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Variations in Reproductive Toxicant Identification

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Introduction

Reproductive toxicants are a very important class of compounds. They present unique hazards to those of child bearing ages, perform their "dirty work" using a wide variety of mechanisms on a number of different organs, and are regulatorily important. Because of all of this, properly identifying reproductive toxicants is important, but fraught with difficulty. In this paper we will describe types or reproductive toxicants, their importance, and both mistakes and good practices that people who are not experts in reproductive toxicology may use in their attempts to identify them. Additionally, this paper will focus on chemical reproductive toxicants and will not address biological agents that could affect reproductive toxicity although many principles outlined here could be applied to that endeavor.

Reproductive Toxicants – What Are They and Why are they Important?

A reproductive toxin is any agent that causes an adverse effect on the reproductive ability of an organism. The Registry of Toxic Effects of Chemical Substances (RTECS) identifies seven major categories of reproductive toxicants. These major categories are those that cause adverse paternal effects, maternal effects, effects on fertility, effects on embryo or fetus, developmental changes, tumorgenic effects and effects upon the newborn organism. Within these seven major categories are sixty five specific effects such as poor sperm production or motility, altered ovulation cycle, altered hormone levels, altered gestation period, difficulties in egg implantation, low birth weight, abnormally slow development, reduced postnatal survival, etc.

Reproductive toxicants are import from several aspects. From a worker protection perspective, reproductive toxicants are important because each worker needs to be able to protect their offspring from deleterious chemical exposures. Properly identifying reproductive toxicants and informing the employee about them is also required in 29CFR1910.1200, Hazard Communication Program, which applies to non-laboratory workers. Reproductive toxicants are also a class of compound identified in 29CFR1910.1450, Occupational Exposure to Hazardous Chemicals in the Laboratory, as being a "particularly hazardous substance (PHS). Whenever a chemical is designated as

being a PHS, regulations require the use of special controls to protect the worker. These controls can be expensive to implement and can consume limited resources so the proper identification of reproductive toxicants is important from a resource loading perspective.

Difficulties in Reproductive Toxin Determination

There are many difficulties associated with determining if a product is a reproductive toxin. The most significant problem is that there are no defined measures for reproductive toxicity. The easiest way to explain this difficulty is to compare the definition of "reproductive toxin" to the definition of "toxic" and the definition of "carcinogen".

A well known tenet of toxicology is that everything will be toxic when an organism is exposed to a large enough concentration a single acute dose. Because of this principle, numerous regulatory agencies have made definitions of "toxic" based upon the LD_{50} or LC_{50} values for the product. (The LD_{50} is the dose and the LC_{50} is the airborne concentration required to kill 50% of a test population.) For ingestion, the LD_{50} is typically 500 mg/kg. Any product more with a lower LD_{50} is considered to fit the definition of "toxic" and products with a LD_{50} greater are not considered to fit this definition. Having this definition makes it easy to identify those products that are "toxic" and those that are not "toxic". But what also makes this definition work is that the definition uses a well defined endpoint based upon a single acute exposure.

The definition of "carcinogen" differs from that of "toxic" in that carcinogenicity is almost always based upon chronic exposures. Once a population is exposed to a chemical product, then it is declared a "carcinogen" when cancerous tissue appears at a statistically significant increased rate in the population. Because determinations of carcinogens are a statistical event based upon a chronic exposure, no exposure levels are used to determine the equivalent of an LD_{50} or LC_{50} dose or concentration. What makes the determination of carcinogenicity more precise is that the concept of "statistically significant" is defined. If the rate of cancer formation upon exposure to a chemical agent is below the defined statistically significant rate, then the chemical product is not considered to be a "carcinogen". If the rate of cancers is greater than the statistically significant rate, then the chemical product is defined as a "carcinogen".

Many issues are at play which makes reproductive toxin determinations difficult. First, reproductive toxicants can be caused by either an acute or by chronic exposures. Because LD_{50} or LC_{50} values are based upon acute exposures, none can be assigned here due to the chronic nature of some reproductive toxicants. Second, reproductive toxicants do not always result in a clear endpoint. Some potential effects such as low birth weight are statistical events that require a clear definition of "low birth weight". What are not always defined are issues such as when a birth weight is considered to be abnormally low. Third, the statistical significance of event frequency is not always defined. Fourth is the complex nature of reproduction. Sexual reproduction requires two sexes and numerous organ systems to function. These are, in turn, controlled by hormones and emotions which can complicate measurements by increasing confounding factors. Lastly,

many species can be used to determine reproductive toxicity, but not all species respond to toxicants similarly. Taken together, these issues make the absolute determination of reproductive toxicity difficult.

Sources for Reproductive Toxin Determinations

Many sources exist to help with the determination as to whether or not a chemical or chemical product is a reproductive toxin. The use of each has its advantages and disadvantages.

Material Data Safety Sheet (MSDS)

The primary source to determine whether or not a chemical or chemical product is a reproductive toxin is the Material Data Safety Sheet (MSDS). Each MSDS is required to have a section that addresses those health hazards associated with the product covered by the MSDS. Sometimes the MSDS will simply state that the product is a reproductive toxin which makes the determination easy. Some MSDSs will state that the productive is a teratogen or a mutagen, but will remain silent on the reproductive toxin issue. In these cases the determination is again easy since mutagens and teratogens are both subclasses of reproductive toxicants which would cause any product to be classed as a mutagen or teratogen to be a reproductive toxin. The vast majority of MSDSs, however, will either state that the product may be a reproductive toxin or will say nothing about the product's reproductive toxicity. In these cases further research may be required to make an accurate determination.

RTECS

The Registry of Toxic Effects of Chemical Substances is a publication of the National Institute of Occupational Safety and Health (NIOSH) and is a compendium of toxicological data extracted from the scientific literature (1). Reproductive effects data are included in each chemical listing. The primary advantage of RTECS is that it provides the most complete listing of chemicals that have been tested for potential reproductive effects. The primary disadvantage and a commonly misunderstood point of RTECS is that it simply provides a listing or what studies were performed and a synopsis of the studies' data. It does not provide any information concerning significance of the data nor does it make any judgments concerning the studies' results. In other words, RTECS simply states what studies were performed and what the results were; it does not determine or classify chemicals to be reproductive toxicants nor does it attempt to determine the degree of reproductive toxicity.

Catalog of Teratogenic Agents

The Catalog of Teratogenic Agents (2) is an excellent source of information concerning teratogenic agents. It is similar to RTECS in that it provides a listing of chemicals that have been tested for producing teratogenic effects, but it suffers from two limitations. The first limitation is that, like RTECS, it provides only the data and does not provide an

interpretation as to whether or not the teratogenic effects of a chemical are significant or not. Second, it provides only a listing for teratogens which is a subset of all reproductive toxicants.

California Proposition 65

In 1986 voters in California approved Proposition 65 which required California to publish annually a list of chemicals that could cause cancer, birth defects and other reproductive harm in an effort to protect the citizens of that state from chemical exposures (3). The most current list is approximately 250 entries long and contains all chemicals thought to be reproductive toxicants by the state of California. Also, several listings on the list are not individual chemicals but are instead classes of compounds such as barbituates, benzodiazepines, tetracyclines, and mercury compounds. One concern that could be raised about using the California Proposition 65 list is that the legislation was aimed at protecting the consumer and not the worker. A result of this is that a large majority of chemicals listed are pharmaceuticals and not industrial chemicals.

Reprotext®

Reprotext® is a data listing published by Thompson Micromedex (4) that lists reproductive toxicants. This listing not only lists chemicals that have been tested for potential reproductive health effects, but it also provides rankings, as determined by the company's committee of experts, on the relative degree of the reproductive toxicity. Ratings include known and unconfirmed human reproductive toxin (rated "A"), known animal reproductive toxicants (rated "B"), no data available (rated "C"), insufficient data available (rated "D") and not thought to be a reproductive toxin (rated "E"). The focus of this listing is those chemicals that could pose a threat to a worker.

Center for the Evaluation of Risks to Human Reproduction (CERHR)

The Center for the Evaluation of Risks to Human Reproduction was chartered by the national Toxicology Program and the National Institute of Environmental Health Sciences in 1998. Since its inception CERHR has evaluated approximately 25 chemicals for reproductive toxicity. Each evaluation is published as a monograph and these monographs vary from 100 to several hundred pages in length. The monographs present an exhaustive treatment of each investigated chemical and provides a degree of risk associated with the subject chemical. For the purposes of this manuscript, one weakness in this database is that there are so few chemicals that have been analyzed. Another weakness is that varying levels of concern are provided for each chemical so the reader must make up their mind as to whether or not the chemical in question is a reproductive toxin.

Development and Reproductive Toxicology (DART) Database

The Development and Reproductive Toxicology Database is maintained by the United States National Library of Medicine. It is a listing of articles concerning reproductive

toxicology that can be searched by topic. This database has the weakness of primarily being a tool for researchers who are investigating reproductive toxicants. When a chemical is identified using this tool, it retrieves a listing of journal article citations that discuss the chemical. In addition to the journal citation, the database will also display the first 250 words of the article's abstract to give the reader an idea as to the paper's contents. This resource provides little help for those who are attempting to determine if a chemical should be classified as a reproductive toxin since it only lists research articles with a portion of the articles abstract.

Jankovic and Drake (7)

In 1996, Jankovic and Drake (7) developed a method for determining whether or not a product was considered to be a reproductive health hazard and what exposure limits should be set to protect workers from these hazards. Using this method, they identified 213 chemicals that should be considered reproductive health hazards and what "occupational exposure guidelines" should be set for each.

American Conference of Governmental Industrial Hygienists (ACGIH®) (8)

ACGIH® annually publishes a book on Threshold Limit Values (TLVs®) and Biological Exposure Indices (BEIs®) are health-based values that are established by committees that review existing published and peer-reviewed literature in various scientific disciplines. One data element published by ACGIH is "TLV® Basis – Critical Effect(s)". If the ACGIH identifies the chemical to be listed as a reproductive toxin in the "TLV® Basis – Critical Effect(s)" column, then the chemical should be considered as a reproductive toxin. The limitation to this resource is that only a very few chemicals are identified as being a reproductive toxicant.

Haz-Map (9)

Haz-Map is a database provide by the National Library of Medicine via the National Institute of Health that provides the public with information concerning the effects of occupational exposures to hazardous materials. It lists 136 chemicals, chemical classes and physical hazards that are reproductive hazards and provides links that allows one to gain access to more information about each identified hazard. Criteria used to determine if a hazard on the list is not described.

Scorecard (10)

Other sources and methods may be used to help determine if a chemical is a reproductive toxicant. One source is Scorecard. Scorecard is a resource developed by the advocacy group Environmental Defense that provides the public with information about chemicals pollutants in the environment. Scorecard provides numerous links to sites that identify reproductive toxicants and provides their own list of "recognized" and "suspected" reproductive toxicants. The list provided by Scorecard is based upon many of the sources cited above.

Quantitative Structure Activity Relationship (QSAR) Methodologies

Also known as quantitative structure-property relationships (QSPR), QSAR based programs utilize electronic and lipophilicity properties of molecules to predict biological activities. These computational chemistry methods have been used successfully to predict biological activities (11, 12). While these tools are widely used in the pharmaceutical industry to predict which analogs of a particular drug warrants further investigation, they are not widely used in general industry because of the cost and availability of quality data for modeling. The downside is that these data are statistically derived values therefore false correlations are possible. Due to the complicated nature of determining reproductive toxicants and variables in QSAR determinations, it is doubtful that these types of computations could be useful in helping to determine which chemicals might be reproductive toxicants.

Difficulties in Using Reproductive Toxin Sources

Initially, it would appear that an easy method of identifying reproductive toxicants would be to simply use either the MSDS for the product in question of one of the databases cited above. While the use of the MSDS is appropriate, the use of a database is more difficult than one may first realize. To illustrate this difficulty, five databases, California Proposition 65, Reprotext® (using chemicals rated "A" and "B"), ACGIH, Haz-Map, and the list published by Jankovic and Drake (7), were combined into one listing (Table 1, Combined Listing of Reproductive Toxicants). (It should be noted that CERHR chemicals were not included on this combined listing since CERHR provides a general level of concern and does not state which chemicals are considered a reproductive toxin. DART chemicals were also left off this list for similar reasons. Scorecard was also not included since many of those chemicals listed in this database were derived from those lists cited above.)

In this table of reproductive toxicants combined from these five sources, there are 786 specific chemicals listed. Of these 786 chemicals, 133 (16.9%) are listed in two sources, 38 (4.8%) are listed by three sources, 18 (2.3% are listed by all four sources, and 7 (0.9%) were listed in all five sources. This results in 590 (75.1%) specific chemicals that are listed in only one source. Two inferences can be drawn from this. First, there appears to be differing intents which lead to differing starting lists used for reproductive toxin determinations. For example, the list found in California Proposition 65 appears to contain far more pharmaceuticals than any of the other lists. Using a different set of assumptions for which chemicals should be analyzed will also serve to prejudice the information. For example two classic reproductive toxicants, thalidomide and ethanol were only listed in two and three of the five lists respectively A second inference is that diverging evaluation criteria were likely used to generate each listing. Diverging evaluation criteria would likely be a natural outcome from many of the issues described above.

The most significant lesson to be learned from these different reproductive toxin lists is not why they may differ, but that they do differ. Because there is so little overlap between these lists, the use of a single-list based approach must be questioned. Without knowing and approving the selection or evaluation criteria used to generate a reproductive toxicant list, one cannot justify using one list over another. Likewise, if one looks to use a list to assist in determining those chemicals that are reproductive toxicants, then that suggests one does not have sufficient knowledge to make the determination without outside help. This being the case, one does not have the knowledge to claim that one reproductive toxin list is superior to another list and each list must be treated with equal weight.

Recommended Path Forward

Clearly the determination of relative reproductive toxicity is difficult. The question is "How does one make this determination given the complicated nature of the subject and the limited resources that are typically available?" There are several steps one can take to accomplish this task.

1. Use the MSDS

Regulations require that an MSDS for every chemical or chemical product be present and that the MSDS be used to determine hazards for employee training and information. If the MSDS states the product to be a mutagen, teratogen or a reproductive toxin then the determination is complete. If the MSDS does not state that the product is a reproductive toxin or suggests that the product might be a reproductive toxin, then a decision needs to be made as to whether or not a further determination is required.

2. Use Multiple Databases

If the MSDS does not provide adequate information on a chemical's reproductive toxicity, then one should consider using ACGIH®, California Proposition 65, Reprotext®, Jankovic and Drake (7), and, possibly, CERHR. If one chooses to use chemicals identified in CERHR, one would have to perform a bit of reading to determine if the chemical should go into their listing. Similarly, since Reprotext® uses a grading system for evaluating reproductive toxicants, one could simple choose a rating and if the chemical is rated above that level, then it would be considered to be a reproductive toxin. Chemicals listed in ACGIH®, Jankovic and Drake (7), and those identified in Reprotext® could be added to the listing from California Proposition 65 to provide for the final list such as was done in Table 1.

3. Be Wary of Mixtures

When identifying reproductive toxicants in mixtures, the initial inclination is to use the rule from the 29CFR1910.1200, Hazard Communication, to determine if a hazard is present. This rule states that if the mixture container at least 1% of the chemical (0.1% for carcinogens), then the mixture contains the hazard. Attempting to apply this rule to

reproductive toxicants may not be appropriate. For example, oxygen at a 100% concentration is a reproductive toxin to fetuses and newborn infants. If one were to blindly apply the 1% rule, then this would result in air which contains 21% oxygen a reproductive toxin. Other, similar examples are easy to find.

Conclusions

Accurate determination of reproductive toxicants is important to both protect the worker and to ensure that limited resources are not consumed unnecessarily. To accomplish this task, one must first understand the difficulties in making reproductive toxin determinations and then develop a strategy that will make use of available information in a constructive organized manner.

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Table 1. Combined Listing of Reproductive Toxicants. This is a listing of chemical reproductive toxicants taken from California Proposition 65 (3), Reprotext (4), Jankovic and Drake (7), ACGIH (8), and Haz-Map (9). NA indicates that the product listed is either a mixture, chemical class or that there was not enough information present to assign a CASRN.