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The Historical Development of Animal Toxicity Testing

From Its Ancient Origins to the Middle of the
Twentieth
Century

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Harvard Law School
Food and Drug Law

Peter Barton Hutt
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Introduction

Modern regulatory systems contain extensive requirements for safety testing of new chemical products before they enter the stream of commerce. Animal toxicity testing plays a significant role in the evaluation of new chemicals, as well as substances intended for use as food additives, pesticides, pharmaceuticals and cosmetics, while there is considerable current debate over nuances of the exact methods and assumptions of animal toxicity testing, the institution is firmly entrenched as an invaluable tool for protecting public health and evaluating potential hazards.

This paper traces the historical development of animal toxicity testing, from its ancient origins through the period of standardization following World War II. It explores the roots of toxicity testing in physiology and experimental medicine, drug development, and the detection and identification of poisons. The discussion then turns to the shift in focus from acute to chronic toxicity which occurred around the turn of the century. The controversy over the potential toxicity of preservatives and pesticide residues illustrates the evolution of toxicity testing in the early to middle part of the twentieth century, as well as the influence of political and economic factors on its development. The paper concludes with the emergence of standardized protocols for toxicity testing during and immediately following the Second World war.

Ancient Origins

Rowan hypothesizes that the earliest cases of animal toxicity testing date back to the dawn of domestication, when primitive man fed a new candidate

foodstuff to camp dogs or other animals to see if it was poisonous.¹ More organized, recorded studies on animals date back at least as far as Aristotle (384-322 B.C.), who dissected animals to reveal anatomical differences among them.² Shortly following, Blyth cites Erasistratus and the Alexandrian school for having dissected both the living and the dead thereby performing the first vivisection some 300 years before Christ.³ The results of these explorations were unknown to the great Roman and Greek writers, and it was the Roman physician Galen of Pergamum whose work in the second century A.D. laid the foundations for animal research for the next fifteen-hundred years.⁴

Since dissection of the human body was illegal in Rome, Galen based his learning on observations made on apes and pigs. As founder of experimental physiology, Galen stressed the value of anatomy and established that the arteries carry blood and not merely air (a topic of contention at the time).⁵ Upon the fall of Rome, however, learning was no longer held in high esteem and experimentation was actively discouraged. Animal research was thus held in abeyance through the Dark Ages until the thirteenth century brought stirrings of the Renaissance and with it the revival of science and philosophy.⁶

Human experience with poisons similarly stretches back into the haze of prehistory. Blyth proposes that early warriors found that when their weapons were soiled with the blood of prior victims the wounds they inflicted were fatal.⁷ Such a revelation may well have inspired early man to experiment with a variety

1 Andrew Rowan *OF MICE, MODELS, AND MEN* Albany: State University of New York Press (1984) at 194 2 on the Use of Laboratory Animals in Biomedical Research *USE OF LABORATORY ANIMALS IN*

BIOMEDICAL AND BEHAVIORAL RESEARCH Washington, D.C: National Academy Press (1988) at 12.

2 A.W. Blyth *POISONS: THEIR EFFECTS AND DETECTION* London: Charles Griffin and Co. (1884) at 7

3 Rowan, *OF MICE, MODELS...* at 42

4 id.

5 id.

6 id.

7 A.W. Blyth *POISONS...* at 1

of noxious substances as enhancements to his arsenal.⁸ Lending support to this hypothesis is that fact that the root tox of the word toxicology can be traced back to a very ancient word meaning bow or arrow, or, more broadly, a tool used for slaying.⁹

Poison lore has its roots in Greek mythology. According to legend, the sorceress Hecate was the discoverer of poisonous herbs, which she could utilize for ends both healing and hurtful. Her knowledge passed to Medea, who narcotized the dragon, guardian of the golden fleece, and thereby laid the groundwork for Jason's undertakings involving the Argonauts.¹⁰ Poets of the day described Hecate's garden as castle guarded by terrible creatures, impenetrable except to the initiated few, implying that the action of poisons was imbued with a mysterious, mystical quality.

The Egyptian experience with poisons dates back to Menes, the oldest Egyptian king, who, along with some of his contemporaries, drew on his extensive knowledge of plants for the preparation of complex poisons and medicines.¹¹ Both the Romans and Greeks apparently made some use of poisons, although their knowledge in this area was rudimentary.¹² Nicander of Colophon (204-138 B.C.) is credited with authoring the earliest treatises on the subject. His

8 id.

⁹id.

10 id.

11 id.at2

12 id.

13 id. at 3. In the reign of Artaxerxes II [B.C. 405-359] Phrysa poisoned the queen Statira by cutting food with a knife poisoned on one side only. Although this has been treated as an idle tale, yet two poisons, aconite and arsenic, were at least well known; either of these could have been in the way mentioned introduced in sufficient quantity into food to destroy life. id. at 6. One can only presume that the participants to the above drama did not adhere to the current custom that one cuts and the other chooses.

two volumes describe the effects of snake venom as well as the properties of opium, henbane, certain fungi, colchium, aconite, and conium.⁴

Animal Testing from the Renaissance through the Nineteenth Century

After the fall of Rome, the knowledge of the ancients lay dormant for well over a thousand years, until the Renaissance reestablished man's quest for scientific learning. During this period and through the nineteenth century, the foundations for the use of animals in modern toxicity testing were laid in three parallel areas: advances in physiology and medicine fueled by experimental research on animals; the emergence of animal testing in drug development and pharmacology; and the use of animals in the detection of poisons and poisoners. Each of these is addressed in turn.

Physiology and Experimental Medicine

The Renaissance brought with it a resurgence of interest in discovery and new ideas, including a revival of animal research. Among a number of contemporaries who experimented on animals, Andreas Vesalius of Padua, Italy (1514-1564) challenged some of Galen's results and created the first modern anatomy.⁵ To the extent that the alternative route of inquiry was to experiment on human subjects, Francis Bacon argued in his *De Augmentis Scientiarum* for the value of replacing them with animals:

*T*⁴_{idat3}

15 Rowan , OF MICE, MODELS., at 42-3

Wherefore that utility may be considered as well as humanity, the anatomy of the living subject is not to be relinquished altogether...since it may be well discharged by the dissection of beast alive, which, notwithstanding the dissimilitude of their parts to human, may with the help of a little judgment, sufficiently satisfy this inquiry.⁶

The seventeenth and eighteenth centuries witnessed the growth of the scientific revolution, and during that time animal research progressed slowly and with little publicity. Widespread and systematic animal research began in earnest during the early part of the 19th century. It was then, as it advanced from a relatively obscure activity to the forefront of medical research, that opposition to animal experimentation developed.⁷

This opposition had multiple sources. Most widespread among them was the anti-vivisection movement, fueled by popular disdain for the perceived the cruelty of animal research. Centered in Britain, but also present in the United States, the movement was keyed to the alleviation of suffering incurred by test animals. It was influenced by the Utilitarian philosophers, especially Jeremy Bentham, from whom the following passage was particularly compelling to animal welfare organizations:

.a full grown horse or a dog is beyond comparison a more rational, as well as a more conversable animal, than an infant of a day, of a week, or even a month old. But suppose the cause were otherwise, what would it avail? The question is not, can they *reason*? Nor, can they *talk*? But can they suffer?⁸

⁶id.at43.

⁷id. at 43-4

⁸from INTRODUCTION TO THE PRINCIPLES OF MORALS AND LEGISLATION (1789) qtd in Rowan at 48

Anything resembling a complete treatment of the anti-vivisection movement and its effect on animal research is beyond the scope of this undertaking. Suffice to say that during the nineteenth century great public debates raged over the value and ethical acceptability of animal experimentation. In Britain, the passage in 1876 of the Cruelty to Animals Act struck a compromise between experimentalists and anti-vivisectionists. The Act regulated painful research but did not abolish it, an ultimate result that was strongly opposed by anti-vivisectionists.¹⁹ In the U.S., the growth of animal research labs in the 1880s and 1890s coalesced a strong anti-vivisection movement which culminated in 1896 with the introduction of a bill in the Congress by Representative McMillan of Michigan that would have regulated vivisection.²⁰ Endorsed by six supreme court justices, leading clergymen, eminent academics and other upstanding citizens, the bill none-the-less withered in the full House under opposition from the National Academy of Sciences, the American Medical Association, and several other medical and scientific bodies. Debate over the bill occurred just two years after development, through animal research, of diphtheria antitoxin, which reduced the mortality rate among those afflicted with the disease from 40% to 10%.²¹ Such a breakthrough result likely had a significant impact on the public's perception of the benefits of animal research. In the years following, voluntary measures on the part of the experimental community to ensure the humane treatment of test animals placated the mainstream foes of animal testing, such as the SPCA.²² Thereafter, though avid pockets of opposition to animal testing

¹⁹ Rowan, OF MICE, MODELS... at 49

²⁰id.at50

²¹ id.

²² id.

remain, they have largely been marginalized as the value of animal testing for medical research has become generally accepted.

What made animal experimentation ultimately so difficult for the public to oppose was that it delivered spectacular medical advances in the second half of the nineteenth century. The first use of ether as an anesthetic in 1846, Lister's revolutionary introduction surgical antisepsis in the 1860's, and breakthroughs in bacteriology by Pasteur and Koch in the 1880's all had their foundations in animal testing.²⁴

But in addition to challenges to animal experimentation from a concerned and sometimes outraged general public, experimentalists experienced staunch opposition from certain pockets within the medical establishment itself. Experimentalists faced professional competition from both anatomists fearing for the primacy of their field and advocates of improved sanitation as the preferred means of enhancing public health.²⁵ The case of Marshall Hall, (1790-1857) a British experimentalist, is illustrative of the former. According to commentators, many of Hall's papers were rejected by the Physiological Committee of the Royal Society because most members of the committee were anatomists who developed their theories of function from the spatial arrangement of organs and were anxious to preserve the supremacy of their own subject on medical education. Experimental medical scientists, feeling that they were riding the wave of the future, garnered further hostility in the medical profession by cultivating an overbearing attitude toward their less up-to-date colleagues.²⁷

²⁴id.at46-7

²⁵id.at45

²⁶ id.

²⁷ This new laboratory doctor (medical experimenter) often disturbed other physicians as much as laymen.

Finally, experimentalists faced opposition from those in the medical community who found animal research distasteful and instead offered to improve public health by providing a cleaner water supply and greater sanitation.

In the end, the experimentalists prevailed. Despite the resentment of many physicians of the experimental methods, and of the opinion of many that they were distasteful, most doctors defended animal research when their profession came under fire from the antivivisection movement. By the turn of the century the medical successes of animal experimentation were so numerous and significant that, though the antivivisection movement has yet to go away, it ceased to be a viable threat.

Drug Development and Pharmacology

The emergence of animal experimentation with regard to drug testing and pharmacology parallels that in the medical field outlined above. The medicine / poison duality has its roots in Greek mythology, and it is widely held that the fields of pharmacology and toxicology developed jointly. The use of toxic substances as medicines therefore predates the lives of two French pioneers of experimental pharmacology, Francois Magendie (1783-1855) and Claude Bernard (1813-1878). Both employed animal tests to investigate the physiological effects of poisonous substances.³⁰ Using dogs, Magendie's research on Java arrow poisons demonstrated that the site of action was the spinal cord. Magendie's student

Occasionally uneasiness burst into open hostility. Most doctors understood clinical investigation and comparative anatomy but, if trained before the 1880's, knew little of the new scientific medicine and its experimental methods. Not surprisingly, they often resented it, not least because of the contemptuously superior bearing that medical scientists too often seemed to adopt toward their less up to date colleagues. Tuner, quoted by Rowan at 45. ²⁸id.at47
29 id.

30 Anne-Marie Coles *Protecting the Consumer: the development of animal tests for evaluating toxic hazards* 155

IMPACT' OF SCIENCE ON SOCIETY 241 (1989) at 242

Bernard continued his physiological tests on animals despite his wife's activism in the French vivisection movement.³¹

Commentators of the age credited Magendie with bringing pharmacology out of the realm of metaphysics and into the world of experimental science. As a

Professor Fraser pronounced in an address delivered at the International Medical

Congress in London in 1881:

The introduction of this method is due to Bichat, and, by its subsequent application by Magendie, pharmacology was originated as the science we now recognize. Bichat represents a transition state, in which metaphysical conceptions were mingled with the results of experience. Magendie more clearly recognized the danger of adopting theories, in the existing imperfections of knowledge; and devoted himself to the supplementing of these imperfections by experiments on living animals. Magendie's approach must have taken hold, as an 1883 text espousing the

virtues of animal testing, ostensibly in response to the antivivisection movement, reveals that by that time the testing of drugs on animals prior to human application was common.³² Indeed, the text claims a number of impressive achievements for animal experimentation to that date:

The use of atropin to check the flow of saliva was thus learnt, and great relief can now be given to a most distressing feature in some cases of paralysis and fracture of the skull. The manner in which belladonna acts as a poison has been shown by experiments; and so also has it been proved

³¹ id.

³² Quoted in Stephen Paget *EXPERIMENTS ON ANIMALS* New York: William Wood & Co. (1900) at 199

- Another way in which experiments upon living creatures have contributed to improvements in treatment, is by the testing of various drugs, whose effects are tried upon the lower animals, in order to judge of their probable value or uselessness to man. Philanthropos *PHYSIOLOGICAL CRUELTY: FACTS AND FANCY* New York: John Wiley and

that a substance extracted from calabar bean is its antidote. The stimulating effects of strychnia upon the spinal chord were made out by Magandie's experiments; by the same means it was tested for practice, and established as a valuable nerve tonic; and by the same means also, Professor Haughton introduced nicotin as its antidote...~

A final use of animal experimentation that bears mentioning here is in the development of an antidote for snake venom. In 1897 alone, 20,959 royal subjects were killed in India as a result of snake bite. Sewall showed in 1887 that animals could be rendered immune, by repeated inoculation with minute quantities of rattlesnake-venom, to a dose seven times as large as would kill an unprotected animal.³⁵ Animal experiments led to the production of a serum by 1896 that saved all snake bite victims to whom it was administered in time.³⁶

While pharmaceutical advances resulting from animal testing saved many patients, the shift from human to animal testing also saved many experimenters. An 1899 article in the *British Medical Journal* catalogued the victims of such self-experimentation:

Dr. Angelo Knorr, *Privat-docent* in the Veterinary School of Munich, died on February 22nd from acute glanders, contracted in the course of an experimental research on mallein. Helmann, the Russian investigator who discovered mallein, himself fell a victim to accidental inoculation of the glanders virus. Some time afterwards, another Russian, Protopopow, died of glanders contracted in a French laboratory. An Austrian physician,

Sons (1883) at 79. As revealed by the title and purported author, this is quite clearly a propaganda piece. None-theless it is indicative of the trends in animal testing at the time.

³⁴idat8O

³⁵Paget EXPERIMENTS ON ANIMALS at 207

³⁶id at 211

Dr. Koffman-Wellendorf, died of the same disease, contracted in the Institute of Hygiene at Vienna. On January 17th of the present year Dr. Guiseeppe Bosso, of the University of Turin, died of infection contracted in the course of cultivations of tubercle-bacilli made in his laboratory. Not long before, Dr. Lola, assistant to the maternity department at the Czech University Hospital of Prague, died of tetanus caused by an experimental inoculation made on himself. Some fourteen or fifteen years ago, a medical student of Lima proved that 'Verruga Peruana' is an infectious disease by inoculating himself with it, an act of scientific devotion which cost him his life. Besides those who have died, there are many who have only escaped with their lives after long and painful illness. Professor Kouloff contracted anthrax in a laboratory at Munich, and was saved only by vigorous surgery. Dr. Nicolas supplied, in his own person, the first example of tetanus produced in man by inoculation of the pure toxin of the bacillus of Nicolaier.³⁷

Thus, its spectacular advances, medical experimentation in the nineteenth century was a dangerous business. From there we now turn to the what has been described as [tiruly, a grim business,~ the sordid world of poisons and poisoners, the need and desire for whose detection was the third foundational element in the development of animal toxicity testing.

The Detection of Poisons and Poisoning

The previous two sections, the antivivisection movement notwithstanding, conjure up an image of men in white coats working diligently in laboratories for

³⁷BRITISH MEDICAL JOURNAL March 18.1899. Quoted in Paget EXPERIMENTS ON ANIMALS at 204-5

the betterment of humanity. In the path to the current state of animal toxicity testing there is, however, a darker side. A significant parallel motivation for the development of toxicology was the detection of poisons and poisonings. Between the fourteenth and nineteenth centuries, the ability of certain individuals to concoct and surreptitiously administer poisons far outstripped the ability of would-be victims to detect them and to differentiate between deaths caused by poisoning and those brought about by natural causes. This imbalance allowed the depraved and the mercenary poisoners to strike fear in the hearts of important figures and common folk alike, as royalty and entire families could be wiped out without leaving behind identifiable marks of foul play. Death by poisoning was indeed the primary means of assassination, as Blyth pointed out in his late-nineteenth century treatise:

The numerous attempts of the Italian and Venetian poisoners on the lives of monarchs and eminent persons, cast for a long time a cloud over regal domestic peace. Bullets and daggers were not feared, but in their place the dish of meat, the savoury pastry, and the red wine were regarded as possible carriers of death.~

While contract and self-motivated poisonings were undertaken throughout Europe, the history of the Venetian poisoners is especially poignant because it represents not merely the depravity of individuals, but of a government formally sanctioning, through routine deliberations, the removal by poison of any number of public figures whom it found disagreeable.⁴⁰ Blyth's retelling is worthy of direct, if lengthy, quotation:

38 A.W. Blyth POISONS... at 9

³⁹idat 12

~ id at 9

41 idat 10

the dark communings of *the council of ten* were recorded in writing, and the number of those who voted for and who voted against the proposed crime, the reason for the assassination, and the sum to be paid, still exist in shameless black and white...One example here will suffice. On the 15th of December, 1513, a Franciscan brother, John of Ragubo, offered a selection of poisons, and declared himself ready to remove any objectionable person out of the way. For the first successful case he required a pension of 1500 ducats yearly, which was to be increased on the execution of future services. The presidents, Girolando Duoda and Pietro Guiarini, placed the matter before the ten on the 4th of January, 1514, and on a division (10 against 5) it was resolved to accept so patriotic an offer, and to experiment first on Emperor Maximillian. The bond laid before the ten contained a regular tariff - for the great Sultan, 500 ducats, for the King of Spain 150 ducats, but the journey and other expenses were in each case to be defrayed; the Duke of Milan was rated at 60, the Marquis of Mantua at 50, the pope could be removed at 100 ducats...The council appear to have quietly arranged thus to take away the lives of many public men, but their efforts were only in a few cases successful. When the deed was done, it was registered by a single marginal note, *fact urn*

This was indeed no laughing matter. The iniquitous Toffana, the Italian record holder, is believed to have poisoned upwards of 600 people, including two popes - Pius III. and Clement XIV. She was brought to justice in 1709 but escaped punishment by taking refuge in a convent, which conferred immunity, and where she continued to sell poisonous concoctions to visitors for the next

twenty years.⁴² Finally, it bears mention that the threat of poison caused such dread during the reign of Henry VIII that the infant Prince of Wales was kept in as close to a bubble as was technologically feasible at the time.⁴³

The foregoing should provide ample motivation for the development of a field of toxicology quite apart from medical and pharmacological research. By the time of Blyth's writing, learning in the discrimination of symptoms, distinguishing between those caused by disease and those by poisons, as well as the development of post-mortem pathology had rendered poisoning among the forms of death least feared by eminent political figures, as poisoners could no longer kill with virtual impunity.⁴⁴

Among the first applications of animal toxicity testing was the feeding to animals of the extant remains of the food last eaten by victims of suspected poisoning. If the animals also died, the existence of foul play was strongly implicated.⁴⁵ Forensic medicine became intertwined with toxicology, as feeding to animals food suspected to have been tainted was seen as a means to determine the presence of foul play. The results of such *expost* experiments constituted proof presented at trial, and those practicing forensic medicine spent a good deal of their time testifying as expert witnesses.⁴⁶ A treatise on forensic medicine from 1832 states that, while such animal experiments had by then fallen into some disrepute, it would be incorrect to dismiss outright their probative value.⁴⁷ The technique is illustrated by the following vignette:

⁴²idat 11

⁴³idat 12

⁴⁴idat 13

⁴⁵idat 14

⁴⁶ See, e.g. William F. Boos THE POISON TRAIL Boston: Hale, Cushman & Flint [1939]

⁴⁷ Robert Christison A TREATISE ON POISONS Edinburgh: Adam Black, North Bridge (1832) at 62

[I]n the case of Mary Bateman, an infamous fortune-teller and charm-worker, who after cheating a poor family for a series of years, at last tried to avoid detection by poisoning them, it was justly accounted good evidence, that a portion of the pudding and the honey, supposed to have been poisoned, caused violent vomiting in a cat, killed three fowls, and proved fatal to a dog in four days, under symptoms of irritation of the stomach such as were observed in the people who died.⁴⁸

While the selection criteria for the sacrificial menagerie are unclear, the result speaks for itself.

These *expost* animal tests shed light on the demise of several unfortunate individuals, but it was J.M.B. Orfila, a Spanish scientist working in Paris, who is credited with undertaking the first systematic study of poisons using animals as test subjects. Orfila was trained in both medicine and chemistry, leading to an interest in the action of poisons on the body.⁴⁹ Through numerous experiments on dogs, he established for the first time the differential absorption and distribution of poisons within the body.⁵⁰ His magnum opus, *Traité de Toxicologie* was first published in 1814. Through several revisions, it ushered in the era of modern toxicology.⁵¹

Learning through the use of animals in medical experimentation, drug development, and the detection of poisons set the stage for the emergence of systematic animal toxicity testing towards the dawn of the twentieth century. The conception of poisons was expanded to that of substances that kill with a single

⁴⁸id. at 66

⁴⁹Coles PROTECTING THE CONSUMER... at 242

⁵⁰Blyth POISONS..., at 16

dose, and the desire for the detection of more subtle effects motivated the development of more sophisticated methods of testing.

The Shift in Focus from Acute to Chronic Toxicity

The approach of the twentieth century brought with it a shift in emphasis with regard to the nature of poison and poisoning. Up to and including the eighteenth hundreds, a poison was conceived as a substance that caused a lethal or deleterious effect by ingestion of a single dose. Toward the turn of the century, however, this notion was augmented with the more diffuse conception that health can be impaired as a result of exposure to small chemical doses over an extended period of time.⁵² The advent of concern over chronic toxicity paralleled improvements in biochemical analysis and greater interest in public health in the early years of the twentieth century. The result was a major conceptual shift in toxicology, from that of *expost* categorization of substances as toxic if they were known to produce a lethal effect, to the use of experimental techniques to *predict* harmful effects.¹⁷

In addition to advances in laboratory technique, four factors contributed to the shift in focus from acute to chronic toxicity: the development of synthetic chemistry which introduced new materials of unknown toxicity into commerce; concern about the population's exposure to small quantities of additives not naturally occurring in food; a general improvement in public health; and the statutory requirement of the Food and Drugs Act of 1906 that food must be considered adulterated if it is injurious to health.

⁵¹id.

⁵²Coles PROTECTING THE CONSUMER., at 242

The development of the chemical industry resulted in the synthesis of many novel organic chemicals that did not occur in nature, or had not previously resulted in human exposure even if they were naturally occurring.⁵⁴ The entry into commerce of these substances of unknown toxicity created the need for predictive testing of their potentially hazardous effects.⁵⁶ Newly created compounds presented categorically different toxicological issues than naturally occurring ones, as over the course of evolution it is expected that humans would have either adapted to natural toxins or learned to avoid ingesting them. With synthetic substances, however, there was no time for adaptation, so greater attention had to be paid to learning, and learning on animals was generally a more acceptable way to proceed than learning by exposing people to new products.⁵⁶ Such an evolution is evident in the 1884 and 1920 editions of Blyth's treatise on poisons; the latter mentions the need for premarket testing as a result of the development of synthetics, while the former does not.

The addition of colorings and preservatives to foods created a situation similar to the introduction of synthetic chemicals in that it resulted in popular ingestion, in small amounts, of substances not historically part of the human diet. Coal tar dyes were frequently added to food as colorings in this period. Theodore XVeyl, a German scientist, began experimenting in 1893 on the safety of these dyes. Convinced that animals could be used to investigate the effects of chemicals on the body, XVeyl fed coal tar dyes to dogs.⁵⁷ And in what may have

~ id. at 244

~ id. at 242

⁵⁵A. W. Blyth and M. W. Blyth *POISONS: THEIR EFFECT'S AND DETECTION* London: Charles Griffin & Co. (1920) at 43

⁵⁶ Failure to follow this prescription with regard to the use of diethyl glycol as the solvent in elixir sulfanilomide was responsible for over 100 deaths among the U.S. population, and in large part for the premarket testing requirement of the 1938 Federal Food, Drug, and Cosmetic Act.

⁵⁷Coles *PROTECT'ING THE CONSUMER...*, at 242

been the first use of animals in carcinogenicity testing, coal tar dyes were found to induce tumors in rats in a 1916 study.⁵⁶ In a study published in 1899, British researcher H.E. Annet used four-week-old kittens to investigate the toxicity of the preservative boric acid. Dividing kittens of the same weight into groups of five, Annet dosed them for four weeks with small amounts of the preservative. From his findings, published in *The Lancet*, he drew the conclusion that boric acid did have an effect at small doses and could be implicated in the high infant mortality rates found in the cities.⁵⁹

While such studies may have been compelling viewed in isolation, the generally poor state of public health and the rapid spread of disease due to poor sanitation and overcrowded living conditions made it impossible to isolate specific effects on health caused by ingestion of small quantities of toxics. A similar kitten study of boric acid undertaken in response to Annet's work by a Dr. Fullerton at the Public Health Laboratory drew opposite conclusions, finding boric acid to be safe.⁵⁰

Such contradictory results highlight the fact that in the absence of improvements in public health, low-grade chronic effects of food additives were difficult to verify in the general public and were of secondary concern. As a testament to the abominable state of public health and sanitation in major U.S. cities in the first half of the nineteenth century, Lemuel Shattuck documented that life expectancy at birth actually *declined* between the second and fifth decades of the century, falling from 27.85 to 21.43 years in Boston and from 26.15 to 19.69

⁵⁸ Rowan ,OF MICE, MODELS... at 231

⁵⁹ Coles PROTECTING THE CONSUMER... at 243

years in New York.⁶¹ While Shattuck included food adulteration as a cause for poor public health, the lack of pure water and common sanitation were recognized as more pressing problems.⁶² On the food front, the chief concern was microbial contamination. Under such circumstances, focused concern over chronic low-level exposures to chemicals in food misplaced.

From this perspective, then, the shift in focus to chronic low-level exposures was a reflection of the public health gains achieved in the U.S. following the turn of the century. Concern over long-term effects is only worthwhile if the target population can be expected to live long enough to experience them. The regulatory fixation on cancer in the post-WWII era follows this pattern, as it was preceded by a significant increase in life expectancy, and cancer is generally a disease of old age.

An additional factor behind the shift in focus from acute to chronic toxicity at the turn of the century was the statutory mandate of the U.S.D.A.'s Bureau of Chemistry under the 1906 Food and Drugs Act. The Act provided that food must be considered adulterated if it contain any added poisonous or other added deleterious ingredient which may render such article injurious to health.⁶³ According to Whorton,

the question of which particular ingredients should be designated poisonous or deleterious proved a Pandora's box once opened. At the head of the host of perplexities that emerged was the difficulty...of detecting chronic injury and relating it to the ingestion of a specific adulterant. The large majority of food adulterants with which the Bureau would have to

61 Peter Barton Hutt and Peter Barton Hutt II *A History of Government Regulation of Adulteration and Misbranding of Food* 39 FOOD, DRUG AND COSMETIC LAW JOURNAL 2(1984) at 39.

62 id. at 39. n321

deal, [director Harvey] Wiley realized, were substances present in too small amounts to produce acute illness; when deleterious, they were chronic poisons⁶⁴

Many of the issues in the developing field of chronic toxicity testing were played out through the scientific concern and politicized response to widespread exposure through the food supply to preservatives and pesticides.

Preservatives and Pesticides

The second half of the nineteenth century witnessed the development of national markets in the food industry, prompting food manufacturers to perceive a need to add chemical preservatives to their products to protect them from spoilage during shipment to distant points. The leading candidates were benzoic, boric, salicylic and sulfurous acids, substances that would be present in food in very small quantities.⁶⁵ Responding to this emerging issue, Congress in 1900 appropriated funds to investigate the character of proposed food preservatives and coloring matters; to determine their relation to digestion and health; and to establish the principles which should guide their use.⁶⁶ The responsibility for this undertaking fell on the USDA Division of Chemistry, headed by Dr. Harvey W. Wiley.⁶⁷ Although food manufacturers were quick to assure that preservatives would be present in food in amounts too small to be of health concern, Wiley was viscerally skeptical. He abhorred deceit, and was deeply disturbed by the presence of synthetic chemical preservatives in foods that, unlike the salt, vinegar, and

63 34 Stat. 758 et. seq. (1906)

~ James Whorton BEFORE *SILENT SPRING* - PESTICIDES AND PUBLIC HEALTH IN PRE-DDT AMERICA Princeton:

Princeton University Press (1974) at 102-3

65 id. at 103

spices traditionally used as preservatives, could not be detected by taste or odor. Thus, Wiley perceived that the defenseless consumer of modern preserved foods was in danger of being fed synthetic chemicals without his knowledge.⁶⁸

As a scientist, Wiley recognized the need for experimental data to substantiate his innate disdain for synthetic food additives, but believed that data obtained from lower animals might not be recognized as probative for human beings. He therefore convened his fabled Poison Squad upon which to perform his investigations. The Poison Squad was a group of young, originally healthy volunteers from the civil service, who, in the fall of 1902, began to answer Wiley's call for test subjects to undergo a feeding program to determine the effects of various preservatives on digestion and health.⁶⁹ In all, 12 USDA employees acted as human subjects between 1902 and 1904 in testing the safety of boric acid and borax, salicylic acid and salicylates, sulfurous acid and sulfites, benzoic acid and benzoates, and formaldehyde.⁷⁰ Although serious injury to the volunteers was carefully guarded against, the potential dangers could not be hidden from the press and the public. Soon the diners at Wiley's dosing table were revered nationally as the heroic Poison Squad and were even endowed with an anthem:

*O we're the merriest herd of hulks that ever the world has seen;
We don't shy off from your rough on rats or even from Paris green:
We're on the hunt for a toxic dope that's certain to kiss, sans fail,
But 'tis a tricky, elusive thing and knows we are on its trail'
For all the things that could kill we've downed in many a gruesome wad,*

~ 31 Stat. 191, 196(1900), Qtd. in Hutt and Hutt 67 Hutt and Hutt
A History... at 51

~ Whorton BEFORE *SILENT SPRING* at 103

69 id. at 104

70 Hutt and Hutt *A History...* at 51

*And still we're gaining a pound a day, for we are the Pizen Squad.*⁷¹ The study is described in detail elsewhere.⁷²

Upon careful physical examination of each volunteer as well painstaking physical and chemical analysis of each man's excretions, Wiley concluded that each preservative caused changes of an injurious nature. The Bureau of Chemistry's report on the studies denounced the chemicals and called for their exclusion from food products.⁷³

Wiley recognized the drawbacks of using human volunteers instead of animals for his study. The Squad members could not be confined in a controlled environment like animals could be, although their behavior was regulated to the extent possible (they took all their meals in Wiley's laboratory). The volunteers were also subject to psychosomatic disturbances, given the nature of the undertaking involved in being a member of the Poison Squad. Animals would have been free from such effects. Upon the conclusion of the study, the volunteers retained their internal organs, unlike test animals who would have been sacrificed for necropsy.⁷⁴ Wiley claimed that he none-the-less preferred to experiment on people rather than animals because of the immediate applicability of his results to the general population, as opposed to the more assailable task of extrapolating between species.⁷⁵

However, an alternate and parallel explanation also avails itself. Since Wiley set out to prove the harmfulness of these preservatives, he was looking for any experimental effect that would further buttress his convictions. The most

⁷¹ Written by S. W. Gillian and quoted in Wiley, *An Autobiography* at 217, as well as in Whorton at 104

⁷² See Hutt and Hutt *A History...* at 51; Whorton *BEFORE SILENT SPRING* at 104

⁷³ U.S. Department of Agriculture *Influence of Food Preservatives and Colors on Digestion - IV. Boric Acid and*

Digestion Bureau of Chemistry Bulletin No. 84, Part IV. (1908) at 1293-4

⁷⁴ Whorton *BEFORE SILENT SPRING* at 105

prevalent symptom among his test subjects was a general feeling of malaise,⁷⁶ which is not surprising given the dosing regimen involving multiple grams of the preservatives taken in concentrated amounts. Wiley also identified a decrease in weight in his test subjects and concluded that the administration of these drugs in food tends to derange the normal activities of the body and to cause a loss of tissue.⁷⁷ However, there is no indication that this admittedly slight weight loss was statistically significant, and even if it was, it is a natural outgrowth of the general feeling of malaise induced by the preservatives.

The point of the foregoing discussion is to illustrate that Wiley's choice of human over animal test subjects may well have been motivated by his having prejudged the toxicity of the preservatives tested. The diffuse health effects reported were easily detectable upon humans told that they were being fed poisons, but would have been much more difficult to demonstrate on animal subjects. There is no indication that Wiley considered the prior animal studies of boric acid conducted in England.

Threatened by Wiley's conclusions, the food manufacturers initiated political maneuvering that resulted in the commissioning by President Roosevelt of an alternative, Wiley-free panel to investigate the safety of preservatives. The Referee Board of Consulting Scientific Experts convened a second poison squad, this time comprised of medical students, and after a feeding study that eliminated some of the psychosomatic bias of the Wiley study concluded in 1909 that sodium benzoate was safe in its proposed use.⁷⁸

~ id.

⁷⁶ U.S. Department of Agriculture *Influence of Food Preservatives...*, at 1285

~ id at 287

⁷⁸Whorton BEFORE *SILENT SPRING* at 106-7

These investigations were imbued with direct regulatory relevance by the adulteration provisions of the of the 1906 Food and Drugs Act.⁷⁹ Although distrustful of even the smallest amount of a poisonous substance, Wiley acceded to toxicological opinion which recognized the existence of a threshold amount, below which a substance was not believed to be harmful. Accordingly, the enforcement procedure of the Division of Chemistry included a tolerance policy under which potentially harmful additives were permitted in foods up to certain quantitative limits.~ For the new preservatives, the tolerance levels were set based on the results of the two poison squad studies, with ample room for disagreement over the amount that constituted a minimum toxic dose.⁸¹

Like chemical preservatives at the turn of the century, pesticide residues on fruit sparked considerable concern and controversy in the 1920's and '30s. As in the former case, politics played a key role in how the toxicity of substances in question was eventually determined. But unlike the poison squads of the 1900's, pesticides were tested on animals, at least until Congressional fiat put an end to the studies.

The U.S. Food and Drug Administration established a new section, the Division of Pharmacology, in 1935. The intended function of the Division was to develop the institutional capacity to determine the safety of chemicals found in food and in the workplace.⁸² The first task of the new Division was to address the concern that was developing over lead and arsenic compounds that were being used in agriculture as pesticides and making their way into the food supply as residues. Skeptical of the claims of potential harm stemming from exposures to

~ See text accompanying note which one? above.

⁸⁰Whorton BEFORE *SILENT SPRING* at 110

⁸¹ id.

⁸²Coles PROTECTING THE CONSUMER... at 244

~ Whorton BEFORE *SILENT SPRING* at 223

~ Memorandum attached to letter from H.A. Wallace to F. Lillie, November 10, 1936. Quoted in Whorton at 224

⁸⁵Coles PROTECTING THE CONSUMER... at 244

small amounts of pesticides, farmers had since the late 1920's been calling for experimental verification of claimed toxicity. FDA personnel shared the desire for comprehensive experimental data on lead and arsenic toxicity, but for the opposite reason. FDA believed that the residues were harmful, and experimental proof would justify enforcement of strict tolerances on treated foods.⁸³

The first task of the Division was thus the determination of the chronic toxicities of arsenic and lead spray residues. The Division planned a rigorous, pioneering set of experiments:

Groups of animals, chiefly white rats, are to be subjected to varying doses of lead, arsenic, and lead arsenate, respectively, in amounts beginning at the levels of the present tolerances and building up to amounts clearly and relatively promptly toxic. The plan is to examine the animals in this group from every possible angle to get leads as to the methods of recognizing toxic effects, and then with these leads to study chronic poisoning in a second group. A second group of animals will be given relatively small doses over longer periods of time to determine the effects of chronic and repeated injury.⁸⁴

As the study got under way, food consumption, growth, activity, appearance, longevity, storage, consumption and excreta were among the attributes examined. From these metrics it was soon obvious that some evidence of toxicity was emerging.⁸⁵ By 1938, several tests had registered that those dosages

of arsenic and lead comparable to those consumed as spray residue had produced physiological damage in rats and dogs.⁸⁶

Threatened by these developments, fruit growers and their representatives took up the position that the results from animal studies would not be applicable to humans actually using and consuming the pesticides.⁸⁷ Strong opposition from California fruit growers eventually resulted in sufficient political pressure to quash the animal toxicity tests, through the work of Clarence Cannon, an apple grower-turned-U.S. Representative who served on the House Appropriations Committee. When FDA personnel perused the U.S.D.A. appropriation bill for the fiscal year beginning July 1, 1937, they were greeted with a rider requiring that "no part of the funds appropriated by this Act shall be used for laboratory investigations to determine the possibly harmful effects on human beings of spray insecticides on fruits and vegetables."⁸⁵ The experiments, and all of the animals involved in the study, were terminated.⁸⁹

None-the-less, the studies established that controlled experiments on animals could be useful in examining the toxic effects of chemicals, and in the Division of Toxicology the FDA now possessed the institutional capacity for the development and refinement of relevant animal toxicity tests. The stage was set for the development of systematic toxicity testing.

⁸⁶Whorton *BEFORE SILENT SPRING* at 225

⁸⁷Coles *PROTECTING THE CONSUMER...*, at 244

⁸⁵ *52 U.S. Statutes at Large 135* (1938); qtd. in Whorton at 230. Cannon's motives may not have been entirely nefarious; as chairman of the subcommittee in charge of agricultural appropriations. He balanced the deduction from FDA's budget represented by the liquidated toxicity studies with an addition to that of the Public Health Service for studies of the effects of the pesticides on humans. See Whorton Chapter 7.

⁸⁹Coles *PROTECTING THE CONSUMER...* at 244

The Movement Toward Standardization

Political maneuvers notwithstanding, the FDA now had in the Division of Pharmacology an applied research unit dedicated to the specific problem of safety assessment and with its own technical and experimental facilities.⁹¹ Some large U.S. firms had opened their own laboratories for testing chemicals, and turned to the Division for technical advice. The initial trickle of such requests turned to a deluge with the dramatic expansion of the chemical industry up to and following the Second World War.~ The Division was the central institution in the development of guidelines for toxicity testing. During World War II, it was entrusted with three main responsibilities: the determination of the potency of drugs; the pharmacological examination of possible deleterious components of food, drugs, and cosmetics; and the general improvement and development of pharmacological techniques for undertaking toxicity investigations⁹²

The Elixir Sulfanilamide disaster helped impress upon the public the need for toxicity testing, driving home the point that the alternative to animal testing was de facto testing on humans. In response, the 1938 version of the Federal Food, Drug, and Cosmetic Act required that drugs be tested for safety before being introduced into the stream of commerce.⁹⁴

During this time, the LD₅₀ test became accepted as the standard metric of toxicity. The test was developed during the 1920's by J. W. Trevan, a British biologist, for the purpose of precisely standardizing such important but potent drugs as digitalis extracts, insulin, and diphtheria antitoxin. The potency of

91 *id.* at 246. One can only assume that the funding freeze was limited to fiscal year 1938.

92 *id.* at 244

⁹⁴Whorton BEFORE *SILENT SPRING* at 238-9

these drugs varied from one batch to another, and it was vital to assess the potency of each batch for appropriate dosimetry.⁹⁵ Before development of the LD₅₀ test, the toxicity of a compound was usually expressed as the lowest dose that had been observed to kill an animal. In 1927, Trevan injected several thousand frogs with digitalis extract and plotted the mortality percentage as a function of dose level. From this curve, he determined the LD₅₀ dose as that which killed 50% of the animals – hence the abbreviation for lethal dose - 50%.⁹⁶ This method became universally adopted in the risk assessment policy of many countries as the standard index of toxicity.⁹⁷

Widespread application of the Draize test followed a similar pattern. The test was developed in 1944 by FDA on request from British scientists for the testing of eye irritants considered for use in chemical warfare.⁹⁸ The test was then adopted by the FDA as a more general test for eye irritancy under a requirement of the 1938 Food, Drugs, and Cosmetics Act that cosmetics be free of poisonous substances.⁹⁹ The test has since been used for premarket screening of cosmetics.

The explosion in the development and marketing of synthetic chemicals during World War II made clear the need for a standardized, systematic process of toxicity testing. By 1943 the presumed solution was for public health officials to evaluate all available information on each new chemical, determine the exposure amounts that could adversely affect public health, and establish tolerances and regulations where necessary.¹⁰⁰

~ Rowan *OF MICE, MODELS...*, at 203

~ *id.* at 206

~ Coles *PROTECTING THE CONSUMER...*, at 243

98 Rowan *OF MICE, MODELS...*, at 217

~ *id.* This requirement followed incidents involving harmful cosmetics. For example, in 1933 a woman was blinded by mascara.

Coles *PROTECTING THE CONSUMER...*, at 245

Between 1942 and 1944 the Division of Pharmacology spent between 70 and 90% of its resources on war-related problems. The large number of requests from industry for regulatory clearance for the use of new chemicals resulted in the establishment of general guidelines for obtaining information about the toxicity of chemicals.¹⁰¹ A 1944 FDA report recommended an extensive battery of tests, which relied heavily on animal toxicity studies.¹⁰² FDA scientists justified such a detailed testing protocol by the inevitability of exposure to novel synthetic chemicals, which called for a considered evaluation of safety.¹⁰³

Following on these recommendations, the FDA in 1949 published the first comprehensive guide to testing chemicals on animals. The article, *Procedures for the Appraisal of the Toxicity of Chemicals in Foods*, appeared in the September issue of the *FOOD, DRUG, AND COSMETIC LAW QUARTERLY*¹⁰⁴ and embodied novel concepts developed by the Division of Pharmacology since the untimely termination of its first safety assessment involving pesticide residues. The procedures included tests for acute, sub-acute, and chronic poisoning, and were accepted by government, industry and academic scientists who adopted them in developing their own test programs.¹⁰⁵ With this initial establishment of a set of

101 id.

102 Among the tests the Division suggested as being necessary were:

A. Pharmacodynamics, which include measurements of blood pressure, respiration, and heart rate. The results of these studies could be used to plan further tests;

B. Acute toxicity; the determination of this index involved compiling a dose response curve, using a minimum of three species for comparison of symptoms;

C. Subacute toxicity; daily doses to be given to one or more species for 6 to 12 weeks to be used as a guide in the design of chronic experiments;

D. Chronic tests, three or more species to be used, one for the duration of its lifetime (rats suggested);

E. External effects, sensitization, skin irritation, etc.;

F. Special studies such as reproduction, haematology, absorption, excretion, distribution and storage through which one may often find that, due to the preventative measures that can be taken to avoid some of the results of the toxic actions of a particular substance. Qtd. in Coles *PROTECTING THE CONSUMER...*, at 245.

103 id.

104 pp.⁴¹²⁻⁴³⁴

¹⁰⁵Coles *PROTECTING THE CONSUMER...* at 246

standardized procedures, animal toxicity testing became firmly entrenched in both regulatory requirements and industry practice.