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 B6C3F$_1$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-napthylamine) 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 exit. 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 equivocals are treated. (The term "equivocal" is used when test officials cannot decide if results are positive or negative.) In the present analysis equivocals were treated as missing data and not used. Treating the equivocals 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 equivocals (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 highthoughput 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).

References

Abraham J. 1998. Regulating the cancer-inducing potential of non-steroidal anti-inflammatory drugs: some lessons from the 1970s and 1980s. Soc Sci Med 46:39-51.

American Council on Science and Health. America's War on "Carcinogens": Reassessing the Use of Animal Tests to Predict Human Cancer Risk. 2005.

Ames, BN and Gold, LS. Chemical carcinogenesis: too many rodent carcinognes. Proc Natl Acad Sci 97:7772-7776, 1990.

Balansky R, Ganchev G, Iltcheva M, Steele VE, D'Agostini F and DeFlora S. 2007. Potent carcinogenicity of cigarette smoke in mice exposed early in life. Carcinogenesis 28:2236-2243.

Bittner JJ. 1938. The genetics of cancer in mice. Quarterly Review of Biology 13:51-64.

Bucher JR. 2002. The National Toxicology Program rodent bioassay: designs, interpretations, and scientific contributions. Ann N Y Acad Sci. 982:198-207.

Bucher, JR and Portier C. 2004. Human Carcinogenic Risk Evaluation, Part V: The National Toxicology Program Vision for Assessing the Human Carcinogenic Hazard of Chemicals. Toxicological Sciences 82: 363–366.

Cameron TP, Hickman RL, Kornreich MR and Tarone RE. 1985. History, survival and growth patterns of B6C3F1 mice and F344 rats in the National Cancer Institute carcinogenesis testing program. Fundamental and Applied Toxicology 5:526-538.

Cogliano VJ, Baan RA, Straif K, Grosse Y, Secretan MB, Ghissassi FE, and Kleihues P. 2004. The science and practice of carcinogen identification and evaluation. Environmental Health Perspectives 112:1269-1274.

Collins FS, Gray GM and Bucher JR. 2008. Transforming environmental health protection. Science 319:906-907.

.

Collins VJ, Gardner WE and Strong LC. 1943. Experimental gastric tumors in mice. Cancer Res 3:29-35.

Crump KS. 1999. Estimates of the proportion of chemicals that were carcinogenic or anticarcinogenic in bioassays conducted by the national toxicology program.

Davies T and Munro A. 1995. Marketed human pharmaceuticals reported to be tumorigenic in rodents. J Am Coll txicol 14:90-107.

Devereux TR, Wiseman RW, Kaplan N, Garren S, Foley JF, White CM, Anna C, Watson MA, Patel A, Jarchow S, Maronpot RR and Anderson MW. 1994. Assignment of a locus for mouse lung tumor susceptibility to proximal chromosome 19. Mamm Genome 5:749-755.

Dragani TA, Sozzi G and Porta GD. 1984. Spontaneous and urethane-induced tumor incidence in B6C3F1 versus B6CF1 mice. Tumori 70:485-490.

Dragani TA, Manenti G, Gariboldi M, De Gregorio L and Pierotti MA. 1995. Genetics of liver tumor susceptibility in mice. Toxicology Letters 82:613-619.

Efron E. The Apocalyptics: How Environmental Politics Controls What We Know About Cancer. Simon Schuster. 1985.

Ennever FK, Noonan TJ and Rosenkranz HS. 1987. The predictivity of animal bioassays and short-term genotoxicity tests for carcinogenicity and non-carcinogenicity to humans. Mutagenesis 2:73-78.
EPA (US Environmental Protection Agency) 2005. Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001F.

Evans JT, Shows TB, Sproul EE, Paolini NS, Mittelman A, and Hauschka TS. 1977. Genetics of colon carcinogenesis in mice treated with 1, 2-dimethylhydrazine. Cancer Res. 37: 134-136.

Falconer DS and Bloom JL. 1962. A genetic study of induced lung tumours in mice. Brit. J. Cancer 16, 665-685.

Festing MFW, Yang A and Malkinson AM. 1994. At least four genes and sex are associated with susceptibility to urethane-induce pulmonary adenomas in mice. Genet Res 64:99-106.

Festing MF. 1995. Use of a multistrain assay could improve the NTP carcinogenesis bioassay. Environ Health Perspect.103:44–52. 1995.

FDA (US Food and Drug Administration). 2006. Toxicological Principles for the Safety Assessment of Food Ingredients. Redbook 2000. IV.C.6. Carcinogenicity Studies with Rodents.

Flaks A. 1968. The susceptibility of various strains of neonatal mice to the carcinogenic effects of 9,10-dimethyl-1,2-benzanthracene. Eur J Cancer 4:579-585.

Goldfeder A., Kauffman SL, and Ghosh AK. (1966) Carcinogenesis in naturally tumour-resistant mice. X-irradiation versus urethan as a carcinogenic agent. Brit. J. Cancer 20: 361-374

Goodman G and Wilson R. 1991. Predicting the carcinogenicity of chemicals in humans from rodent bioasssay data. Environmental Health Perspectives 94:195-218.

Gori GB. 2001. The costly illusion of regulating unknowable risks. Regulatory Toxicology and Pharmacology 34: 205-212.
Graham HF. 1936. The influence of heredity in cancer. Ann Surg 104:952-956.

Haseman J and Johnson FM. 1996. Analysis of National Toxicology Program rodent bioassay data for anticarcinogenic effects. Mutat. Res. 350: 131–141.

Haston C. K., Amos C. I., King T. M., and Travis E. L. 1996. Inheritance of susceptibility to bleomycin-induced pulmonary fibrosis in the mouse. Cancer Res. 56, 2596-2601.

Hecht SS. 2005. Carcinogenicity studies of inhaled cigarette smoke in laboratory animals: old and new. Carcinogenesis 26:1488-1492.

Hennings H., Glick AB, Lowry DT, Krsmanovic LS, Sly LM, and Yuspa SH. 1993. FVB/N mice: An inbred strain sensitive to the chemical induction of squamous cell carcinomas in the skin. Carcinogenesis 14:2353-2358.

Heston WE. 1952. The bearing of mouse genetics on our understanding of human cancer. Am J Hum Genet 4:314-331.

Heston WE. 1965. Genetic factors in the etiology of cancer. Cancer Research 25:1320-1326.

Huff J. 1999. Value, validity, and historical development of carcinogenesis studies for predicting and confirming carcinogenic risks to humans. In Kitchin,K.T. (ed.) Carcinogenicity Testing, Predicting, and Interpreting Chemical Effects. Marcel Dekker, pp. 21–123.

Huff J. 2007. Industry influence on occupational and environmental public health. Int J Occup Environ Health. 13:107-117.

Hutt JA, Vuillemenot BR, Barr EB, Grimes MJ, Hahn FF, Hobbs CH, March TH, Gigliotti AP, Seilkop SK, Finch GL, Mauderly JL and Belinsky SA. 2005. Life-span inhalation exposure to mainstream cigarette smoke induces lung cancer in B6C3F1 mice through genetic and epigenetic pathways. Carcinogenesis 26:1999-2009.

IARC. 1986. IARC Monographs 39:247-276.

IARC. 1999. IARC Monographs 71:383-400.

IARC. 2007. Overall Evaluations of Carcinogenicity to Humans. Group 4: Probably not carcinogenic to humans. http://monographs.iarc.fr/ENG/Classification/crthgr04.php.

Johnson FM. 2000. The rodent carcinogen dilemma, formidable challenge for the technologies of the new millennium. Ann NY Acad Sci. 919:288-299.

Kirkland D, Pfuhler S, Tweats D, Aardema M, Corvi R, Darroudi F, Elhajouji A, Glatt H, Hastwell P, Hayashi M, Kasper P, Kirchner S, Lynch A, Marzin D, Maurici D, Meunier JR, Muller L, Nohynek G, Parry J, Parry E, Thybaud V, Tice R, van Benthem J, Vanparys P And White P. 2007..How to reduce false positive results when undertaking in vitro genotoxicity testing and thus avoid unnecessary follow-up animal tests: Report of an ECVAM (European Centre for the validation of Alternative Methods)workshop. Mut Res 30:31-55.

Knight A, Bailey J, and Balcombe J. Which drugs cause cancer? BMJ 331:E389-E391. 2005.

Knight A, Bailey J and Balcome J. Animal carcinogenicity studies: I. Poor human predictivity. Altern Lab Anim 34: 19-27.

Lathrop AEC and Loeb L. 1913. Incidence of cancer in various strains of mice. Proc Soc Exptl Biol Med 11: 34-38.

Lee GH, Bennett LM, Carabeo RA and Drinkwater NR. Identification of hepatocarcinogen-resistance genes in DBA/2 mice. Genetics 138:387-395.

Loeb L and Lathrop AEC. 1919. The effect of continued inbreeding on the cancer rate in mice: the cancer rate in hybrid strains. J Cancer Res 4: 65-66.

Lynch CJ. 1926. Studies on the relation between tumor susceptibility and heredity. III. Spontaneous tumors of the lung in mice. J Exp Med 43:339-355.

Lynch HT and Lynch JF. 2002. Hereditary cancer: family history, diagnosis, molecular genetics ecogenetics and management strategies. Biochimie 84:3-17.

MacDonald JS. 2004. Human carcinogenic risk evaluation, part IV: Assessment of human risk of cancer from chemical exposure using a global weight-of-evidence approach. Toxicological Sciences 82:3-8.

Malkinson AM. 1989. The genetic basis of susceptibility to lung tumors in mice. Toxicology 54:241-271.

Manenti G, De Gregorio L, Pilotti S, Falvella FS, Incarbone M, Ravagnani F, Pierotti MA and Dragani TA. 1997a. Association of chromosome 12p genetic polymorphisms with lung adenocarcinoma risk and prognosis. Carcinogenesis 18:1917-1920.

Manenti G, Gariboldi M, Florino A, Zedda AI, Pierotti MA and Dragani TA. 1997b. Pas 1 is a common lung cancer susceptibility locus in three mouse strains. Mamm Genome 8:801-809.

Manenti G, Stafford A, De Gregorio L, Gariboldi M, Falvella FS, Avner P and Dragani TA. 1999. Linkage disequilibrium and physical mapping of Pas1 in mice. Genome Research 9:639-646.

Manenti G and Dragani TA. 2005. Pas1 haplotype-dependent genetic predisposition to lung tumorigenesis in rodents: a meta-analysis. Carcinogenesis 26:875-882.

Maronpot RR, Flake G, Huff J. 2004. Relevance of animal carcinogenesis findings to human cancer predictions and prevention. Toxicol Pathol. 32 (Suppl 1):40-48.

Mauderly JL, Gigliotti AP, Barr EB, Bechtold WE, Belinsky SA Hahn FF, Hobbs CA, March TH, Seilkop SK and Finch GL. 2004. Chronic inhalation exposure to mainstream cigarette smoke increases lung and nasal tumor incidence in rats. Toxicol Sci 85:447-459.

Monro A. 1993. How useful are chronic (life-span) toxicology studies in rodents in identifying pharmaceuticals that pose a carcinogenic risk to humans? Adverse Drug React Toxicol Rev 12:5-34.

Monro A. 1996. Are lifespan rodent carcinogenicity studies defensible for pharmaceutical agents? Exp Toxicol Pathol 48:155-166.

NAS (National Academy of Sciences). 2007a. Applications of Toxicogenomic Technologiess to Predictive Toxicology and Risk Assessment .National Academies Press.

NAS (National Academy of Sciences). 2007b Toxicity Testing in the Twenty-first Century: A Vision and a Strategy. Nat. Acad. Press.

NTP (National Toxicology Program). 1983. Fiscal Year 1983 Annual Plan. U.S . Department of Health and Human Services. Public Health Service.

NTP (National Toxicology Program). 2004a. Toxicology in the 21st Century: The Role of the National Toxicology Program.. http://ntp.niehs.nih.gov/ntp/main_pages/NTPVision.pdf

NTP (National Toxicology Program). 2004b. A National Toxicology Program for the 21st century: Roadmap to achieve the NTP vision. http://ntp.niehs.nih.gov/files/NTPrdmp.pdf.

NTP (National Toxicology Program). 2007. NTP vision and roadmap. http://ntp.niehs.nih.gov/index.cfm?objectid=EE4AED80-F1F6-975E-7317D7CB17625A15.

NTP (National Toxicology Program). 2008a. Nominations to the Testing Program. http://ntp.niehs.nih.gov/ntpweb/index.cfm?objectid=25BC6AF8-BDB7-CEBA-F18554656CC4FCD9.

NTP (National Toxicology Program). 2008b. NTP Technical Reports Index. http://ntp.niehs.nih.gov/index.cfm?objectid=070E4402-959F-C0C5-9BB56CDB50750337.

Nebert DW. 1981. Genetic differences in susceptibility to chemically induced myelotoxicity and leukemia. Environmental Health Perspectives 39:11-22.

OECD (Organization for Economic Cooperation and Development). 1981. Guidelines for testing of chemicals. Carcinogenicity studies. No. 451. Paris, France.

OECD (Organization for Economic Cooperation and Development). 2002. Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies. Paris, France.

Porta GD, Capitano J, Parmi L and Colnaghi MI. 1967. Urethan carcinogenesis in newborn, suckling and adult mice of C57BL, C3H, BC3F1, C3Hf and SWR strains. Tumori 53:81-102.

Portier CP. 2004. Future Directions of the National Toxicology Program. http://www.toxforum.org/Documents/Past_Meetings/Summer_Meetings/2004_Summer_Meetings/2.7_Portier.pdf . 2004.

Rall DP. 1988. Laboratory animal toxicity and carcinogenesis testing: Underlying concepts, advantages and constraints. Ann NY Acad Sci. 534:78–83.

Rall DP. 2000. Laboratory animal tests and human cancer. Drug Metab Rev. 32:119-128.

Robson JM and Bonser GM. 1938. Production of mammary carcinomasin mice of a susceptible strain by the synthetic oestrogen, tripheyl ethylene. Nature 142:836.

Saffiotti U. 1976. Validation of short-term bioassays as predictive screens for chemical carcinogens. In" Screening Tests in Chemical Carcinogenesis. R Montesano, H Bartsch and L Tomatis, Eds. IARC Scientific Publications No. 12, International Agency for Research on Cancer, Lyon, France, 1976.

Saffiotti U. 1977. Identifying and defining chemical carcinogens. In: Origins of Human Cancer. H Hiatt, JD Watson and J Winsten, Eds. Cold Spring Harbor Conferences on Cell Proliferation, Vol 4, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Saffiotti U. 1978. Experimental identification of chemical carcinogens, risk evaluation, and animal-to-human correlations. Environmental Health Perspectives 22:107-113.

Sellers C. 1997. Discovering environmental cancer: Wilhelm Hueper, post-world war II epidemiology, and the vanishing clinician's eye. Am J Public Health 87:1824-1835.

Sellers TA, Bailey-Wilson JE, Elston RC, Wilson AF, Elson GZ, Ooi WL and Rothschild H. 1990. Evidence for Mendelian Inheritance in the Pathogenesis of Lung Cancer. J Natl Cancer Inst 82:1272-1279.

Storer RD. Current status and use of short/medium term models for carcinogenicity testing of pharmaceuticals—scientif perspective. Toxicology Letters 112:557-566.

Takahashi K, Dinse G, Foley JF, Hardisty JF and Maronpot RR. 2002. Comparative prevalence, multiplicity, and progression of spontaneous and vinyl carbamate-induced liver lesions in five strains of male mice. Toxicologic Pathology 30:599-605.

Tomatis L. 2006. Experimental chemical carcinogenesis: Fundamental and predictive role in protecting human health in the 1930s-1970s. Eur J Oncol 11:5-13.

Trosko JE and Upham BL. 2005. The emperor wears no clothes in the field of carcinogen risk assessment: ignored concepts in cancer risk assessment. Mutagenesis 20:81-92.

Vesselinovitch SD, Rao KVN, Mihailovich N, Rice JM and Lombard LS. 1974. Development of broad spectrum of tumors by ethylnitrosourea in mice and the modifying role of age, sex and strain. Cancer Res 34:2530-2538.

Ward JM. 2007. The two-year rodent carcinogenesis bioassay—will it survive?. J Toxicol Pathol 20:13-19.

Weisburger EK. 1983. History of the bioassay program of the Nactional Cancer Institute. Prog Exp Tumor Res 26:187-201. 1983.

Whelan EM. Toxic Terror: The Truth About the Cancer Scare. Jameson Books. 1985.

Zeng AA, Higashi S, Kitayama W, Denda A, Yan Y, Matsuo K, Konishi Y, Hiai H and Higashi K. 2000. Genetic resistance to chemical carcinogen-induce preneoplastic hepatic lesions in DRH strain rats. Cancer Research 60:2876-2881.

Table 1

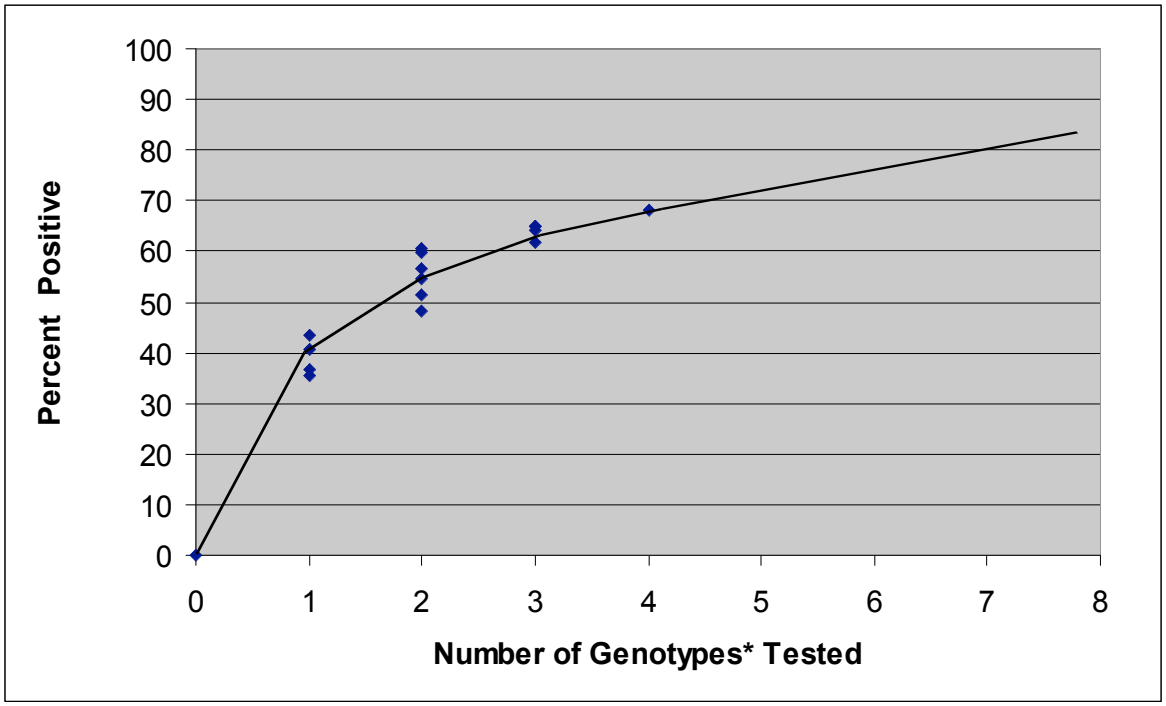
Percent of chemicals testing positive (carcinogenic) according to genotype based on results from 490 NTP rodent cancer bioassays.

	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 evidcence) =.
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


Percent Positive
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Number of Genotypes* Tested