Ann. Rev. Pharmacol. Toxicol. 1987. 27:157–67

SAFETY EVALUATION OF POLYMER MATERIALS

Thomas D. Darby

Department of Pharmacology, School of Medicine, University of South Carolina, Columbia, South Carolina 29208

INTRODUCTION

Polymer materials have made important contributions to human well-being during the last fifty years. The different chemical formulas and syntheses of polymers supply an enormous number of materials of widely differing composition. Continuous research and development in the health industry have led to an increased life span and improved quality of life for many patients by providing improved materials for medical devices. Both elastomers and plastics have been devised to furnish materials with every conceivable type of physical response. Yet, ideas for new and better materials to replace existing materials are proposed each day.

As with other products, safety evaluation of polymer materials and the devices made from them begins with concerns for toxic or adverse reaction associated with the raw materials for the product and ends with the safe disposal of the end materials from product use. One has only to review the problems associated with the production of vinyl chloride monomer to be concerned about manufacture of products with polyvinyl chloride (1, 2). If the monomer had been removed from production, many polyvinyl chloride products would no longer be in the market place. In addition, traces of contaminants from the manufacture of raw materials often contribute to the potential toxicity of a product (3). Naturally, most concern in safety assessment is directed toward the manufacture and use of products. However, shipping and storage can also contribute to toxic effects at time of use. Also, disposal of end materials of product use causes environmental concerns. Polyvinyl chloride is also a good example in this case because the release of hydrochloric acid during pyrolysis causes adverse reactions ranging from eye irritation to pulmonary problems including asthma (4, 5).

0362-1642/87/0415-0157$02.00

This review analyzes the test systems used to evaluate the toxic risk potential for polymer materials and discusses the general concepts used in these evaluations. The test systems are generally intended to assure safety as well as to investigate the range of toxicity for plasticizers and other substances contained in the polymer material.

In vitro test systems and in vivo animal studies are often considered indicative of the risks to man. Therefore, these investigations require well-designed standard operating procedures. Also important are protocol studies that consider all relevant parameters prior to actual test, so that the resulting data can be analyzed and applied to human safety concerns.

Tests used in safety assessment of plastic materials need to be sensitive and inexpensive, and must provide comparative data for competing materials. The time in which
vestigators have devised a series of sensitive screening tests that attempt to eliminate false-negative safety data while allowing a fairly substantial number of false-positive results. These simple screening tests that include both chemical and biological measurements are not intended to eliminate valuable new materials but to indicate, when positive, that additional protocol tests must be designed. The object of these additional tests is to determine whether the plastic material will be safe to manufacture and use and whether its end products can be disposed of safely (6).

SCREENING TEST

The tests listed in the *United States Pharmacopeia* (USP/NF Volume I, 1980) are frequently used to screen materials for safety. The description of the sample preparation and the test protocol is not detailed enough to allow the user to determine many important parameters of the test. Therefore, review of the data generated by these standard USP tests depends on the standard operation procedures (SOP) of the laboratory where the test is conducted.

Table 1 illustrates the test procedures used for medical-grade plastic materials. The tests are listed in order of procedure. The sample preparation is important to the SOP of the test procedures. Plastic materials are usually a mixture of ingredients. Substances involved in the manufacture of the plastic may appear as contaminants (7). Tests are most useful when the test specimen is as close as possible in composition to the final material that will be used in device manufacture. In any case, raw materials for devices should be screened at periods that allow statistical assurance of safety for the material or materials used in current manufacture of devices.

Physico-Chemical Test

USP tests are designed to determine physical and chemical properties of plastics and their extracts. When testing extracts, the designated amount of

Table 1 Screening test sequence

Chemical Screening Tests
USP analysis
Nonvolatile residue
Residue on ignition
Heavy metal analysis
Buffering capacity
Other analyses
IR spectrum analysis
Ammonia test for nitrogen
Decision to stop or continue testing
Biological Tests
USP procedures
Systemic injection test
Intracutaneous test
Implantation test
Other procedures
Ames test
Cell culture and Ames test with enzyme additives, e.g. S-9 liver cell fraction
Decision to stop or continue testing
Protocol Tests
Safety studies
With the device
With chemical compounds knows to be potentially toxic
Toxicity Studies
With extracts from the device
With chemical compounds known to be potentially toxic
Safety and risk assessment associated with decision to market device

the plastic must be used and the specified surface area must be available for extraction at the designated temperature (8, 9). The ingredients of a plastic material migrate to the surface in a complicated process controlled by several factors. Rate of removal from the surface is one such factor (7).

In these tests the extract is usually tested. Naturally, a certain amount of the plastic may often be tested directly. These tests are designed to estimate potential toxic effects, based on the known potential toxic characteristics of extracts. Nonvolatile residue and ash provide an estimate of the amount of extractable material contained in the specimen. The quantity of extractable substances is related to the dose upon patient exposure. Since dose is important to toxicity, especially in the case of functional toxicity, these tests are extremely important. Heavy metals are toxic, especially lead and tin. Certain organotin compounds cause extensive toxic reactions (10, 11).

Changes in buffering capacity of the extraction medium indicate that acids or bases have been extracted from the specimens. These potentially reactive compounds could cause adverse reactions. Likewise, a simple test for ammonia can indicate the presence of nitrogen-containing compounds that are

leached from the specimen. Infrared spectra indicate activity sites such as double bonds. As more chemically reactive substances are extracted from the plastic sample or contained in the plastic matrix, potentials for toxic reactions in the biosphere are greater.

These data, along with a list of chemical substances in the formulation, are most helpful when selecting from a list of potential materials. During manufacture of a medical device, however, other chemicals may be incorporated into the plastic. Substances such as solvents, detergents, lubricants, sterilizing agents, etc are often found in the finished product (8, 9, 11).

As Table 1 indicates, a choice between competing materials can often be made at this early point in the testing procedure. Since a major goal of each product is to provide the maximum benefit at a minimum risk, the material with the lowest amount of extractable material and the least potential for chemical reaction is the most favorable.

Biological Tests

These USP tests examine a plastic's suitability for incorporation into a product. These tests of the plastic or its extracts provide information that is often useful in assessing worker safety and evaluating concerns for environmental pollution effects following disposal.

USP tests are preliminary in nature and are screening tests only. Protocol tests are necessary to determine safety. These tests are based on the interpretation of data in making toxicity and safety judgments about plastics in a particular device. Many factors must be analyzed, including plastic composition, processing, and cleaning procedures, contacting media, inks, adhesives, absorption, preservatives, conditions of storage, stability of the plastic, and specific conditions of use (8, 9).

The tests procedures (except for the implantation test) are based on the use of extracts. Temperature determines the class designation for the plastic material. USP Class I plastics are extracted at 50°C. The class of the plastic and the temperature of extraction are usually included in the test results; for example, Class IV has a temperature extraction of 121°C. Systemic injection and intracutaneous tests are carried out with predetermined volumes of the extraction medium containing plastic extracts. Extraction medium is used as a negative control (8, 9).

SYSTEMIC INJECTION TEST The USP systemic injection test is insensitive and therefore fails to meet the criteria for a screening test. It was designed to detect toxic substances that might be leached from a container used for products intended for large volume parental administrations. The use of this test for other purposes is doubtful.

In the systemic injection test five mice comprise the test group and five control mice are used for comparison. The dose administered is usually 50 ml

of the extract per kilogram of body weight of the mouse. The rate of injection is important and is adjusted to approximately 0.1 ml per second.

Extracts from plastics obtained under the conditions established above are injected either intravenously or intraperitoneally. The extraction medium can be physiological saline, ethanol in saline, or an oil such as vegetable oil or cotton seed oil. The extracts provide both hydrophilic and lipophilic extraction of chemicals from the plastic matrix. The total time of the extraction can be varied depending on the use of the medical device. However, in the general screening test it is desirable to use a standard procedure and build a data base on a particular raw material. Thus, the data base becomes generic and can be used to compare materials for many different intended uses.

The mice are observed during the first hour (hr) following the test and then for short periods at 24, 48, and 72 hr. This three-day observation is time-consuming and only overt signs of toxicity can be observed. Certainly if the mice die the material tested is very suspect. Because of the small number of animals used in the test, euthanasia and examination of the tissues are of little value.

INTRACUTANEOUS TEST The USP intracutaneous test is essentially an in vivo cell culture toxicity test. Extracts from the plastic are injected and the site of injection is observed for tissue changes such as edema and erythema. One major problem with both this test and the implantation test is that the reaction to the plastic extract is judged against a negative control called the "blank." The blank is a sample of the extraction medium. Both false negatives and false positives result from this procedure. Edema and erythema can be randomly caused by the injection so that positive tissue reactions can occur at blank injection-sites. Since these screening tests emphasize positive reactions to the specimen, a large number of samples would be needed to reduce the likelihood of false-negative results confounding the data.

The intracutaneous test uses New Zealand White rabbits. At least two rabbits of either sex are used for each type of extraction media. The test area on the back of the rabbit is prepared by closely clipping the hair and carefully cleaning the area with dilute alcohol. Ten injection sites on each side of the spinal processes are used. The volume of the extraction fluid injected is 0.2 ml. Control sites and test sites may be divided into areas. The injected sites are examined at 24, 48, and 72 hr. The sites are graded on a numerical scale from zero, for no reaction, to four for lesion formation. If longer periods of observation are needed care must be taken in removing hair from the site of the injection before observation. The multiple injections allow averaging of the test scores for the test medium and the control medium. When undetermined differences in the test and control medium give positive results, retest is neccssary. The retest should contain data from at least three rabbits.

IMPLANTATION TEST The implantation test is an excellent tissue toxicity test in that the tissue fluids extract substances from the plastic. The test is similar to the tissue culture or cell culture tests in which the culture overlays the plastic material.

The negative control material implanted with the specimen material is used for a comparison of the tissue effects of implantation. The use of the negative control plastic material can lead to false-negative results when the time of reading is three days or less from the time of the implant. Seven-day readings appear to give better results.

Table II illustrates this point. Usually trauma of implantation clears up quickly. However, longer leaching times correlate with increased toxicity from the implant.

Naturally, surface extraction of toxic substances may be higher at the time of the implant. The time required for migration of toxic substances to the surface can affect the degree of toxic effects. If the delivery time is sufficiently long the reaction resembles the slow release of active ingredients from time-released dose forms. Cell culture toxicity tests where larger concentrations of extracted substances can be obtained have the advantage of fewer false-negative responses.

The intramuscular injection sites are similar in location to the sites chosen for the intracutaneous test. The methods used for reading the test results are also similar. Here again the SOP for the laboratory is needed to make judgments regarding the test. Procedural differences are possible, and round robin test results demonstrate both false-positive and false-negative results.

The injection site is best evaluated by removal of the tissue at the site of the implant and microscopic examination of the tissue. Staining preparations may be used to aid in determining the degree of tissue injury.

Table 2 False positive and false negative responses[a]

	Scores		
	3-day Reading	5-day Reading	7-day Reading
Material A	+2	+4	+4
Control A	+3	+1	0
Material B	+4	0	0
Control B	+1	+1	0
Material C	0	0	+3
Control C	+2	+1	0

[a]Three examples of types of readings of implant and intracutaneous scores. Material A could be considered nontoxic at day 3, but at days 5 and 7 the readings are positive. Material B is toxic at day 3, but would be judged nontoxic at days 5 and 7. Material C failed to show toxicity until day 7. If Control B were used to judge Control A or Control C, these two control nontoxic materials could be judged toxic.

Cell Culture and Tissue Culture Tests

Cell culture and tissue culture tests best meet the criteria of the screening test procedures. These tests are very sensitive to cell toxicity, and they screen for potential toxic effects rather than measure directly the toxicity occurring under conditions of use. When a specimen material passes the screening test under standard operating conditions, it is very unlikely that it will cause adverse effects under use conditions because the concentration of the extractants and the sensitivity of the test should be sufficient to provide this assurance (10, 12).

However, when a specimen material fails a screening test, protocol safety evaluations are necessary to assure that the screening test result was a false-positive result and that the material is not toxic in the intended application. The positive screening test results also alert the investigators to the possibility of toxic responses at points other than the end use of the product.

The in vitro test sensitivity can be improved by choice of cell lines and choice of cell culture response evaluated. Biochemical methods can measure such cell functions as DNA synthesis, protein synthesis, and ATP activity. Cell function tests such as adhesion and phagocytosis are highly sensitive but have low toxicity-ranking agreement and reproducibility. Measuring ATP activity with CCL76 or mouse-embryo cell cultures, DNA synthesis with CCL76 or CCL 1 cultures, and protein synthesis with CCL 1 or mouse-embryo cultures might be appropriate for biomaterial toxicity screening. Each of these assays demonstrates high relative sensitivity in discernment of test materials with known toxicity. Reproducibility and predictability of cellular response were good with these tests (10). Less complicated and more subjective means of measuring cellular response are also functionally acceptable as a screening system (12).

For example, the Ames test allows evaluation of specimen materials for genotoxic responses (13). When the S-9 liver enzyme fraction is added to the culture medium, the metabolic alteration of the parent compounds can be tested (14). The metabolic system can also be used with other enzyme testing methods. The choice of the SOP is based on the chemical structure of the ingredients of the material and on their metabolism. While false-negative responses can also occur here, the concentration of the extractable materials can be increased to levels that overrule low dose considerations. A positive Ames test warrants further tests for potential chemical carcinogenesis as well as for potential effects on reproduction.

PROTOCOL TESTS

The USP tests and the cell culture and tissue culture tests will likely fail to detect function toxicity, especially that related to metabolites of the leached

compound. Since patient exposure dose can be estimated from the chemical tests and these doses are generally very low, concerns for functional toxicity related to phase I biotransformations or to failure of the phase II detoxication processes are not important (15). However, both toxicological testing and safety testing frequently require test procedures other than those in the screening test for benefit-risk assessments. These test protocols can be used to discover functional toxicity as well as tissue toxicity. As for devices with blood-material interactions, special protocol tests may be needed to evaluate effects on blood functions, clotting, calcification of the materials, and other hematological responses (16). Toxicity due to large-dose exposures (such as those occurring with feeding) in which doses of two to four percent of the diet weight is common, where the toxicity is related to biotransformations or failure of the phase II reactions (15). Often toxicity occurs when the capacity of detoxication mechanisms is exceeded. For example, large oral doses of di(2-ethylhexyl)phthalate (DEHP) cause an increase in hepatic peroxisome proliferation in rat feeding studies (17). Hypolipidemic responses associated with hepatic peroxisome proliferators are reported to form a novel class of chemical carcinogens (18, 19). The response following DEHP administration is actually a response to MEHP, a metabolite of DEHP (20, 21). In cases where DEHP is used to plasticize materials contained in medical devices the exposure dose is low even for hemodialysis patients, and MEHP, which is water soluble, is rapidly cleared by the body. However, human patients differ in their hepatic and other reaction to DEHP exposure according to the lipid level of their plasma.

Laboratory test systems provide us with excellent products, but noticeable failures have occurred after long-term human exposure in the ability of these test systems to predict adverse effects.

Immunological responses and allergic reactions are the most difficult to detect with animal systems. Yet, with long-term hemodialysis, anaphylactic-type reactions have occurred (22). These reactions are thought to be related to residual ethylene oxide from sterilization procedures. Although these patients have elevated levels of immunoglobulins, animal test systems fail to demonstrate a cause-effect relationship for any known chemical found in devices used for human dialysis.

These and other laboratory protocol test systems used to assess the risk of human exposure to chemical substances need to be validated. The method used and the means of data analysis should consistently detect a response directly related to a human response. Whole-life carcinogenesis tests, with their multiple organ examinations at regular intervals during the test period, are often biologically confounded (23). Unlike pharmacological or toxicological response detection, where drug receptor response can be determined to occur in each animal at some dose, the animal carcinogenesis test is like an

epidemiology test. Prediction of carcinogenesis in other species has been less than exciting.

With each standard operation procedure or test protocol, dose form, route of exposure, whole body distribution, species metabolism, and elimination differences are important. Studies conducted with DEHP have certainly proven this importance (24). The use of whole body autoradiography to indicate in which organs or tissues chemical-receptor responses are possible can certainly aid in protocol study design (25).

Improved biochemical procedures for study of chemical-receptor binding have greatly improved our understanding of receptor occupation responses (26). Application of these techniques to toxicity mechanism studies can improve dose-response assessments of human risk. Stephenson (27) supplies the following postulates:

1. A maximum effect can be produced by an agonist when it occupies only a small proportion of the receptors.
2. The response is not linearly proportional to the number of receptors occupied.
3. Different drugs may have varying capacities to initiate a response and may consequently occupy different proportions of the receptors when producing equal responses. This property was referred to as the efficacy of the drug. In this setting, a pure antagonist would have zero efficacy.

These postulates help to clarify some of the problems associated with toxicity assessment. In cases of toxicity both efficacy or intrinsic activity and affinity are important (28). Binding and metabolism are also useful detoxification mechanisms. In carcinogenesis studies the genetic specific target (GST) is recognized as the receptor, and certainly "a maximum effect can be produced by an agonist when occupying only a small proportion of the receptors" (1). Naturally the higher the number of people exposed to a given concentration of a toxic substance the greater the probability of an agonist receptor reaction.

The most important aspect of protocol design for safety and toxicity assessment is the analytical chemical determinations. The formulation of the material must be known. Also, analytical procedures can provide qualitative and quantitative data about substances that become incorporated in the polymer matrix during manufacturing processes. These investigations of risk assessment are greatly improved over previous procedures.

CONCLUSIONS

A group of screening tests has been suggested that allows comparisons of materials proposed for use in medical devices. Whereas certain organizations

have proposed the division of medical devices into classes and the selection of the screening procedure based on the intended use of the device, the generic use of these tests must be considered. When these screening tests are considered as a battery, the sensitivity of the system is sufficient to prevent false-negative results. Because of the ubiquity of medical devices, the diversity of biological states of the patients, and the range of benefit to risk ratios, protocol studies are needed that provide convincing evidence for the safety and efficacy of medical devices. The risk reported in animal toxicity studies involving monomers, polymers, plasticizers, and other substances contained in a material must be evaluated in light of the multiple factors that affect human response during the beneficial use of the device.

ACKNOWLEDGMENTS

The author thanks R. Van Essche, DVM, and R. F. Wallin for their assistance in the preparation of this review.

Literature Cited

1. Infante, P. F., Wagoner, J. K., Waxweiler, R. J. 1976. Carcinogenic, mutagenic and teratogenic risks associated with vinyl chloride. *Mutat. Res.* 41:131–32
2. Selikoff, I. J., Hammond, E. C., eds. 1975. *Ann. N.Y. Acad. Sci.* Vol. 246
3. Darby, T. D., Johnson, H. J., Northup, S. J. 1978. An evaluation of a polyurethane for use as a medical grade plastic. *Toxicol. Appl. Pharmacol.* 46:449–53
4. Clark, C. A. 1972. Gases from burning PVC. *Soc. Plastics Eng. Tech.* 18:623–26
5. Sokol, W. N., Aelony, Y., Beal, G. N. 1973. Meat Wrappers Asthma. *J. Am. Med. Assoc.*, 226:639–41
6. Darby, T. D. 1978. Pharmacological considerations in the design of toxicology experiments. *Clin. Toxicol.* 12:229–38
7. Henne, W., Dietrich, W., Pelger, M., von Sengbusch, G. 1984. Residual ethylene oxide in hollow-fiber dialyzers. *Artif. Organs* 8:306–9
8. Henry, T. J., ed. 1985. Guidelines for the preclinical safety evaluation of materials used in medical devices. *HIMA Rep.* 85–1, Washington, DC: Health Ind. Manufactures Assoc.
9. Committee on Standards. 1982. Standard practice for selecting generic biological test methods for materials and devices. *Ann. Book ASTM Standards*, Vol. 13.01. F-748-82
10. Johnson, H. J., Northup, S. J., Seagraves, P. A., Garvin, P. J., Wallin, R. F. 1983. Biocompatibility test procedures for materials evaluation, in vitro. I. Comparative test system sensitivity. *J. Biomed. Mater. Res.* 17:571–86
11. Autian, J. 1980. Plastics. In *Toxicology, The Basic Science of Poisons,* ed. J. Doull, C. D. Klaassen, M. O. Amdur, p. 531. New York: Macmillan
12. Johnson, H. J., Northup, S. J., Seagraves, P. A., Atallah, M., Garvin P. J., Darby, T. D. 1985. Biocompatibility test procedures for materials evaluation, in vitro. II. Objective methods of toxicity assessment. *J. Biomed. Mater. Res.* 19:489–508
13. McCann, J., Choi, E., Yamasaki, E., Ames, B. N. 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 30 chemicals. *Proc. Natl. Acad. Sci. USA* 72:5135–39
14. Maron, D. M., Ames, B. N. 1983. Revised methods for the Salmonella mutagenicity test. *Mutat. Res.* 113:173–215
15. Neal, R. A. 1980. Metabolism of toxic Substances. See Ref. 11, p. 56
16. McIntire, L. V., ed. 1985. Guidelines for blood-material interactions. *US Dep. Health Human Serv. NIH Publ.* No. 85-2185
17. Reddy, J. K., Moody, D. E., Azarnoff, D. L. 1976. Di-(2-ethyl) phthalate: An industrial plasticizer induces hypolipidemia and enhances hepatic catalase

and carnitine acetyltransferase activities in rats and mice. *Life Sci.* 18:941–45
18. Reddy, J. K., Azarnoff, D. L., 1980. Hypolipidemic hepatic proliferatiors form a novel class of chemical carcinogens. *Nature* 283:397–98
19. Reddy, J. K., Warren, J. R. 1981. Toxicological implications of hepatic peroxisome proliferation in rodents: Possible role of oxygen radical toxicity in hypolipidemic drug-induced carcinogenesis. *Toxicologist* 1:131
20. Albro, P. W., Thomas, R., Fishbein, L. 1973. Metabolism of diethylhexyl phthlate by rats. Isolation and characterization of the urinary metabolites. *J. Chromatogr.* 76:321–30
21. Albro, P. W., Corbett, J. T., Schroeder, J. 1981. The fate di-(2-ethylhexyl) phthalate in rats. *Toxicologist* 1:55
22. Grammer, L. C., Roberts, M., Nicholls, A. J., Platts, M. M., Patterson, R. 1984. IgE against ethylene oxide-altered human serum albumin in patients who have had acute dialysis reactions. *J. Allergy Clin. Immunol.* 74:544–46
23. Hart, R. W., Turturro, A., Weisburger, E. 1985. Chemical carcinogens; A review of the science and its associated principles. *Fed. Reg.* March 14, p. 49–52
24. Thomas, J. A., Darby, T. D., Wallin, R. F., Garvin, P. J., Martis, L. 1978. A review of the biological effects of di-2(ethyl hexyl) phthalate. *Toxicol. Appl. Pharmacol.* 45:1–27
25. Waddell, W. J., Marlowe, C., Miripol, J. E., Garvin, P. J. 1977. The distribution in mice of intravenously administered 14C-di-2-(ethyl hexyl) phthalate (14C DEHP) determined by whole-body autoradiography. *Toxicol. Appl. Pharmacol.* 39:339–53
26. Limbird, L. E. 1986. *Cell Surface Receptors: A Short Course on Theory and Methods*. Boston: Nijhoff 196 pp.
27. Stephenson, R. P. 1956. A modification of receptor theory. *Br. J. Pharmacol.* 11:379–93
28. van Rossum, J. M., Ariens, E. J. 1962. Receptor reserve and threshold phenomena. II. Theories on drug-action and a quantitative approach to spare receptors and threshold values. *Arch. Int. Pharmacodyn.* 136:385–13