

Nomination of the National Toxicology Program Rodent Bioassay for ICCVAM Review and Validation

This nomination is made in accordance with Public Law 106–545

SEC. 3. INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS. (b) PURPOSES.—The purposes of the ICCVAM shall be to—

(1) increase the efficiency and effectiveness of Federal agency test method review; (2) eliminate unnecessary duplicative efforts and share experiences between Federal regulatory agencies; and (e) DUTIES.—The ICCVAM shall, consistent with the purposes described in subsection (b), carry out the following functions: (1) Review and evaluate new or revised or alternative test methods, including batteries of tests and test screens, that may be acceptable for specific regulatory uses, including the coordination of technical reviews of proposed new or revised or alternative test methods of interagency interest, (5) Consider for review and evaluation, petitions received from the public that—(A) identify a specific regulation, recommendation, or guideline regarding a regulatory mandate; and (B) recommend new or revised or alternative test methods and provide valid scientific evidence of the potential of the test method;. And others as may apply.

Purpose of this Nomination

This nomination asks ICCVAM to review and validate the National Toxicology Program Rodent Bioassay (NTPRB) as a method for accurately predicting human carcinogens and noncarcinogens. Secondly, this nomination asks ICCVAM to evaluate the suitability of results obtained from the NTPRB as a standard against which the predictive performance of alternative short and medium term in vivo and in vitro tests can be objectively measured. Thirdly, if after reviewing existing data and in the event that ICCVAM is unable to validate the NTPRB, this nomination asks ICCVAM to describe what new data and approaches would be needed in order for ICCVAM to conduct a proper validation in the future.

To date, neither ICVAAM/NICEATM nor any other organization, has conducted an independent technical evaluation of the NTPRB as a method for predicting rodent/human carcinogens and noncarcinogens or as a standard for evaluating the performance of alternative short and medium term tests.

This document provides representative citations in support statements made and does not claim to include all relevant references that ICVAAM might want to consider.

Others have also called attention to the need for validation of long term rodent bioassays as conducted by the NTP (e.g., Ennever et al 1987; Storer 2000; Kirkland et al 2007)

Justification and Background

Epidemiological studies are generally regarded as the best source for evidence of carcinogenicity in humans, but unfortunately evidence is found only after the damage has been done (e.g., Cogliano 2004; EPA 2005). The National Toxicology Program Rodent Bioassay (NTPRB) is a standardized 2-year test to determine if an agent can cause cancer in laboratory rodents, traditionally F344 rats and B6C3F₁ mice, and by extrapolation in humans (Rall 1988, 2000; Bucher 2002). Although unvalidated, the NTPRB thus serves as a predictive alternative for retrospective human studies. The NTPRB and similar long-term tests for carcinogenicity have become an integral component of the regulatory apparatus in the US and other countries around the world (OECD 1981, 2002; EPA 2005; FDA 2006; Tomatis 2006). As a result, chemical manufacturers and pharmaceutical companies conduct rodent cancer tests, among others, for purposes of product registration. The NTP, operating under the National Institute of Environmental Health Sciences (NIEHS), also conducts a limited number of rodent bioassays in response to a variety of concerns from outside agencies and the public (NTP 2008a). Studies conducted by the NTP are reported in detail and published as a series of technical reports (NTP 2008b). Rodent cancer studies conducted by chemical manufacturers and pharmaceutical companies are seldom reported in similar detail in the open literature. The NTP has published the results of more than 500 rodent bioassays (NTP 2008b). The total number of agents that industry has tested in rodents is unknown.

The NTPRB has been criticized for being too costly and time consuming, for employing doses that are too high, for exposures that are of too long in duration and generally out of range of actual human exposures, for seldom being repeated, for never having been objectively validated as a predictive test, and for not being relevant, among other reasons (Efron 1985; Whelen 1985; Ames and Gold 1990; Carr and Kolbye 1991; Monro 1993, 1996; Cohen and Lawson 1995; Johnson 2000; Gori 2001; MacDonald 2002; Cohen et al 2004; ACSH 2005; Trosko and Upham 2005; Knight et al. 2005, 2006. Haseman and Johnson (1996) report almost as much chemically related anticarcinogenic as carcinogenic effect in NTP studies, often with improved survival, see also Crump (1999). Davies and Munro (1995), Abraham (1998) and Ward (2007) point out that in many cases drugs are approved for human use after being demonstrated to be carcinogenic in rodent tests, suggesting that regulatory agencies often perceive the benefits of exposure to a substance to outweigh the harm associated with the cancer the agent might induce. Some critics of the bioassay reportedly have a vested interest in industry (Huff 2007), but most of the criticisms, on the surface at least, appear to be valid. There are of course advocates of the bioassay who argue passionately in its favor, e.g., "...it is hard to believe that anybody with any sense of responsibility and with even only minimal interest in public health could have discarded or ignored or belittled the role that long term carcinogenicity tests (could have)...in the adoption of preventive measures for the protection of human health," Tomatis (2006), but do so without answering the criticisms.

Evaluating the accuracy of the bioassay is made difficult in part because evidence of human noncarcinogenicity is rarely reported; the IARC, for instance, classifies only one chemical as probably not carcinogenic to humans (IARC 1986, 1999, 2007). The lack of reporting means that negative results when demonstrated in rodents cannot be easily compared and confirmed with high quality human data. Human experimental studies to distinguish carcinogens from noncarcinogens would of course be unethical. Another difficulty with evaluating the predictive performance of the bioassay is that many test agents were selected for testing on the basis of known or suspected carcinogenicity in humans, especially in the early days of the program at NCI. (Weisburger 1983; Huff 1999). Indeed, Weisburger (1983) describes the bioassay program as being "highly research oriented with emphasis on structures and structural classes," rather than having a testing focus, and the reports themselves typically advise that results apply only to the conditions of the bioassay. Such academic caution stands in contrast with policy "to consider all agents, for which the evidence is not clearly negative under accepted minimum conditions of observation, as if they were positive..." (Saffiotti 1978) and generally advocating testing for carcinogenicity for safety assessment in a regulatory framework. (Saffiotti 1976, 1977, 1978; NTP 1983; 2008; Rall 1988, 2000; Huff 1999; Maronpot et al 2004).

Susceptibility to Tumor Development is Determined by Genotype, Sex and Test Conditions

The genetics of susceptibility to spontaneous and induced tumors in experimental animals and humans has been under increasingly intense study since the early 1900s (e.g., Lathrop and Loeb 1913; Sly 1913; Loeb and Lathrop 1919; Lynch 1926; Strong 1935; Graham 1936; Robson and Bonser 1938; Bittner 1938; Collins et al 1943; Falconer and Bloom 1962; Heston 1952, 1965; Goldfeder et al 1966; Evans et al 1977; Henning et al 1993; Haston et al 1996). Gradually, over time a number of chromosome regions and specific genes and have become associated with susceptibility and resistance to tumors induced by different agents, particularly in mice and in some cases humans (e.g., Porta et al 1967; Flaks 1968; Vesselinovitch et al 1974; Dragani et al 1984, 1995; Nebert 1981; Malkinson 1989; Sellers et al 1990; Gariboldi et al 1993; Devereux et al 1994; Festing et al 1994; Lee et al 1995; Manenti et al 1997a,b, 1999, 2005; Zeng et al 2000; Lynch and Lynch 2002; Takahashi et al 2002; Hecht 2005).

A general implication of all the genetic work on susceptibility in the context of testing is that for the carcinogenic response to develop, genetic factor(s) conferring susceptibility (or absence of resistance) must be present in the DNA of the test animals and if not, results in test animals will be negative. For many known human carcinogens (cigarette smoke, arsenic, asbestos, benzene and 2-naphthylamine) it proved to be quite difficult to find a rodent model to respond the same as humans (eg, Mauderly et al 2004; Hutt et al 2005; Balansky et al 2007). Wilhelm Hueper (author of the first textbook on occupational cancer) long refused to believe tobacco could be a human carcinogen due to his inability to induce tumors in rodents (and perhaps because of his own addiction to nicotine) (Sellers 1997). Of course, dose and duration of

exposure might also be determining factors in the response, since resistance mechanisms might at some point be overcome, and as Goodman and Wilson (1991) have stated, “it might be helpful to assume all chemicals are carcinogenic with the important variable being potency”. However, potency might sometimes be trumped by route of exposure since if the agent is unable to reach the susceptible target tissue, a positive response may not be possible. Likewise for genotype, since the absence of susceptibility factors in the animals chosen for testing might prevent absolutely the occurrence of a carcinogenic response.

Perusing the results of NTP studies, one rarely, if ever, sees the same carcinogenic response to an agent in all four genotypes (sex-species groups) (NTP 2008b). This observation undoubtedly reflects random variability to some degree but also the effects of genotype on the carcinogenic response. In the absence of repeated studies, which NTP almost never conducts, it is not possible to separate the effects of chance and genotype with any confidence, though we know from many independent investigations of mice and humans in past decades that multiple susceptibility genes exist. Since NTPRB utilizes one inbred rat strain and one hybrid mouse strain (comprised of the progeny resulting from the cross of two inbred strains), individuals within a sex-species group are virtually identical genetically, i.e., of the same genotype. Males and females of the same strain of course differ by their sex chromosomes, XX in females, and XY in males. Compared to populations of rats, mice and humans, each of which contain millions of different genotypes, the four sex-species groups used in rodent bioassays represent only four genotypes. In testing agents with the rodent bioassay, we are thus asking the four rodent genotypes used in the bioassay to represent millions of human genotypes, some of which may not occur at all in rats or mice. Developers of the NTPRB (cf. Cameron et al 1985) decided to use hybrid mice believing the animals “...would more closely represent the genetic diversity of human populations...” apparently not realizing that one heterozygous genotype does not represent any diversity at all. Thus, picking a couple of strains of rodents for carcinogenicity testing, hoping to match genetic susceptibility factors in all, or some average human being, probably did not demonstrate the highest level of thinking on the part of test advocates from the very beginning.

Table 1 shows the effect of number of genotypes tested on the carcinogenic response in the NTPRB data set. Thus, if the results are considered in only one rat or mouse genotype, values range from 35.47 to 43.40 percent of tested chemicals showing a carcinogenic response or a 39.10 percent average positive result. The four rodent genotypes generally do not respond the same. Thus, when combinations of two, three and four genotypes are considered, the proportion of agents testing positive in one or another genotype progressively increases to 68.1 percent.

This simple descriptive analysis depends on how the equivocal results are treated. (The term “equivocal” is used when test officials cannot decide if results are positive or negative.) In the present analysis equivocal results were treated as missing data and not used. Treating the equivocal results as positive would of course add apparent positivity (more carcinogens) while treating them as negative would reduce it (fewer carcinogens). Various reports have indicated around 50 percent of tested agents are carcinogenic (cf., MacDonald 2004). The lower value generally comes up when the equivocal results (and sometimes missing data) are regarded as negative.

Figure 1 is a graphical representation of the same data with the addition of a trend line which reaches the vicinity of 80% positive or more if 8 rodent genotypes would be used. In the real world, no one knows how many genes determine susceptibility to chemically induced cancer; conceivably hundreds of genes will eventually be identified. Thus, perhaps a bioassay utilizing a population of diverse rodent genotypes with many susceptibility genes represented would identify a much larger proportion of tested agents as carcinogens, perhaps approaching 100%.

If there are so many susceptibility genes present in populations, one might ask why is the proportion of detected carcinogens so high in tests using only four genotypes? Two factors may explain the high proportion. One, test developers initially selected agents they already knew or strongly suspected would be carcinogenic (Weisburger 1983; Huff 1999) and even today nominations of agents for which there is no reason to suspect carcinogenic activity might not receive high priority consideration. Two, the number of susceptibility factors may be so great that even restricting the number test genotypes to four, still provides a genetic constitution sufficient to enable a carcinogenic response to most agents, at least under the MTD and

near MTD conditions used for testing. If this is the case, negative results in the bioassay might merely signal a comparatively rare absence of necessary susceptibility factors in the test animals and say nothing particularly relevant to human susceptibility and safety.

Festing (1995) recommended that NTP test a number of chemicals with an increased number of rodent genotypes to see what effect this would have on test performance. Festing predicted the power of the test would improve, i.e., detect more carcinogens. Of the course, as anyone of Festing's scientific stature must be aware, the problem with the bioassay is not that it does not detect enough carcinogens, the problem is that its predictive performance appears, from available evidence, to be little or no better than arbitrary.

So far NTP has not acted to implement Festing's suggestion, not announced plans nor indicated any need to validate the rodent bioassay. NTP, under political steam from NAS, continues to promote development of alternative technologies (most recently highthroughput systems and toxicogenomics) and indicates diminished reliance on long term rodent testing in the indefinite future (Bucher 2002; Bucher and Portier 2004; Portier 2004; NTP 2004 a,b; NTP 2007; NAS 2007a,b; Collins et al 2008).

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Table 1

Percent of chemicals testing positive (carcinogenic) according to genotype based on results from 490 NTP rodent cancer bioassays.

		Number of Genotypes*									
		0		1		2		3		4	
	0	MR	43.40	MRFR	51.29	MRFRMM	64.77	MRFRMMFM	68.10		
		FR	35.47	MRMM	60.57	FRMMFM	61.68				
		MM	36.83	MRFM	59.85	MRFRFM	64.09				
		FM	40.71	FRMM	54.50	MRMMFM	64.80				
				FRFM	56.58						
				MMFM	48.10						
AVG	0		39.10		55.15		63.83				68.10

*Genotypes refers to sex-species groups, MR = male rats, FR = female rats, MM =male mice, FM = female mice. Mice generally used were B6C3F1 strain hybrids, rats generally used were F344.

The data for this table were taken directly from NTP reports; reduced as follows for purposes of tabulation.

IS (insufficient or inadequate data) =.

NT (not tested) =.

E, EE (equivocal evidence) =.

SE (some evidence) = P (positive) = 1

CE (clear evidence) = P (positive) =1

NE (no evidence) = N (negative) = 0

Note: A dot or period represents "missing data."

Figure 1

