is often used interchangeably with ‘intrinsic toxicity’ in risk assessment guidelines. Hazard is a qualitative, risk is a quantitative term which includes both intrinsic toxicity and the circumstances specific to exposure. Highly toxic substances can be used safely provided one controls the environment to prevent exposure and absorption of sufficient quantities of the substance to produce toxicity. Although the chemical is highly toxic, it must not be of high risk in the manner in which it is used. Depending on the conditions under which it is used, a very toxic chemical may be less risky than a relatively nontoxic one.

The activities of toxicologists are classified by Klaassen and Eaton [8] as descriptive, mechanistic and regulatory:

The descriptive toxicologist is concerned with toxicity testing, which provides necessary information for safety evaluation and regulatory requirements. The appropriate toxicity tests in experimental animals are designed to yield information that can be used to evaluate the risk posed to humans and the environment by exposure to specific chemicals.

The mechanisms by which chemicals excert their toxic effects on living organisms are investigated by the mechanistic toxicologist. Results of these studies lead to the development of sensitive predictive tests useful in risk assessment. An understanding of the mechanisms of toxic action contributes to the knowledge of physiology, pharmacology, cell biology and biochemistry.

A regulatory toxicologist has the responsibility of deciding if a chemical poses a sufficiently low risk to be marketed and used for a stated purpose. In the United States, the Environmental Protection Agency (EPA) is responsible for regulating chemicals according to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the Toxic

Substances Control Act (TSCA), the Resource Conservation and Recovery Act (RCRA), the Safe Drinking Water Act, and the Clean Air Act. The Consumer Product Safety Commission has the responsibility of protecting the consumer from hazardous substances, whereas the Department of Transportation ensures that materials shipped in interstate commerce are labeled and packaged in a manner consistent with the degree of hazard they present. Regulatory toxicologists are also involved in the establishment of standards for the amount of chemicals permitted in ambient air, in industrial atmospheres or in drinking water.

In the European Union there is a definite trend towards European regulations in many fields relating to chemicals. This pertains to risk assessment of new and existing chemicals, regulation of cosmetics, drugs, occupational exposure limits, etc.

Inhalation toxicology

Environmental and occupational exposure to a variety of chemicals may result in a variety of adverse effects. It is necessary to investigate the processes which contribute to the toxicity of a chemical. An essential component of risk assessment of potential harmful chemicals is the determination of toxicokinetic processes. Many agents are transformed into a chemically active form, a metabolite, that might be able to interact with DNA, RNA and proteins. The partition of the agent in the body of experimental animals is a first step of the chemical and biochemical pathway of the formation of DNA adducts, and in consequence, to mutations. Very frequently, a non-linearity of the relationship between applied dose and tumor response is connected with the kinetic processes involved in the formation of DNA adducts, therefore an important step to assess the risk of an agent is to investigate the kinetic processes of its uptake (absorption), distribution, exhalation (if the chemical is volatile), metabolism and excretion (“ADME”).

Selinski et al. [12] have applied the theory of hierarchical Bayes models, which incorporate modelling of the variability structure and estimation of population parameters. Their methods were applied to an inhalation study with ethylene, which is a colorless, flammable gas. Ethylene is one of the basic petrochemical industrial compounds, a constituent of tobacco smoke and a plant hormone, involved in the process of ripening. As explained by Bolt [3], ethylene is not only an exogenous toxic chemical, but also, to some extent, a natural body constituent. This aspect has a potential influence for legal regulations of weak genotoxic chemicals. Possible endogenous sources of ethylene are given by Bolt [4]: Lipid peroxidation, oxidation of free methionine, oxidation of haemine in haemoglobin and metabolism of intestinal bacteria. In mammalian organisms, ethylene is partly transformed by hepatic metabolizing enzymes to its reactive epoxide, ethylene oxide. Ethylene oxide is a colorless, flammable gas dissolving in water, alcohol, acetone, benzene, ether and most organic solvents. It is used in the production of industrial chemicals and as sterilizing agent for stored agricultural products, in health product and medical fields.

Thier and Bolt [14] report that ethylene oxide is detoxified by two enzymatic systems. The polymorphism of the glutathione S-transferase (GST) isoenzym GSTT1 has an impact on the background sister chromatid exchange (SCE) rate in human lymphocytes. There are two individual genetic states, GSTT1 +/-, which influence the SCE response of human lymphocytes towards exogenous ethylene oxide. The influence of the GSTT1

–status on the background SCE rate can be viewed as an indication of a biological significance of the endogenous ethylene oxide for a physiological background genotoxicity. Therefore, Bolt [3] questions current regulatory procedures of assessing the risk of minute doses of exogenous carcinogens and calls for reevaluation of risk assessments for ethylene oxide and related compounds.

Studies of the absorption, distribution, metabolism and elimination of chemicals from organisms are performed by using toxicokinetic methods and compartmental modelling. Modelling aspects and statistical approaches for estimating kinetic parameters are given by Becka [2], Urfer and Becka [15], Selinski et al. [12] and Gilberg et al. [6]. Weller et al. [17] discuss design and analysis principles for the study of air toxins, including dose rate effects. They present a case study of ethylene oxide and outline areas for further statistical research.

Proof of hazard versus proof of safety

A toxicological safety assessment has to be performed before the first dose of a new medicinal compound can be administered to human beings. The null hypothesis of no difference in the effect between the treatment and a negative control group is tested and failure to reject this null hypothesis often leads to the conclusion of evidence in favor of safety. The major drawback of this approach is that we can only control the probability of erroneously concluding hazard. The primary concern of safety assessment is the control of the probability of erroneously concluding safety. Thus, the adequate test problem should be formulated by reversing the null hypothesis and the alternative incorporating an a priori- or a posteriori-defined threshold. This direct approach is demonstrated by Hauschke and Hothorn [7] for the two sample and k-sample many-to-one problem relative to non-monotonic and monotonic response relations.

Genetic toxicology

Sen and Margolin [13] address the manner in which genetic toxicology can be used to predict various disease processes. They define genetic toxicology as the study of agents that damage DNA and related genetic material. Such agents have the capability to alter the human gene pool with unknown but potentially deleterious consequences for future generations. The focus is on the processes of mutagenesis, which include the induction of DNA damage and all kinds of genetic alterations, ranging from changes in one or a few DNA base pairs to gross changes in chromosome structure or in chromosome number. Any agent that causes mutation is a mutagen.

In toxicological studies and mutagenicity testing often more than two treatment groups are used. In this situation it is quite common that there is an intrinsic order in the treatments, for example increasing doses of the same compound. Therefore, trend tests which are particularly sensitive for detecting order restricted alternatives are of special interest.

In practice, the assumption of normality is frequently violated. Hence, Neuhäuser et al. [10] consider a non-parametric model, i.e. the underlying distribution is unknown. To be precise, they investigate the location model in which there is one control group (negative or vehicle group) and k treatment groups. In the i-th group there are n_i,

$i=0,...,k$ observations $\left(N = \sum_{i=0}^{k} n_i\right)$. Let $Y_{i1},...,Y_{in}$, $i=0,...,k$, be k+1 independent random samples with Y_{ij}, $j=1,..., n_i$, having continuous distribution functions $F_i(t)=F(t-\theta_i)$, i.e. the distribution functions are the same except perhaps for a shift in their location parameters. The index 0 denotes to the control group.

The null hypothesis states that all location parameters are identical, i.e. $H_0:\theta_0=\theta_1=...=\theta_k$. Neuhäuser et al. [10] assume a non-decreasing dose-response relationship and, in order to specifically design a test for trend, they take the ordering of the groups into account. Therefore, they restrict the alternative to the following one-sided ordered alternative hypothesis

$H_A : \theta_0 \leq \theta_1 \leq ... \leq \theta_k$ with at least $\theta_0 < \theta_k$.

This alternative still allows very different dose-response shapes. An extreme concave shape results when there are no differences between the location parameters of the treated groups and only the control group has a lower location parameter: $\theta_0 < \theta_1 = \theta_2 =...= \theta_k$. In an extreme convex shape only the group with the highest dose would differ from the others: $\theta_0 = \theta_1 =...= \theta_{k-1} < \theta_k$. In practice, knowledge about the pattern is mostly lacking and the shape is a priori unknown.

The power of a single trend test strongly depends on both the a priori unknown shape of the dose-response curve and the a priori unknown underlying distribution. Nevertheless, trend tests which are routinely used to analyze toxicological assays should be robust. Neuhäuser et al. [10] investigate non-parametric contrast tests which are powerful for different shapes and different distributions. In order to get a robust test they propose to use the maximum of the contrast test statistics as a new test statistic and to perform a permutation test.

The role of Ras proto-oncogene in carcinogenesis and the future of statistics in toxicology

In the process of carcinogenesis, point mutations may activate, functionally alter, or silence critical genes in a cell type-specific and carcinogen-specific manner. Carcinogenic risk caused by the conversion of DNA-damage into mutations is inversely correlated with the capacity of target cells for DNA-repair. Differential repair of structurally distinct mutagenic lesions in critical genes may influence the cellular risk of malignant conversion. For instance, Engelbergs et al. [5] have investigated rat mammary tumorigenesis induced by N-ethyl-N-nitrosourea (EtNU) versus N-methyl-N-nitrosourea (MeNU) with respect to tumor incidence, Ras gene mutation and gene-specific repair. Their model of mammary tumorigenesis permits to investigate carcinogenic risk as a function of the formation and repair of defined miscoding DNA-lesions in specific genes.

Bailer and Piegorsch [1] mention the increasing understanding of biological mechanisms leading to cancer and the complexity of statistical models in associated areas such as bioinformatics. Tumors arise through stepwise mutations in proto-oncogenes and tumor-suppressor genes. The initial identification of these genes and their functions suggested that they affect discrete pathways, each making distinct contributions to the development of the full malignant phenotype.

Vetter et al. [16] describe Ras as a major regulator of cell growth and development. Oncogenic mutants of Ras induce transformation of cells. Ras is a signal switch molecule that cycles between the GDP-bound inactive and the GTP-bound active form (GDP = guanosine diphosphate, GTP = guanosine triphosphate). Several effectors have been identified each of which is believed to initiate a cascade of signal transduction reactions. The structure of the complex of Ras with the Ras-binding domain of its effector Ral GDS (Ral guanine nucleotide dissociation stimulator) has been solved by Vetter et al. [16] using X-ray crystallography.

Liu et al. [9] provide a detailed sequence analysis of the GTPase family of protein sequences using the hidden Markov model. A major restriction of their statistical model is that the sequences to be aligned are treated as having evolved along independent pathways. There is a need for further statistical research on protein modelling and prediction in terms of gene function and gene product structure to learn more about the complex process of carcinogenesis.

An interesting review on statistical problems in toxicology is given by Ryan [11]. This review contains details on long term carcinogenicity studies, teratology, developmental and reproductive toxicity studies. I would like to repeat some comments of Ryan [11] on the future of statistics in toxicology:

‘Although statistical research over the past several decades has made valuable contributions to the field of toxicology, many of us shy away from the really difficult problems, such as biologically based models and exposure assessment, that require sophisticated biological as well as statistical knowledge. Many are predicting that this will change over the next several decades. Our field will need to become even more

interdisciplinary to stay competitive with emerging disciplines such as computational biology'.

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