The Quantity That Was Chosen

A History of Toxicology and the Measure It Left Out

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Preface

This book follows one question through the whole history of a science.

The question is simple. When a substance harms a living body, what exactly should be measured?

Toxicology answered that question once, in the sixteenth century, and has not revisited it since. The answer was: the amount. Every instrument the field has built since then measures an amount, or estimates one, or sets a limit on one. The apparatus has become extraordinarily sophisticated. The quantity has not changed.

This is not a complaint about carelessness. The people in this book were careful. Many of them were brilliant. The argument of this book is narrower and, I think, harder to dismiss: a science that measures one quantity can only see what that quantity registers. Everything else becomes invisible, and the invisibility is systematic rather than accidental.

The book proceeds in six parts. The first traces how the amount was chosen and why the choice looked obvious. The second recovers two episodes, in 1924 and in 1948, when a different quantity was written down and then reduced to a correction factor. The third follows the building of the regulatory apparatus around the amount. The fourth shows that the great modernisation of the last twenty years changed the vessel and kept the quantity. The fifth examines a class of substance for which the amount is the wrong measure, and shows what happened when the instruments were pointed at it. The sixth states what the missing measure is, and what changes if it is adopted.

Each chapter asks the same three questions of its period. What was measured. What became visible because of that. What became invisible because of that. The third column is the argument. It accumulates.

The reader needs no prior knowledge of toxicology. Every technical term is explained where it first appears. The annotated references at the end are arranged so that any chapter can be pursued further by someone who wishes to check the account or go deeper.

PART I — THE CHOICE

Chapter 1. One Question, Three and a Half Thousand Years

A man drinks from a cup and dies. Something in the cup did it. That is where this story begins, and in an important sense it is also where it still is.

The oldest surviving document that catalogues poisons is Egyptian. The Ebers papyrus, dated to around 1500 BC, runs to a hundred and ten pages on anatomy, physiology, toxicology, spells and treatment. It contains hemlock, aconite — an arrow poison from the buttercup family — opium, lead, copper and antimony. It mentions more than seven hundred substances. It is a list of what is dangerous.

Fifteen hundred years later, a Greek physician at the court of the emperor Nero wrote the first attempt to order plants by their poisonous and therapeutic effect. His name was Pedanius Dioscorides and the book was De Materia Medica. His classes of poison were still in use in the nineteenth century. That is a remarkable span. It is also a sign of how slowly this particular science moved while medicine around it changed repeatedly.

Between Galen, who died around the year 200, and Paracelsus, who was born in 1493, there were few advances in either medicine or toxicology worth recording. For thirteen centuries the field consisted of knowing which things were dangerous and in some cases what to do about it.

Then one man asked a different question, and the field has been answering it ever since.

The question Paracelsus asked was not *which substances are poisons*. It was *how much*. That shift is the subject of chapter 3, and it is the hinge of this entire book. Everything before it is a catalogue. Everything after it is a measurement.

What this book sets out to show is that the measurement, once chosen, closed a door. Not deliberately, and not visibly. A measuring instrument does not announce what it cannot detect. It simply returns a value, and the value is believed, and the thing the instrument cannot register stops being discussed because it stops being measurable. After several centuries it stops being thinkable.

Three and a half thousand years after the Ebers papyrus, the European Union sets a legal limit on four per- and polyfluoroalkyl substances in food. The limit is four point four nanograms per kilogram of body weight per week. It is an amount. The reasoning that produced it is sophisticated beyond anything Paracelsus could have imagined, involving benchmark dose modelling, physiologically based kinetic models and an international scientific committee. And it answers his question, not a different one.

This book asks what the other question would have been.

Chapter 2. Before the Dose

It is worth dwelling on what toxicology looked like before it had a variable, because the shape of the earlier practice explains why the later one was such an advance.

The Egyptian and Greek material is a catalogue of identities. This plant kills. That mineral kills. This one kills quickly and that one slowly. The Ebers papyrus gives formulations, which implies that quantity was understood in practice — a formulation is a recipe and a recipe has proportions. But quantity is not the object of study. Identity is.

The practical knowledge was considerable. The earliest humans used natural toxins for hunting, warfare and assassination, and in parts of South America, Borneo and the Pacific islands that knowledge is still in use. Shen Nung, the legendary father of Chinese medicine, is said to have tasted three hundred and sixty-five herbs. The method there is the body itself, and the record is oral.

What could this practice see? A great deal. It could see which substances were dangerous, which were useful, which were both. It could see that some killed in minutes and others over months. It could see antidotes, or at least believed it could. For a world in which the main question was whether to eat the berry, this was adequate.

What could it not see? It could not compare. Without a measured quantity there is no way to say that this substance is more dangerous than that one, except by counting bodies. There is no way to establish a safe use for something known to be harmful. Above all, there is no way to be wrong in a useful fashion — no way to make a claim precise enough that an experiment could refute it.

That is what the dose gave the field. It converted a catalogue into a science. The cost of that conversion is the subject of this book, but the gain should not be understated. Before the dose, toxicology was lore. After it, toxicology could be checked.

Chapter 3. Paracelsus and the Choosing of the Variable

Theophrastus von Hohenheim, who called himself Paracelsus, lived from 1493 to 1541. He is remembered for a single sentence, usually rendered as: all substances are poisons, there is none which is not a poison, the right dose differentiates a poison from a remedy.

That sentence does three things at once, and it is worth separating them.

First, it abolishes the category of poison. If everything is a poison, then poison is not a property of substances. The old catalogue collapses. There is no longer a list of dangerous things and a list of safe things.

Second, it installs a variable in the gap. What distinguishes harm from benefit is no longer *what* but *how much*. Quantity becomes the carrier of the distinction.

Third, and least often noticed, it asserts that every substance has a right amount. The sentence does not say that a sufficiently small dose of anything is harmless, though it is usually read that way. It says something stronger: that for each substance there exists a dose at which it is a remedy. Paracelsus meant this literally. He used mercury for syphilis for the next three hundred years of medical practice, and he used ether as an analgesic.

Paracelsus also established two further principles that the field kept. He held that experimentation is essential to examining the response, and he determined that specific chemical substances are responsible for the toxicity of a plant or an animal poison. He studied the health of miners and produced what is generally regarded as the first book on the diseases of a specific occupational group.

So the package handed to posterity contains: a variable, a method, and a commitment to chemical specificity. All three were productive. All three are still in force.

What was measured. The amount administered.

What became visible. Comparison between substances. Safe use of dangerous materials. Therapeutic windows. The whole of pharmacology, in time, since a medicine is simply a poison used at the right amount. The experiment, because an experiment requires a variable you can set.

What became invisible. Anything that is not a quantity of substance. Where the substance goes. How long it stays. In what order events happen. Whether the body gets rid of it. Whether the system returns to where it was.

None of these were denied. They were simply not what the instrument returned. And because Paracelsus's formulation was so powerful and so obviously correct within its range, there was no pressure to ask whether its range had an edge.

The edge is where this book eventually arrives. But first the field had to build the instruments, and the instruments were built for a courtroom.

Chapter 4. Orfila and the Courtroom

Mateu Josep Bonaventura Orfila i Rotger was born in Menorca in 1787 and died in Paris in 1853. He is regarded as the father of modern toxicology. He became Dean of the Faculty of Medicine in Paris. His Traité des poisons of 1813 identified and analysed poisonous substances and divided them into six principal classes: the corrosive, the astringent, the acrid, the narcotic, the narcotico-acrid and the stupefying.

Orfila's lasting contribution was not the classification. It was the test.

In 1832 James Marsh, an English chemist, developed a method for detecting arsenic. The method exploits the formation and ready decomposition of arsine gas, and it was sensitive enough to find arsenic in very small quantities. Marsh first presented it publicly in the context of a murder trial.

Orfila took the Marsh test, modified the apparatus to handle larger volumes of liquid, and applied it to human tissue. That step created forensic toxicology. He used it in criminal cases, among them the Lafarge and Mercier trials.

Pause on what that means. The founding instrument of modern toxicology was built to answer the question: did this person poison that person, and can we prove it from the body?

The question has a definite grammar. There is an agent — a substance. There is an act — administration. There is a doer — the person who administered it. There is a victim. And there is a trace, left in the tissue, that connects them. The method succeeds when the trace is found and the chain is closed.

This grammar did not stay in the courtroom. It became the grammar of the field.

Consider how much of modern toxicology still has this shape. A hazard is identified by finding the agent. A dose-response curve quantifies the act. A threshold establishes the amount below which the agent is not culpable. A tumour signature is a trace connecting agent to victim. An adverse outcome pathway, as we shall see in chapter 14, begins with a molecular initiating event — which is to say, with the moment the agent acts. Litigation against a chemical manufacturer asks a court to find a doer. A resident near a factory asks whether the plant caused her cancer, which is a request for exactly the chain Orfila constructed.

What was measured. The presence and amount of a specific agent in tissue.

What became visible. Proof. Legal certainty. The possibility of convicting a poisoner, and later of holding a manufacturer responsible. An immense public good; it should not be sneered at.

What became invisible. Harm without a doer. If the method is built to connect an agent to a victim through a trace, then harm that leaves no trace, or that arises from something an agent failed to do rather than something it did, falls outside the method entirely. It is not judged unproven. It is not formulated as a question.

This is, I will argue, the deepest reason why a certain class of substance has resisted two centuries of investigation. The field has been asking who did it. For some substances, nobody did it.

Chapter 5. Ramazzini and Pott: the Trail That Could Not Be Followed

Two physicians, a generation apart, found something the dose could not explain.

Bernardino Ramazzini lived from 1633 to 1714. He is often described as the father of occupational medicine. His De Morbis Artificum Diatriba, the dissertation on workers' diseases, outlined the health hazards of chemicals, dust, metals, repetitive or violent motions, odd postures and other disease-causing agents encountered by workers. He observed in 1713 that nuns suffered high rates of breast cancer, which he attributed to their celibate life.

Percivall Pott, an English surgeon, lived from 1714 to 1788. In 1775, in his Chirurgical Observations, he described the occurrence of cancer of the scrotum among men whose common history was employment as chimney sweeps when they were boys. He attributed it to a lodgement of soot in the folds of the scrotum. It is the first reported occupational cancer, and the first time a specific disease was causally linked to a specific substance in the environment.

Pott's observation contains something the framework of his time could not hold.

The boys were exposed in childhood. The cancers appeared in adulthood. Between the exposure and the effect lay a gap of years or decades, during which nothing visible happened. The soot was not present when the disease appeared. There was no dose to measure at the moment of harm, because the harm became apparent long after the exposure had ceased.

Pott reasoned correctly anyway, by a route that is worth noticing: he used the shape of the population, not the state of any individual. He had a group of men with a shared history and an unusual disease. That is epidemiology before the word existed, and it worked precisely because it did not require a dose.

Ramazzini's nuns are the same shape. There is no agent at all. There is an occupation, a way of living, and a raised rate.

What was measured. The co-occurrence of an occupation and a disease, in a population, over a lifetime.

What became visible. Delayed harm. The idea that a substance encountered long ago can act now. The beginnings of a method that does not need the agent to be present at the moment of measurement.

What became invisible, or rather what was not taken up. Time as a quantity in its own right. Pott had in his hands a phenomenon whose defining feature was a long interval. He could have asked how long, and whether the interval varied with the amount of soot, and whether the relation between amount and interval was lawful. He did not, and neither did the field for another hundred and seventy years.

When somebody finally did ask that question, the answer turned out to be a law. That is the subject of Part II, and it is the part of this history that has been most thoroughly forgotten.

PART II — THE LAW OF TIME, TWICE WRITTEN

Chapter 6. Haber, 1924

Fritz Haber was a German physical chemist who lived from 1868 to 1934. He is known to history for two things: the synthesis of ammonia from atmospheric nitrogen, which feeds a large part of the world, and the development of chemical weapons for the German army in the First World War. The second of those is the context in which he entered toxicology.

Studying the acute lethality of war gases in the early 1900s, Haber contended that there was a constant relationship between the concentration of the gas and the length of time leading to the death of an animal. The formulation is simple:

$$C \times t = k$$

Concentration in milligrams per cubic metre, multiplied by the time in minutes the animal had to breathe the air before death ensued, equals a constant. The constant depends on the gas and on the effect.

The practical reading is that doubling the concentration halves the time to a given toxic effect. The product is what matters, not either factor alone.

This is the first time in the history of the field that time enters a toxicological law as an equal partner to amount. One commentator put it exactly: the rule states that time and not only concentration is an important factor in toxicological considerations, going beyond Paracelsus, who considers only the dose.

Going beyond Paracelsus. In 1924, briefly, the field had a second variable.

Haber's rule has been used in various settings by regulatory bodies ever since, and it has supporters and critics. It was used, among other things, to set maximum permissible limits for chemicals in spacecraft. It has real limitations, which the critics are right about. Animal experiments show that the rule does not always apply. When the effects of phosgene on pulmonary gas exchange were measured over intervals from five minutes to eight hours, there was no indication that concentration contributed more than exposure time, and the rule held. Under other conditions it did not. The product tends to

increase as concentration falls, which means the rule overestimates the danger of long, low exposures — or, read the other way, that something other than the simple product is going on at low concentrations.

The field's response to this was instructive. It did not conclude that the relationship between concentration and time needed a better theory. It generalised the formula to

$$C^n \times t = k$$

where the exponent n, fitted per chemical and per endpoint, absorbs the discrepancy. Haber's rule becomes the special case where n equals one.

That is a reasonable engineering move and it preserved the usefulness of the rule. It also quietly converted a candidate law of nature into a curve-fitting parameter. The exponent is not interpreted. It is estimated.

And the use to which the rule was put reveals the deeper decision. Haber's rule is used in regulatory practice almost exclusively for *time-scaling*: for converting a toxicity value measured over one exposure duration into a value for a different duration, so that the result can be expressed as a concentration. Time enters the calculation in order to be eliminated from the answer.

What was measured. The product of concentration and exposure time, against a fixed endpoint.

What became visible. That duration matters as much as amount, at least for acute inhalation. A principled way of comparing exposures of different lengths.

What became invisible. The possibility that time was the primary quantity and concentration the secondary one. The exponent n contains that information and nobody reads it. A value of n far from one is telling you something about how the substance and the body interact over time. In practice it is treated as a nuisance parameter.

That reading — the one nobody took up — was supplied twenty-four years later, in German, by two men who worked out what the exponent means.

Chapter 7. Druckrey and Küpfmüller, 1948 and 1949

This chapter is the pivot of the book. The material in it is well documented, has never been refuted, and is almost entirely absent from contemporary regulatory toxicology.

Hermann Druckrey was a German pharmacologist and cancer researcher. Karl Küpfmüller was a communications engineer and control theorist — a man from a different discipline entirely, whose working material was systems, signals and time constants. Between them, in two papers published in 1948 and 1949, they produced a theory of dose and effect that contains time as a structural element rather than as a correction.

The starting observation was Druckrey's own, from 1943. Working with the azo dye known as butter yellow, he found that the carcinogenic action was determined by the total dose and was completely independent of the daily dose. Feed an animal a large amount quickly or a small amount slowly, and when the same total had been delivered, the tumour appeared. The product of daily dose and time was constant.

That is Haber's rule, appearing in chronic carcinogenesis rather than acute gas poisoning, which was remarkable given the long latency involved.

Druckrey and Küpfmüller then did what nobody had done with Haber's rule: they asked what would have to be true of the underlying mechanism for the relation to hold.

Their analysis is built on receptor kinetics with two time constants. The first, which they denoted T_R, is the time constant for the reversibility of receptor binding: how quickly the substance lets go of the target it has bound. The second, T_r, is the time constant for the reversibility of the effect: how quickly the consequence of that binding undoes itself.

From these two constants they derived three classes of dose-response behaviour, depending on whether each constant tends towards zero — fully reversible — or towards infinity — fully irreversible.

Their central inference is the sentence this book has been working towards. If both receptor binding and the effect are irreversible, exposure time reinforces the effect.

The resulting relation is written

$$c \times t^n = constant, \text{ with } n \geq 1$$

and the exponent n, which in Haber's generalisation was a fitted nuisance, now has a meaning. It is an exposure-time reinforcement factor. It measures the degree to which the system fails to undo what has been done to it.

Three consequences follow, and all three are consequential.

The first is that there is no threshold. If the effect accumulates because it does not reverse, then every exposure contributes, and the total dose required to produce the effect is the same whether it arrives quickly or slowly. There is no amount small enough to be safe; there is only an amount small enough to take a long time.

The second is the inverse relation that the regulatory mind finds hardest. The lower the exposure concentration, the longer the period up to the effect — and therefore the *lower* the daily dose at which the effect eventually appears. Low exposure is not safe exposure. It is slow exposure.

The third is that the governing quantity is a time constant of the host-substance pair, not a property of the substance. T_R and T_r are not chemical constants. They describe how long a particular system takes to let go and to recover.

Druckrey pursued this experimentally for two decades. When Peter Magee and John Barnes linked the carcinogenicity of dimethylnitrosamine to the alkylation of nucleic acids in 1956, Druckrey investigated the dose-time relationship of diethylnitrosamine and found the same structure. The definition of cumulative poisons and the first proof of the irreversibility of carcinogenic effects had, by the assessment of later reviewers, an extraordinary impact on cancer research.

What was measured. The time to effect as a function of daily dose, and the two time constants of reversibility.

What became visible. Cumulative poisons as a class. Irreversibility as a mechanism rather than a metaphor. A principled, mechanistic reason why some substances have no threshold. And, implicitly, a

quantity that is neither amount nor duration of exposure but something else: the rate at which a system undoes what has happened to it.

What became invisible. Nothing, at the time. This was an addition, not a subtraction. The invisibility came later, and it came from what the field did with it.

Chapter 8. What Became of the Time Laws

Two laws containing time were written, in 1924 and in 1948. Neither was refuted. Neither is now a working part of mainstream regulatory toxicology. Understanding how that happened is understanding how a paradigm digests an exception.

Haber's rule survived, but in a reduced role. It became a duration adjustment: a device for converting an experimental result obtained at one exposure length into an equivalent at another, so that a concentration limit can be set. Its use for this purpose has itself been criticised, on the grounds that where toxicity depends on concentration rather than duration, a time adjustment derived from short-term data can overestimate risk. The debate is conducted entirely in terms of how best to arrive at a concentration. Time is the thing being adjusted away.

Druckrey and Küpfmüller fared differently. Their framework did not become a tool at all. It became a historical citation.

Part of the reason is linguistic and political — the papers are in German, published in 1948 and 1949 by a researcher whose wartime career invited no enthusiasm. Part is institutional: the regulatory apparatus that was built in the following decades, which Part III describes, needed numbers that could be divided by a safety factor, and a reinforcement exponent does not divide.

But the deepest reason is that their conclusion is incompatible with the structure of regulation itself. A regulator issues limits. A limit is a quantity below which something is permitted. Druckrey and Küpfmüller say that for a genuinely cumulative substance there is no such quantity — only a slower arrival. A finding that cannot be converted into a limit cannot be used by an institution whose only instrument is a limit.

The framework did not vanish entirely. It resurfaced in the 2000s in the work of Henk Tennekes, who found that the dose-response characteristics of neonicotinoid insecticides in arthropods were strikingly similar to those of genotoxic carcinogens, with the Druckrey-Küpfmüller form and an exponent around four and a half. Tennekes's conclusion — that the toxicity of these compounds is time-cumulative, so that very low exposures become lethal given long enough — was fiercely contested, which is itself evidence of how badly the result fits the apparatus. A 2013 paper in Toxicology reconstructed the molecular basis of the Druckrey-Küpfmüller equation in terms of the association and dissociation time constants of the substance at its target. The mechanism stands. It is simply not what anybody measures.

There is one more thing to notice, and it is the hinge into the rest of this book.

Druckrey and Küpfmüller's two constants describe *letting go*. T_R is how long the substance takes to release the receptor. T_r is how long the effect takes to subside. Both are return times. Their entire theory is built on the proposition that what determines harm is not how much arrived but how slowly the system gets back.

That proposition was available to toxicology in 1949. The field chose the other road, and the other road is Part III.

PART III — THE APPARATUS

Chapter 9. Lehman and Fitzhugh: Dividing by One Hundred

In 1949 the United States Food and Drug Administration published a volume titled Appraisal of the Toxicity of Chemicals in Foods, edited by Arnold Lehman, director of its Division of Pharmacology. In 1954 and 1955, Lehman and his colleague O. Garth Fitzhugh set out the method that still governs chemical safety worldwide.

The problem they faced was real and remains real. Toxicity is measured in animals. Limits must be set for humans. Two gaps have to be crossed: the gap between species, and the gap between one human and another.

Their solution was arithmetic. They introduced two safety factors, each of magnitude ten, to account for those two limitations in what could be learned from experimental toxicity studies. Ten for the step from animal to man. Ten for the variation among men. Multiplied together, one hundred. The experimentally determined value, divided by one hundred, became the Acceptable Daily Intake: the safe dose for humans.

It is worth being clear about what kind of number one hundred is. It is not derived. It is a judgement about how much ignorance to allow for, expressed as a number so that it can be used in a calculation. Lehman and Fitzhugh were candid about this. Their successors have been less so, and the factor has acquired an air of precision it never had.

Lehman also wrote the guidelines for toxicity testing that the pharmaceutical industry followed: acute, subacute and chronic studies, with chronic toxicology running two years in the rat and one year in the dog. The durations are conventions. They were chosen to be long relative to the life of the animal, and they have barely changed in seventy years.

The approach laid out by Lehman and Fitzhugh remained in place and is still used widely to establish limits on human exposure to chemicals. The European tolerable weekly intake for four per- and polyfluoroalkyl substances, which chapter 17 examines closely, is a direct descendant.

What was measured. A no-observed-adverse-effect level in an animal, divided by a factor representing uncertainty.

What became visible. A number a regulator can use. This cannot be overstated as an administrative achievement. Before the ADI there was no principled way to permit a substance at all; afterwards there was.

What became invisible. Two things.

The first is the structure of the uncertainty. A single factor of one hundred absorbs species differences, individual differences, duration differences and mechanism differences into one scalar. Anything the factor absorbs stops being studied, because it has already been handled.

The second is duration. The test runs two years in a rat. The resulting number is expressed per day or per week for a human lifetime. The conversion assumes that what matters is the rate of intake, and that a steady rate produces a steady state. For a substance the body clears, that assumption is sound. For a substance it does not clear, the assumption is false in a way the arithmetic cannot reveal.

Chapter 10. Thalidomide, Delaney and the Birth of Regulatory Toxicology

Two events, two years apart, gave the field its modern institutional shape.

In 1960 the Delaney clause was added to the United States Federal Food, Drug and Cosmetic Act. It prohibited food additives shown to be carcinogenic in animals or humans. The clause is notable because it is absolute. It contains no dose. A substance that causes cancer in an animal at any dose may not be added to food. For a brief period, in one narrow domain, the law abandoned the quantity paradigm entirely — and it was eventually eroded precisely for that reason, criticised as scientifically unreasonable because it admitted no threshold.

In 1961 thalidomide, developed by Grünenthal in the 1950s and marketed as a completely safe sedative, was withdrawn after causing severe birth defects. The Society of Toxicology was founded in the same year. The disaster established reproductive and developmental testing as a mandatory part of drug evaluation, and it established regulatory toxicology as a permanent institution with statutory authority.

Thalidomide also carried a lesson about timing that the field absorbed only partially. The harm depended not on how much was taken but on *when* in the pregnancy it was taken. A given dose on day twenty did something a far larger dose on day sixty did not. This produced the concept of the critical window: a period during which the system is vulnerable in a way it is not before or after.

The critical window is a genuinely temporal idea. It says that the same amount means different things at different times, because the state of the system differs. It entered developmental toxicology and stayed there. It did not generalise.

What was measured. Presence or absence of a defined adverse outcome, under dose and, in the developmental case, timing.

What became visible. Vulnerability as a property of system state rather than of dose. The institutional machinery of modern drug and chemical regulation.

What became invisible. The generalisation. If the state of a system determines what a dose does in an embryo, the same is true of an adult, whose state also varies — with age, with prior exposure, with what it is already carrying and has not yet cleared. That thought was available in 1961 and was confined to pregnancy.

Chapter 11. Ames, 1973: the Agent Must Touch the DNA

In 1973 Bruce Ames, a biochemist at the University of California at Berkeley, published a bacterial test system for detecting mutagens. It used strains of Salmonella typhimurium carrying mutations in the histidine operon, combined with a liver extract to simulate mammalian metabolism. A mutagenic compound causes reverse mutations that restore the bacteria's ability to make histidine, and the colonies can be counted.

The test was simple to perform, took two days, was relatively inexpensive and transferred easily between laboratories. It became and remains the most frequently conducted of all genotoxicity assays, now standardised as OECD test guideline 471. The majority of available structural alerts for chemical hazard derive from Ames test data.

Its intellectual foundation is a specific hypothesis: that DNA damage is the first step towards cancer. The title of the founding paper states the position — carcinogens are mutagens.

This is the Orfila grammar in molecular form. There is an agent, an act — the chemical modification of a base — a victim, and a trace that can be found. The test asks whether the agent did it, and answers in two days.

The effect on the field was enormous and double-edged. It made screening possible at scale. It also silently redefined what a carcinogen is. A substance that scores positive is a suspect. A substance that scores negative is not, in the operational sense of the word, a carcinogen at all — not because anyone decided so, but because the screen is where the inquiry starts and a negative result ends it.

Ames himself later argued that concern about synthetic chemicals was overblown, after his assays showed that many natural compounds in ordinary foods were as mutagenic as synthetic ones. That controversy is beside the present point. The point is structural: for fifty years the primary question asked of a new substance has been whether it damages DNA.

What was measured. Mutation frequency in bacteria, as a proxy for the agent's capacity to act on genetic material.

What became visible. Rapid, cheap, transferable screening. A mechanistic account of one route to cancer that is correct for a large and important class of substances.

What became invisible. Every other route. A substance that causes cancer without touching DNA has no signature in this system. It does not fail the test; it passes, and passing is reassurance. Chapter 17 examines a group of substances classified by the World Health Organization's cancer agency as carcinogenic to humans on grounds that explicitly exclude genotoxicity. They pass the Ames test. They have been passing it for fifty years.

Chapter 12. The Red Book, 1983: Procedure Replaces Judgement

In 1983 the United States National Research Council published Risk Assessment in the Federal Government: Managing the Process. It is universally known as the Red Book, for its cover.

It described a four-step analytic process for human health risk assessment: hazard identification, dose-response assessment, exposure assessment and risk characterisation. It also made a recommendation that is cited more often than any of the four steps — the separation of the science of risk assessment from the policy of risk management.

The Red Book's recommendations shaped risk policy and practice at regulatory agencies, and the four-step framework remains the backbone of chemical regulation in the United States, the European Union and most of the world. Later frameworks, including the modernisation described in Part IV, are explicitly designed to be compatible with it.

Look at the four steps as a sequence of questions. Which substance is dangerous. How much of it does what. How much are people getting. Therefore, what is the risk.

Every step takes an amount as input or produces one as output. The framework is a machine for turning observations into a permitted quantity. It is extremely good at this. It is not capable of anything else, and that is not a flaw in its construction but its specification.

The separation of assessment from management had an unintended consequence worth naming. By declaring the first part science and the second part policy, it placed the choice of what to measure on the science side, where it is treated as a technical matter and therefore not debated. The question *should we be regulating an amount at all* has no home in the structure. It is too fundamental for the assessment step and not a policy question in the sense the framework means.

What was measured. The same quantity as before, now embedded in a procedure with defined inputs and outputs.

What became visible. Consistency. Auditability. The possibility of comparing assessments across substances and agencies. Defensibility in court, which matters more than it sounds.

What became invisible. The choice itself. A procedure makes its own assumptions invisible by making them structural. After 1983 it was no longer necessary for anyone to decide that harm is a matter of amount; the forms assumed it.

PART IV — THE MOVE INWARD

Chapter 13. Tox21c, 2007: the Same Quantity, a Smaller Vessel

In 2004 the United States Environmental Protection Agency asked the National Academy of Sciences to review existing strategies and develop a vision for the future of toxicity testing. A committee of twenty-two experts reported in 2007 under the title Toxicity Testing in the 21st Century: A Vision and a Strategy. The report is usually abbreviated Tox21c, and it is the most significant document the field has produced since the Red Book.

Its vision was radical in method. Virtually all routine toxicity testing would be conducted in human cells or cell lines in vitro, by evaluating cellular responses in a suite of toxicity pathway assays using high-throughput tests, implemented with robotic assistance. Animal testing would recede. Testing would become quicker, cheaper and more directly relevant to human exposures.

The essential premise was that scientists can infer human risk from the perturbation of cellular response pathways, rather than from apical endpoints — death, tumour, malformation — observed in whole animals.

A partnership formalised in 2008 brought together the National Toxicology Program, the National Institutes of Health's Chemical Genomics Centre and the EPA's Computational Toxicology Centre under the name Tox21. The associate director of the National Toxicology Program said at the time that in almost all aspects it looked like a paradigm shift in the field.

Within its own terms it was. The ToxCast and Tox21 databases now contain data from over fourteen hundred assays with numerous biological targets and activity data for over nine thousand chemicals.

Now read the vision's own statement of what risk assessment becomes: a shift towards the avoidance of significant perturbations of these pathways in exposed human populations.

Avoidance of significant perturbations. The regulated quantity is still the concentration at which the perturbation becomes significant. The vessel has changed from a rat to a well in a plate. The question has not.

There is a further consequence, examined in detail in chapter 18. A cell in a well is a system with a very short memory and no capacity to return to a prior state over a period of weeks. Any harm that consists of a system taking too long to recover is, in such a vessel, structurally undetectable. Not hard to detect. Undetectable, because the vessel does not persist long enough to have the property.

What was measured. Perturbation of a cellular pathway, as a function of concentration, in a human cell.

What became visible. Throughput at a scale that makes the tens of thousands of untested substances approachable in principle. Mechanism rather than mere outcome. A route away from animal testing.

What became invisible. Anything requiring an intact organism over time: clearance, redistribution, enterohepatic recirculation, immune memory, recovery. The move inward bought resolution and sold duration.

Chapter 14. Adverse Outcome Pathways: a Path with a Doer at its Head

The adverse outcome pathway, introduced in 2010 from the field of ecotoxicology, is the organising framework that the Organisation for Economic Co-operation and Development and the European agencies are now standardising. It is the conceptual counterpart to the technical shift of Tox21c.

An adverse outcome pathway is a formalised sequence of key biological events that links a molecular initiating event to an adverse outcome at the level of the organism. The components have names: the molecular initiating event, abbreviated MIE; a chain of key events, KEs; and the key event relationships that connect them.

This is, in form, a considerable advance. It is a path. It acknowledges that harm is a process with stages rather than a single transaction, and it allows evidence from different levels of biological organisation to be assembled into one structure.

Consider, then, what the existing pathways for one substance family look like. In the published pathway numbered 529, the molecular initiating event is the binding of a perfluoroalkyl sulfonate to a peroxisome proliferator-activated receptor. In pathway 60, it is the activation of the PXR/SXR receptor leading to liver injury. For reproductive toxicity, a meta-analysis of twenty-eight studies gives the initiating event as an increase in reactive oxygen species. For neurotoxicity, a systematic review of two hundred and seventy-one studies gives the same. For non-alcoholic fatty liver disease, a recent computational study gives the downregulation of a particular protein.

In every case the first term is something the substance does.

This is the Orfila grammar again, and it is now built into the first slot of the framework that will govern European and OECD chemical assessment for the next generation. The framework is a path, which is right. It requires the path to begin with an act by the agent, which is an assumption.

If a substance causes harm by failing to leave rather than by acting, there is no molecular initiating event to enter. The framework does not return the answer "no initiating event". It cannot accept the case at all.

What was measured. The chain of events from the agent's act to the organism's outcome.

What became visible. Mechanism as a connected sequence. The integration of in vitro data into regulatory arguments. A shared vocabulary across disciplines and agencies.

What became invisible. Harm without an initiating act. The framework's first term is a filter, and it filters out precisely the class of substance this book is concerned with.

Chapter 15. NAMs, NGRA and the Roadmap of 2026

On 1 June 2026 the European Commission adopted the Roadmap towards phasing out animal testing for chemical safety assessments, accompanied by a detailed staff working document. The roadmap envisages gradually replacing all animal testing for chemical safety assessment across the European Union. It covers fifteen legislative areas, including industrial chemicals, pharmaceuticals and food additives, and sets out more than thirty recommendations.

It arose from a European citizens' initiative, Save Cruelty-Free Cosmetics, which gathered more than 1.2 million signatures.

Its recurring theme is the shift to Next-Generation Risk Assessment: assessing safety from a mechanistic, molecular understanding of toxicity rather than from observed effects in animals. The supporting instruments include a Horizon Europe call worth forty-nine million euros, artificial intelligence tools, the Virtual Human Twins initiative, and links to the European Health Data Space. A Roadmap Steering Team was established in mid-2026 and the call for its expert group closes on 21 December 2026.

The methods it endorses are collectively called New Approach Methodologies: human cell-based in vitro models, computational simulation, omics, read-across, physiologically based kinetic modelling. Taken together they constitute the largest deliberate reconstruction of chemical safety assessment since 1983.

And inside that reconstruction sits a sentence that ought to be read very carefully by anyone who thinks the measured quantity is settled. Among the roadmap's stated tasks is the need to define more clearly what information regulators require from a new, non-animal-based testing system.

That is an open question about what to measure, asked by the body that decides, with a deadline attached.

What is being measured. Not yet fixed. That is the point.

What is becoming visible. A genuine opportunity. The apparatus is open for the first time in forty years, and the people opening it have said in writing that they do not yet know exactly what they want from it.

What is at risk of becoming invisible, again. The default. Every method on the list — in vitro assays, kinetic models, omics, read-across — can return a concentration, and the institutions receiving them are built to consume concentrations. Without an explicit decision to the contrary, Next-Generation Risk Assessment will deliver the same quantity through better instruments. That is the most probable outcome, and the window in which it can be influenced is measured in months.

PART V — THE CASE THAT DOES NOT FIT

Chapter 16. A Guest That Cannot Finish

Per- and polyfluoroalkyl substances are a family of synthetic compounds built around a chain of carbon atoms whose hydrogens have been replaced by fluorine. The carbon-fluorine bond is the strongest single bond in organic chemistry. There are an estimated ten thousand of them. More than fourteen hundred are produced or imported at scale. They are in cookware, textiles, firefighting foam, cosmetics, pesticides, medical devices, semiconductors, construction products and printing ink. They are called forever chemicals.

The standard account of why they are a problem has two parts, and this chapter argues that both are misplaced.

The first part is that they persist in the body because they cannot be destroyed. The elimination half-life of perfluorooctanoic acid in humans is given in years. In a well-conducted study of residents of Ronneby in Sweden, where contaminated municipal water was replaced with clean water on a known date in December 2013, the mean half-life was 2.7 years for perfluorooctanoic acid, 3.4 years for perfluorooctane sulfonate and 5.3 years for perfluorohexane sulfonate.

The explanation offered is indestructibility. The difficulty is that indestructibility does not by itself produce retention. A kidney does not require a solute to be metabolised before excreting it. It filters, and what is not reclaimed leaves. A small, highly water-soluble, chemically inert anion is in principle the easiest thing a kidney ever handles, and many such compounds are cleared within hours. If these compounds stay for years, something is holding them, and inertness is not that something.

What holds them is now well documented. Three organs recapture these substances from fluids that would otherwise carry them out. In the kidney, the organic anion transporters and the urate transporter reabsorb the filtered compound from the tubular lumen; urinary excretion of the protein-bound fraction falls below one tenth of one per cent. In the liver, the organic anion transporting polypeptides and the sodium-taurocholate cotransporting polypeptide take it up from portal blood. In the intestine, the apical sodium-dependent bile acid transporter returns the compound from bile to the circulation, completing a loop.

These transporters are not a defence that is failing. They are the return limbs of a conservation circuit that the body runs deliberately, because bile acids are metabolically expensive and worth recovering. For a genuine bile acid the circuit is sound, and it is sound for one reason: there is a forward limb with an end. What is recaptured is eventually used, modified and voided, so the circuit drains.

Place in that circuit a guest that cannot complete the forward limb and the identical machinery becomes a trap. The compound enters on the strength of its shape — it has the geometry of a fatty acid and the behaviour of a bile acid — and the handling machinery treats it accordingly. What it cannot do is finish the traverse, because the oxidative step cannot run. No mammalian enzyme cleaves a perfluoroalkyl carbon-fluorine bond.

So the position is occupied and the path has no end for its occupant. Each organ performs its function correctly, and because nothing terminates, nothing drains.

The strongest evidence for this reading is not observational but interventional, and it is decisive. If retention were a property of the compound, interrupting bile acid recapture would do nothing to it. A

randomised crossover trial in Denmark gave forty-five participants cholestyramine, an ordinary bile acid sequestrant, three times four grams daily for twelve weeks. Serum perfluorooctane sulfonate fell by sixty-three per cent. The drug does not touch the molecule. It removes one return limb, and the burden drains through the opening.

A modelling result makes the point quantitatively. A physiologically based pharmacokinetic model of the rat kidney, built to explore the role of transporters in renal reabsorption, reproduced the observed sex-specific half-lives from transporter expression and activity. When the transport parameters were set to zero, the predicted serum half-life was 4.4 hours, at every dose.

Four hours, not four years. The difference between those two numbers is not a property of the carbon-fluorine bond. It is the conservation machinery of the host.

The second part of the standard account concerns cancer, and it requires equal care. In 2023 the International Agency for Research on Cancer classified perfluorooctanoic acid as carcinogenic to humans, Group 1. Read the grounds. Evidence in exposed humans for specific cancers was rated limited, for renal cell carcinoma and testicular cancer. Evidence in experimental animals was sufficient. The classification rests on that animal evidence combined with strong mechanistic evidence in exposed humans, and the mechanistic evidence rated strong concerns two characteristics: epigenetic alteration and immunosuppression.

Genotoxicity is not among them. These compounds are not classified as mutagens. They pass the Ames test.

Now consider what epigenetic alteration and immunosuppression have in common. Epigenetic alteration means that the reading settings of a cell do not return to their prior values after a perturbation. Immunosuppression means that an immune response does not run to completion. Both are statements about return, not about damage. The transient component fails to subside within its characteristic time, and the registering component carries an offset.

Transformed cells arise continually in everyone. What determines the outcome is whether the surveillance and repair pathway closes on them. On this reading the compound does not produce the transformed cell. It lengthens the return time of the pathway that would have closed it off.

Three consequences follow, and they predict the shape of the evidence.

There is no signature. A multiplier on an existing background leaves no mark in the tumour.

There is no threshold. A rate multiplier acts at every level of exposure, scaled. There is no dose below which it does not operate, only a progressively smaller multiplier. This is Druckrey and Küpfmüller's conclusion from chapter 7, reached by a different route.

There is no individual attribution. A raised probability cannot be reduced to a single case. The question a resident asks — did the plant cause my cancer — has no answer in principle, and not for want of study.

Chapter 17. What the Instruments Saw

The European point source for these compounds is the DuPont, later Chemours, works at Dordrecht in the Netherlands. The Dutch National Institute for Public Health and the Environment estimated that residents were exposed through air above the chronic exposure limit, in the worst case for twenty-five

years, with the limit no longer exceeded after 2002. Drinking water was not elevated. Liver effects could not be excluded. Blood was measured in residents in 2017.

Cancer incidence in the postcode areas around the plant was counted for 1989 to 2013, with kidney, testis and liver singled out because of the literature. No site was elevated in any of the three areas; the total in one area fell below expectation. A comparable study in a neighbouring municipality in 2018 reached the same conclusion. In Sliedrecht over 1998 to 2017 the total was raised, attributable mainly to skin cancer, while kidney and testicular cancer were not.

Three studies, three decades, no excess.

Compare the investigations in the Mid-Ohio Valley in the United States. A case-control analysis of 25,107 cancer registrations across thirteen counties reported an adjusted odds ratio for testicular cancer of 5.1, with a confidence interval from 1.6 to 15.6, resting on six cases in the highest serum exposure category. Six cases produced a signal where three Dutch studies on far larger counts produced none.

The usual explanation is exposure contrast, and it is part of the answer: the American route was drinking water over decades, with serum concentrations in some districts two orders of magnitude above background.

But the decisive difference is the design. The American studies had a dose axis. Individual cumulative exposure was reconstructed from modelled water concentrations, residential histories and measured serum, and the analysis asked whether risk rose with the amount received. The Dutch studies asked whether a geographic area had more cases than the country.

For a rate multiplier these are not equivalent questions, and the second is far the weaker. A multiplier shows itself in the ordering, not in the total. A gradient analysis can recover it from a handful of cases. A geographic count can miss it in thousands.

Now the third site, which has not been read correctly by anyone including, until recently, the present author.

From the mid-1980s roughly 140,000 people in the Veneto region of Italy were exposed through the public water supply across thirty municipalities. The contamination was discovered and exposure stopped in 2013. Mortality has been followed from 1980 to 2018, and in related work to 2021.

What was found there is not a tumour. An ecological mortality study reported statistically significant raised relative risks in both sexes for all-cause mortality, diabetes, cerebrovascular disease, myocardial infarction and Alzheimer's disease.

That is the shape a multiplier makes. Not a signature in one organ but a small lift across everything that depends on a system recovering properly.

And Veneto has a property no other site has. The exposure stopped on a known date, in a large population, with a complete mortality registry running for decades on either side. That is a single defined perturbation with an aftermath — which is to say, it is a return experiment at population scale, already conducted, waiting to be read as one.

Chapter 18. What the Instruments Could Not See

This chapter records three failures. None of them is a failure of competence. Each is a case of an instrument faithfully reporting that the quantity it measures is not present.

The cell assay. Immunosuppression by these compounds is among the best-established effects in humans. The European tolerable intake is derived from it. And yet most of these substances show weak or no activity in the immune-related assays of the ToxCast programme. The literature records this plainly as a lack of concordance between toxicological and high-throughput data, and treats it as a current limitation of in vitro screening for immunotoxicity.

It is not a limitation of sensitivity. A suppressed antibody response is a property of a response that takes too long to complete, measured over weeks in an organism with memory. A well in a plate has no weeks and no memory. The assay cannot be made sensitive enough, because the thing is not small — it is absent from that vessel.

The incidence count. The Dutch studies asked whether a defined area had more cases than expected. That is one number per area. A closure deficit is not readable at a point; it requires at least two values, because what is measured is a return or a slope. The design had almost no power to detect what it was deployed to detect, and its null result has been treated by both parties to the dispute as though it were decisive.

The regulatory limit. The European tolerable weekly intake for four of these substances is 4.4 nanograms per kilogram of body weight per week. Follow its derivation. The critical study examined the relation between plasma concentrations in 101 one-year-old infants and their antibody response after vaccination. From the diphtheria antibody data a benchmark dose lower confidence limit of 17.5 nanograms per millilitre of serum was modelled. Physiologically based kinetic models then converted that critical infant serum level into a critical milk level of 0.133 nanograms per millilitre, then into a maternal daily intake of 0.63 nanograms per kilogram, and seven times that daily figure gives 4.4 per week.

Look at what happens across those four steps.

The first measurement is a return. A defined perturbation is delivered — a vaccination — and the system's response is tracked over time; a suppressed response means the system did not come back as it should. That is a dynamic quantity with at least two timepoints.

The final number is a concentration in food.

Somewhere in the conversion the time axis is discarded. It is not falsified or rejected. It is simply not carried, because the receiving institution has no slot for it. The whole European food standard for these compounds rests on a return measurement that was converted into an amount at the last step.

This is the single most compact illustration of the thesis of this book. The field measured the right thing once, by accident, because immunologists happen to work with challenge-and-response. Then it translated the result into the only quantity its apparatus can hold.

PART VI — THE MEASURE THAT WAS LEFT OUT

Chapter 19. Closure

This chapter introduces the framework from which the preceding readings were derived. It is presented here, late, deliberately. The argument of chapters 1 to 18 stands on the historical and experimental record alone. What follows is an account of why those findings have the shape they have.

The framework is called the Vacuum.Net compression. The word compression is used advisedly: it is not a rival theory with new entities, but a rewriting of existing physical description in which a small number of relations carry what is normally spread across many separate laws. For the purposes of this book only three of its terms are needed, and each is given here in a form that requires no prior reading.

Path. The fundamental object is not a particle or a field but a closed strand, and what happens, happens along it. There is nothing outside the path. Structure arises because the strand is wound; the windings are what we name as objects.

Depth. A winding has a depth, which is how far along the nesting it sits. An atom is a depth. A molecule is a deeper one. A cell, an organ, a body are further depths. A body, in this vocabulary, is simply a depth. This matters for what follows: there is no point at which the description stops applying because biology has begun.

Crossing and direction. Where the strand crosses itself there is no stored value — not a plus, a zero or a minus. What a crossing carries is a direction: onward or back. This is the term that does the work in the present argument.

From these, closure. A path through a system closes when what entered it has traversed it and the position is released. Closure is a property of a whole path, and of the pair formed by a host and its guest. It is not a property of either one alone, and it is not readable at a point, because a point has no traverse.

Three consequences for toxicology follow directly.

First: half-life is not a property of a substance. It is a property of a pair, set by how many return limbs the host runs and how vigorously. This is not a philosophical claim; it is already half-confirmed in the data without having been read as such. Mice clear perfluorooctanoic acid in hours and humans take years. Male and female rats differ by more than an order of magnitude, and the difference tracks renal transporter expression. In the Ronneby population the most important determinants of a shorter half-life were young age, better kidney function measured by glomerular filtration rate, and the ratio of paired urine and serum concentrations — all properties of the host. The field calls this population variability, which treats it as noise around a compound-level constant. The reading offered here says there is no compound-level constant to be noisy around. The host-specific return capacity is the quantity.

Notice that the modelling literature has already conceded this in practice. The kinetic models used to derive the European intake value are fitted by adjusting the transporter capacity for tubular reabsorption until the model reproduces the reported human half-life. The quantity that this chapter says is the real answer is used as an input. The model therefore cannot discover that it varies.

Second: harm without an act. If closure is a property of the pair, then a guest can cause harm without doing anything. It enters a path by resemblance, occupies a position, and fails to release it. The damage is the host's inability to complete, prolonged by the occupancy. There is no molecular initiating event, because there is no initiating act.

This is why the adverse outcome pathway framework of chapter 14 cannot represent the case. Its first term is reserved for an act.

It is also why the inventory of documented effects for these compounds — receptor occupancy, impaired fatty acid oxidation, uncoupling, bile acid dysregulation, endoplasmic reticulum stress — reads as five separate insults only if you insist on an agent. Read as one condition sampled at five successive positions along one path, it is a single statement. The substance does nothing at any of them. It fails to leave all of them.

Third: the measurable quantity is return time. If what has gone wrong is that a path does not close, the observable is how long the system takes to come back after a defined disturbance, and what offset it retains.

Here the framework and the forgotten history of Part II meet. Druckrey and Küpfmüller's two time constants — for the reversibility of binding and the reversibility of effect — are return times. Their reinforcement exponent measures failure to close. They had the quantity in 1949 and derived it from receptor kinetics. The present framework reaches the same quantity from the structure of a path. Two independent derivations of the same missing measure, separated by seventy-seven years, is not a small fact.

Chapter 20. The Future of Toxicology

What changes if the regulated quantity becomes a return time?

The test changes shape. Present-day toxicity testing administers a substance and measures a state. The replacement administers a defined, reversible perturbation to a system already carrying a given burden, and measures the trajectory back. The output is not a concentration but a time, or a rate, or a residual offset. Any system that admits a defined and ethically deliverable challenge with a measurable recovery is a candidate: glucose tolerance, inflammatory resolution after a standardised challenge, wound closure, and — most obviously, because the field already does it — antibody response after vaccination.

That last one deserves emphasis. The one human effect of these compounds at ordinary exposure levels that is found reliably, and is accepted as a valid regulatory endpoint, is reduced vaccine response. This is not a coincidence about immunology. It is the only endpoint that happens to have been measured in the right form.

The vessel changes. A well in a plate cannot hold a return. An organ-on-chip with perfusion can: it is the only in vitro system that can deliver a disturbance and keep watching. The technology exists and is not being used for this. A virtual human twin, which the European roadmap is funding, is by construction a model that runs in time; a static limit does not fit inside it, whereas a return time is its natural output.

The norm changes. A limit value says: not more than this much. A return norm says: not slower than this. The second is a different kind of law. It cannot be met by dilution, and it cannot be evaded by

producing a substance in small volumes — which, as chapter 15's registration arithmetic implies, is how the great majority of these ten thousand substances currently escape scrutiny altogether. A substance that never leaves accumulates regardless of volume. A tonnage trigger is the quantity paradigm applied to administration, and it fails for exactly the same reason.

The remedy changes, and this is the part with immediate consequences. In a paradigm of amount and agent, the answer to a poison is to stop the poisoner. Every European instrument is built on that verb: limit value, threshold, maximum level, restriction, ban. None of them addresses what is already in the population.

If harm is non-departure, the intervention is departure. Open the exit.

That is not speculative. A bile acid sequestrant, a cheap and long-established drug, removes sixty-three per cent of one of these compounds in twelve weeks by closing a single return limb. Plasma donation every six weeks reduced levels by approximately thirty per cent over twelve months in a randomised trial of 285 firefighters. And the kinetic model says that with the return limbs closed the body would clear the compound in hours.

No study has ever blocked two return limbs at once. The trials conducted to date each address one. An organic anion transport inhibitor was tested alone in an observational analysis and produced modest, non-significant increases — exactly what should be expected when one of three limbs is closed while the others run, since the burden is redistributed rather than evacuated. The prediction that follows is specific and cheap to test: a transport inhibitor combined with a bile acid sequestrant should contribute more than its standalone effect, and more than additively, because the sequestrant supplies the terminus that the renal blockade then prevents the system from circumventing.

Three measurements follow from this book, and none of them has been made.

One. A removal trial blocking two recapture limbs simultaneously. The drugs are old and inexpensive. This is a small trial standing between the current standard of care and a materially better one.

Two. A dose-gradient analysis of the Dutch cohort, linking the serum values measured in residents in 2017 to the national cancer registry. This converts the Dutch question from a count into a gradient, which is the form in which the quantity is detectable at all. It requires no new sampling, no new consent for collection and no new follow-up time — only linkage and the governance to permit it. Until it is done the dispute cannot resolve. The company is correct that no excess has been demonstrated. The residents are correct that exposure above the limit occurred for up to twenty-five years. Both are correct because they are describing different quantities, and the measurement that would adjudicate between them has not been performed on data that already exists.

Three. Return measures across non-immune systems, in populations with known burdens. The prediction is that burden lengthens return time across multiple systems, not immunity alone, and does so at burdens at which cross-sectional measures show nothing.

Each of these is falsifiable and each would weaken the account if it failed. If a combined regimen proves merely additive, the recapture-circuit reading is weakened. If individual-level gradient analysis shows no relation across a genuine exposure range, the multiplier reading is weakened. If return measures show no dependence on burden where cross-sectional measures also show none, the generalisation fails.

There remains the question of why this is hard to hear, and the history in this book answers it.

Modern toxicology was born in a courtroom. Orfila's instrument was built to prove that a person had poisoned another person. The grammar of that instrument — agent, act, trace, doer, victim — has been inherited by everything downstream: the threshold that exonerates below a certain amount, the mutagenicity assay that looks for the agent's fingerprint on DNA, the adverse outcome pathway that begins with a molecular initiating event, the litigation that seeks a liable party, and the resident who asks whether the factory caused her illness.

All of them ask the same question in different vocabularies. Who did it.

For the class of substance described in this book, nobody did it. There is a guest that will not leave, and a host that therefore does not come back on time. The harm is real, large and measurable. It has no author.

A science built to find authors will keep returning empty-handed, and will keep reporting that result as reassurance.

The European apparatus is, for the first time in forty years, explicitly open on the question of what it should measure. The roadmap adopted on 1 June 2026 states among its tasks the need to define more clearly what information regulators require from a new, non-animal-based testing system. That is the question of this book, asked by the institution that decides, with a deadline attached.

Paracelsus chose the quantity in the sixteenth century, and it was the right choice for the substances in front of him. Haber in 1924 and Druckrey and Küpfmüller in 1948 each wrote down a second quantity, and each time the field reduced it to a correction factor on the first. The choice is open again, briefly.

The question is no longer how much. It is how long.

APPENDIX — ANNOTATED REFERENCES

The references are grouped by the chapter whose claims they support. Each entry states what it was used for and, where relevant, what the reader will and will not find in it. Entries marked *consulted indirectly* were available to the author only through secondary summary; that is stated so the reader can weigh it.

Part I — The Choice

Milestones of Toxicology and **Historical milestones and discoveries that shaped the toxicology sciences.**

https://www.researchgate.net/publication/265051156_Milestones_of_Toxicology_English

https://www.researchgate.net/publication/23805736_Historical_milestones_and_discoveries_that_shaped_the_toxicology_sciences

Timeline sources for chapters 1 and 2: the Ebers papyrus of about 1500 BC with its hundred and ten pages and its hemlock, aconite, opium and metals; Shen Nung and the three hundred and sixty-five herbs; Dioscorides at the court of Nero; and the identification of Orfila as the father of modern toxicology. Useful as a chronological spine. Not useful for interpretation, which is this book's own.

A Textbook of Modern Toxicology, third edition.

https://farmacognozie.usmf.md/sites/default/files/inline-files/A%20Textbook%20of%20Modern%20Toxicology.pdf

Source for the judgement that there were few advances between Galen and Paracelsus, for Dioscorides' date of about AD 50, and for Claude Bernard beginning the modern study of mechanisms of toxic action. A standard teaching text; its framing of the history is the orthodox one this book departs from.

Gallo, R. From Murder to Mechanisms: 7000 Years of Toxicology's Evolution. Society of Toxicology, Eminent Toxicologist Lecture Series, 2017.

https://www.toxicology.org/education/docs/07262017/GalloSOTEminentToxLectureSeries2017.pdf

The single most useful source for the twentieth-century chronology used in chapters 9 to 11: the 1949 FDA volume under Lehman, the first Gordon Research Conference in 1956, the Delaney clause of 1960, the thalidomide disaster and the founding of the Society of Toxicology in 1961. Also the source for Paracelsus's use of mercury and ether. The title itself — from murder to mechanisms — concedes the forensic origin that chapter 4 builds on, without drawing the consequence.

Toxicology: Then and now. Clinica Chimica Acta.

https://www.sciencedirect.com/science/article/abs/pii/S0009912006000907

Source for the formulation of Paracelsus's dictum used in chapter 3, for his determination that specific chemicals are responsible for the toxicity of a plant or animal poison, and for Marsh's discovery of the arsenic test in 1832.

Mathieu Orfila. Encyclopaedic entry.

https://en.wikipedia.org/wiki/Mathieu_Orfila

Source for the central fact of chapter 4: that Orfila took the Marsh test of 1836, modified the apparatus for larger liquid volumes, applied it to human tissue and thereby created forensic toxicology, using it in the Lafarge and Mercier trials. The reader who wants the primary account should go to the next entry.

Poisons and the development of Toxicology in the 19th century. Royal College of Surgeons library blog.

https://www.rcseng.ac.uk/library-and-publications/library/blog/poisons-and-toxicology/

Source for Orfila's six classes of poison in the Traité des poisons of 1813 and for his position as Dean of the Faculty of Medicine in Paris.

Chimney sweeps' carcinoma and **Percivall Pott and the Chimney Sweeps' Cancer.**

https://en.wikipedia.org/wiki/Chimney_sweeps%27_carcinoma

https://www.encyclopedia.com/science/encyclopedias-almanacs-transcripts-and-maps/percivall-pott-and-chimney-sweeps-cancer

Sources for chapter 5: Pott's 1775 description, his attribution to soot lodging in the folds of the scrotum, the exposure of the boys in early childhood, and the standing of the observation as the first reported occupational cancer and the first causal link between cancer and an environmental substance.

Pott, P. Chirurgical Observations, London, 1775, as reproduced and discussed in the British Journal of Industrial Medicine.

https://moglen.law.columbia.edu/twiki/pub/EngLegalHist/ArmorieDelamirie/pott_scrotum_article.pdf

Pott's own words, including his reference to Ramazzini. Worth reading directly; it is short and the reasoning is visible.

Historical review of the causes of cancer.

https://pmc.ncbi.nlm.nih.gov/articles/PMC4734938/

Source for Ramazzini's 1713 observation on breast cancer among nuns, used in chapter 5 as the limiting case of a harm with no agent at all.

Occupational safety and health (for Ramazzini's De Morbis Artificum Diatriba and its catalogue of hazards).

https://en.wikipedia.org/wiki/Occupational_safety_and_health

Part II — The Law of Time, Twice Written

Witschi, H. Some Notes on the History of Haber's Law. Toxicological Sciences, 1999.

https://www.farmlandbirds.net/sites/default/files/H%20Witschi%20Haber's%20Law%20Tox%20Sci%201999.pdf

The best short history of Haber's rule. Source for the formulation $C \times T$ = constant, for its origin in Haber's work on war gases, for Haber's dates, and for the phosgene experiments in which the rule held

and those in which it did not. Also the source for the observation that the product tends to increase as concentration falls — the anomaly that chapter 6 argues was never interpreted.

Haber's rule: a special case in a family of curves relating concentration and duration of exposure to a fixed level of response for a given endpoint. Toxicology, 2000.

https://www.sciencedirect.com/science/article/abs/pii/S0300483X00002298

Source for the statement that Haber considered concentration and time equally important, for the original units (concentration in mg/m³ times time in minutes to death), and for the rule's continued use by regulatory bodies despite its capacity to over- and underestimate.

Haber's Rule: The Search for Quantitative Relationships in Toxicology.

https://www.researchgate.net/publication/248944673_Haber's_Rule_The_Search_for_Quantitative_Relationships_in_Toxicology

Source for the generalisation to the family c × t^m = K with variable exponent, and for the explicit statement that the rule goes beyond Paracelsus, who considers only the dose. That sentence is quoted in chapter 6 because it is the field acknowledging, in passing, that it had a second variable.

Appendix G: Example of Time-Scaling Calculations, in Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals, National Research Council.

https://www.ncbi.nlm.nih.gov/books/NBK223440/

Shows Haber's rule in its surviving regulatory role: time-scaling, with C^n × t = k and Haber as the special case n = 1. This is the primary evidence for chapter 6's claim that time enters the calculation in order to be eliminated from the answer.

Haber's rule duration adjustments should not be used systematically for risk assessment in public health decision-making.

https://www.sciencedirect.com/science/article/abs/pii/S0378427411001925

A representative example of the modern debate. Note that the entire argument concerns how best to arrive at a concentration.

Dosis und Wirkung in der Toxikologie: die Haber'sche Regel und Ableitungen.

https://www.researchgate.net/publication/335574269

The key historical source for chapter 7. States that theoretical knowledge of the relationship between dose and tissue response, and in particular of carcinogenic action, derives largely from two papers by Hermann Druckrey and Karl Küpfmüller of 1948 and 1949; that the definition of cumulative poisons and the first proof of the irreversibility of carcinogenic effects had an extraordinary impact on cancer research; and — the sentence on which this book turns — that Druckrey and Küpfmüller inferred that if both receptor binding and the effect are irreversible, exposure time would reinforce the effect. *The original 1948 and 1949 papers are in German and were consulted indirectly through this and the following entries.*

The molecular basis of simple relationships between exposure concentration and toxic effects with time. Toxicology, 2013.

https://www.sciencedirect.com/science/article/abs/pii/S0300483X1300111X

The modern mechanistic reconstruction. Source for the Druckrey-Küpfmüller equation c · t^n = constant, for the reading of n as an exposure time reinforcement factor, and for the two time constants: T_R for the reversibility of receptor binding and T_r for the reversibility of the effect, with three classes of dose-response behaviour depending on whether each tends to zero or infinity. This is the reference for anyone who wants the mathematics rather than the history.

Tennekes, H. The significance of the Druckrey-Küpfmüller equation for risk assessment: the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time. Toxicology, 2010.

https://www.boerenlandvogels.nl/sites/default/files/Tennekes_2010_2.pdf

Source for the modern revival discussed in chapter 8, for Druckrey's 1943 butter yellow result in which the carcinogenic action depended on total dose and was independent of daily dose, and for the key regulatory consequence: the lower the exposure concentration, the longer the period up to effect, and the lower the daily dose at which it occurs. Tennekes's application to neonicotinoids is contested; the contest is itself discussed in chapter 8 and the reader should look at both sides.

The History of the Druckrey-Küpfmüller Equation. Open Access Journal of Toxicology.

https://juniperpublishers.com/oajt/OAJT.MS.ID.555572.php

Supplementary history, including Druckrey's follow-up on diethylnitrosamine after Magee and Barnes linked dimethylnitrosamine carcinogenicity to the alkylation of nucleic acids in 1956.

Influence of exposure time on toxicity — an overview. Griffith University repository.

https://research-repository.griffith.edu.au/server/api/core/bitstreams/6118ac62-683b-4b10-a9ce-8ff596f89789/content

A survey of a century of attempts to relate exposure time to toxicity, including the threshold-corrected forms. Useful for a reader who suspects this book has overstated the neglect; it has not, but the survey shows the work that was done at the margins.

Part III — The Apparatus

In Pursuit of Safety: One-Hundred Years of Toxicological Risk Assessment. RSC Environmental Chemistry Group.

https://www.envchemgroup.com/one-hundred-years-of-toxicological-risk-assessment.html

The principal source for chapter 9. Lehman and Fitzhugh, the two safety factors of magnitude ten each, the derivation of the Acceptable Daily Intake, and the statement that the approach remains in place and is still used widely. Also connects forward to the Red Book.

Science and Judgment in Risk Assessment, National Research Council, student edition 1996, chapter reproduced at

https://facultystaff.richmond.edu/~sabrash/ES%20201/Readings/Reading43.pdf

Source for the four-step analytic process of the 1983 Red Book and for the factor of one hundred in ADI derivation.

Jacobs, A.C. and Hatfield, K.P. History of Chronic Toxicity and Animal Carcinogenicity Studies for Pharmaceuticals. Veterinary Pathology, 2013.

https://journals.sagepub.com/doi/full/10.1177/0300985812450727

Source for the Lehman Guidelines and the study durations — two years in the rat, one year in the dog — discussed in chapter 9 as conventions that have barely changed.

Health Risk Assessment in the Workplace (for the Red Book's adoption as the standard framework).

https://stacks.cdc.gov/view/cdc/226348/cdc_226348_DS1.pdf

Source for the characterisation of the 1983 report as groundbreaking and for its codification of risk assessment across agencies.

Scientific Frontiers in Developmental Toxicology and Risk Assessment, National Academies Press, 2000, chapter 3.

https://nap.nationalacademies.org/read/9871/chapter/5

Source for the Red Book's role as the common framework and for the subsequent EPA developmental toxicity guidelines of 1991.

The test that changed the world: The Ames test and the regulation of chemicals. Mutation Research, 2019.

https://www.sciencedirect.com/science/article/abs/pii/S1383571819300956

Source for chapter 11: the two 1973 publications from Ames's Berkeley laboratory, the test's simplicity, two-day turnaround, low cost and transferability, and its status as a basic component of genetic toxicity testing schemes internationally.

Genotoxicity, WHO food safety guidance, section 4.5, second edition 2020.

https://www.who.int/docs/default-source/food-safety/publications/section4-5-genotoxicity.pdf

Source for the Ames test as OECD test guideline 471, for its position as the most frequently conducted of all current assays, and for the derivation of the majority of structural alerts from Ames data.

The Political Life of Mutagens: A History of the Ames Test.

https://www.academia.edu/29163280/

Source for the founding hypothesis — carcinogens are mutagens — and for the later controversy when Ames tested natural substances. Read for the intellectual history rather than the method.

The Ames Test. Science History Institute, Distillations.

https://www.sciencehistory.org/stories/distillations-pod/the-ames-test/

Accessible account of Ames's own trajectory, including his belief that DNA damage is the first step towards cancer.

Part IV — The Move Inward

Toxicity Testing in the 21st Century: A Vision and a Strategy. National Research Council, 2007.

https://nap.nationalacademies.org/resource/11970/Toxicity_Testing_final.pdf

The primary document of chapter 13. Read the summary pages directly: the vision of toxicity pathways, the reliance on cellular response pathways, and the framing of risk assessment as the avoidance of significant perturbations. The last phrase is the one chapter 13 turns on.

Toxicity Testing in the 21st Century: Bringing the Vision to Life. Toxicological Sciences, 2009.

https://academic.oup.com/toxsci/article/107/2/324/1683303

Source for the explicit statement of what the vision entails: virtually all routine toxicity testing in human cells or cell lines in vitro, high-throughput, with robotic assistance, and risk assessment shifted towards avoidance of significant pathway perturbations.

TOX 21: New Dimensions of Toxicity Testing. Environmental Health Perspectives, 2009.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2721892/

Source for the 2008 memorandum of understanding establishing the Tox21 partnership between the National Toxicology Program, the NIH Chemical Genomics Centre and the EPA Computational Toxicology Centre, and for the quoted judgement that it looked like a paradigm shift.

Hartung, T. A Toxicology for the Twenty-First Century — Mapping the Road Ahead. Toxicological Sciences, 2009.

https://academic.oup.com/toxsci/article/109/1/18/1675018

The clearest statement of the reform programme from inside it. Included because any reader who wishes to argue against this book's thesis should start here: Hartung is the most articulate advocate of the modernisation, and his account of what is being changed makes visible what is not.

Jeong, J. et al. Application of ToxCast/Tox21 data for toxicity mechanism-based evaluation and prioritization of environmental chemicals. Toxicology in Vitro, 2022.

https://www.turi.org/wp-content/uploads/2025/12/Jeong-2022_Application-of-ToxCast_Tox21.pdf

Source for the scale of the databases: over fourteen hundred assays and activity data for over nine thousand chemicals, with the assay platforms and cell lines enumerated.

AOP-Wiki, pathway 529.

https://aopwiki.org/aops/529

The published adverse outcome pathway in which the molecular initiating event is the binding of perfluorooctane sulfonate to the ligand-binding domain of a peroxisome proliferator-activated receptor. Cited in chapter 14 as a representative instance of an agent-initiated first term.

Weighted network analysis of adverse outcome pathways decodes the multiscale mechanisms of environmental toxicity. 2026.

https://www.sciencedirect.com/science/article/pii/S2666498426000086

Source for the definition of an adverse outcome pathway as a formalised sequence linking a molecular initiating event to an adverse outcome at organism level, and for pathways 60 and 377 in relation to liver injury and immune activation.

Adverse outcome pathway for the neurotoxicity of per- and polyfluoroalkyl substances: a systematic review. 2024.

https://pubmed.ncbi.nlm.nih.gov/39605965/

Source for reactive oxygen species generation as the molecular initiating event, derived from 271 studies.

A meta-analysis-based adverse outcome pathway for the reproductive toxicity induced by per- and poly-fluoroalkyl substances in animals.

https://pubmed.ncbi.nlm.nih.gov/41290085/

Source for reactive oxygen species as the initiating event in the reproductive pathway, from 28 studies.

Exploring Toxicological Mechanisms of Typical PFAS-Induced NAFLD through Integrated Computational Toxicology and AOP Frameworks.

https://pubmed.ncbi.nlm.nih.gov/42344598/

Source for the downregulation of THBS1 as a molecular initiating event. Included because it is recent and shows the pattern continuing.

Roadmap towards phasing out animal testing for chemical safety assessments. European Commission, C(2026) 3497 final, adopted 1 June 2026; published as C/2026/3087; staff working document SWD(2026) 144.

https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX%3A52026XC03087

https://single-market-economy.ec.europa.eu/sectors/chemicals/reach/roadmap-towards-phasing-out-animal-testing_en

The primary document of chapter 15 and the target of chapter 20. Source for the intention to replace all animal testing for chemical safety assessment across the European Union, the fifteen legislative areas, the more than thirty recommendations, the origin in the European citizens' initiative with over 1.2 million signatures, the Roadmap Steering Team, and the expert group call closing 21 December 2026.

The end of animal testing? The EU's chemical safety plan explained.

https://www.mcdermottlaw.com/insights/the-end-of-animal-testing-the-eus-chemical-safety-plan-explained/

Source for the supporting instruments: the Horizon Europe call of forty-nine million euros, AI tools, the Virtual Human Twins initiative, the European Health Data Space link, and the characterisation of Next-Generation Risk Assessment as assessing safety from mechanistic molecular understanding rather than observed effects in animals.

Report of the European Commission workshop on the roadmap.

https://transcience.fr/wp-content/uploads/2025/03/report-of-the-european-commission-workshop-on-the-ET0224681ENN.pdf

Source for the stated aim of defining more clearly what information regulators need from a new, non-animal-based testing system. This sentence is the opening identified in chapters 15 and 20.

New approach methodologies. European Food Safety Authority topic page.

https://www.efsa.europa.eu/en/topics/new-approach-methodologies-nams

Source for EFSA's adoption of the roadmap, its 2025 guidance on read-across, and its scoping review of existing in silico and PBK models.

Part V — The Case That Does Not Fit

Forever Chemicals, Finite Defenses: PFAS Burden the Liver, Break Mitochondria, and Outpace Modern Regulation. International Journal of Molecular Sciences, 2026.

https://www.mdpi.com/1422-0067/27/6/2723

Source for the binding and distribution data and the mechanistic inventory in chapter 16: the primacy of protein and membrane partitioning over octanol-water partitioning, and impaired fatty acid oxidation as the best-supported mitochondrial effect.

A state-of-the-science review of interactions of per- and polyfluoroalkyl substances with renal transporters in health and disease.

https://pfascentral.org/science/a-state-of-the-science-review-of-interactions-of-per-and-polyfluoroalkyl-substances-pfas-with-renal-transporters-in-health-and-disease-implications-for-population-variability-in-pfas-toxicokinetics

Source for the transporter inventory and for the attribution of sex and species differences in half-life to transporter activity. The review's own framing of this as population variability is the framing contested in chapter 19.

Li, Y. et al. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occupational and Environmental Medicine 75(1):46-51, 2018.

https://pubmed.ncbi.nlm.nih.gov/29133598/

Source for the Ronneby half-lives used in chapter 16: 2.7 years for PFOA, 3.4 for PFOS, 5.3 for PFHxS, with first-order elimination, an approximately threefold interindividual range and a marked sex difference. Clean water was supplied from 16 December 2013, which is what makes the cohort a cessation study.

Li, Y. et al. Determinants of serum half-lives for linear and branched perfluoroalkyl substances after long-term high exposure. Environment International, 2022.

https://doaj.org/article/b85eef09edbc4b25b4aecbe824acb057

Source for the determinants used in chapter 19: young age, better kidney function measured by estimated glomerular filtration rate, and the ratio of paired urine and serum levels. These are host properties, which is the chapter's point.

Worley, R.R. et al. Application of Physiologically-Based Pharmacokinetic Modeling to Explore the Role of Kidney Transporters in Renal Reabsorption of Perfluorooctanoic Acid in the Rat.

https://pmc.ncbi.nlm.nih.gov/articles/PMC4662604/

The single most important modelling reference in this book. Source for the finding that when transporter activity is set to zero the predicted serum half-life is 4.4 hours at all doses, and for the conclusion that sex-specific half-lives are driven by renal transporter expression and activity. Chapter 16 reads this as a published computation of the thesis.

Mechanistic Middle-Out Physiologically Based Toxicokinetic Modeling of Transporter-Dependent Disposition of Perfluorooctanoic Acid in Humans. Environmental Science & Technology, 2023.

https://pubs.acs.org/doi/10.1021/acs.est.2c05642

The human counterpart: in vitro transporter kinetics scaled to in vivo clearance by quantitative proteomics, constructed specifically to explain persistence mechanistically.

A Human Next Generation PBK Model for PFOA. bioRxiv, 2026.

https://www.biorxiv.org/content/10.64898/2026.02.04.703497v1.full

Source for the identification of the critical processes — plasma protein binding, transporter-mediated enterohepatic circulation, renal elimination and reabsorption — and for the fraction unbound in plasma as the most sensitive parameter.

Advancing PFAS risk assessment: integrative approaches using agent-based modelling and physiologically-based kinetic for environmental and health safety.

https://pmc.ncbi.nlm.nih.gov/articles/PMC11267999/

Source for the statement in chapter 19 that transporter maximum capacity for renal tubular reabsorption is fitted in order to achieve the desired half-life. This is the evidence that the quantity is used as an input.

Carcinogenicity of perfluorooctanoic acid and perfluorooctanesulfonic acid. IARC Monographs evaluation, summarised in The Lancet Oncology, 2023; and the agency's own announcement of 30 November 2023.

https://pfascentral.org/science/carcinogenicity-of-perfluorooctanoic-acid-and-perfluorooctanesulfonic-acid

https://www.iarc.who.int/news-events/iarc-monographs-evaluate-the-carcinogenicity-of-perfluorooctanoic-acid-pfoa-and-perfluorooctanesulfonic-acid

Source for the grounds of the Group 1 classification: limited human evidence for renal cell and testicular carcinoma, sufficient animal evidence, and strong mechanistic evidence in exposed humans for epigenetic alteration and immunosuppression, with genotoxicity not among them. The whole of chapter 16's second half depends on reading these grounds rather than the headline.

Risicoschatting emissie PFOA voor omwonenden locatie DuPont/Chemours, Dordrecht. RIVM.

https://www.rivm.nl/publicaties/risicoschatting-emissie-pfoa-voor-omwonenden-locatie-dupontchemours-dordrecht-nederland

Source for the exposure estimate in chapter 17: air exposure above the chronic limit for up to twenty-five years in the worst case, no elevation in drinking water, limit not exceeded after 2002, liver effects not excludable.

Betekenis resultaten bloedonderzoek PFOA omwonenden DuPont/Chemours. RIVM report 2017-0101.

https://www.rivm.nl/bibliotheek/rapporten/2017-0101.pdf

The 2017 serum measurements in residents. This is the dataset whose linkage to the national cancer registry is proposed as measurement two in chapter 20.

Incidentie van kanker in het gebied rond chemische fabriek Chemours, Dordrecht (1989-2013). Dienst Gezondheid & Jeugd Zuid-Holland Zuid.

https://www.dienstgezondheidjeugd.nl/assets/uploads/2016-03-23-Rapportage-incidentie-kanker-rondom-Chemours.pdf

The principal Dutch incidence study: observed against expected for kidney, testis and liver in three postcode areas, no site elevated, one area's total below expectation. The design analysed in chapter 18.

Het optreden van kanker in Sliedrecht (1998-2017). Dienst Gezondheid & Jeugd Zuid-Holland Zuid, 2019.

https://www.dienstgezondheidjeugd.nl/assets/uploads/2019-11-25-Rapport-Het-optreden-van-kanker-in-Sliedrecht-in-de-periode-1998-2017.pdf

The later analysis in which total incidence was raised through skin cancer while kidney and testicular cancer were not.

Vieira, V. et al. Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. Environmental Health Perspectives, 2013.

https://foodpackagingforum.org/news/high-exposure-to-perfluorinated-compound-linked-to-kidney-testicular-cancer-in-humans

Source for the case-control analysis of 25,107 cancer registrations across thirteen counties, the testicular cancer odds ratio of 5.1 with interval 1.6 to 15.6 on six highly exposed cases, and the approximate doubling of kidney cancer odds in the high categories. The decisive example in chapter 17 of a gradient design recovering what a count design cannot.

Winquist, A. et al. Design, methods, and population for a study of PFOA health effects among highly exposed Mid-Ohio Valley community residents and workers. Environmental Health Perspectives, 2013.

https://escholarship.org/content/qt54q143pp/qt54q143pp.pdf

Source for the cohort figures: 32,254 participants, 28,541 community members and 3,713 workers, distinguishing the follow-up cohort from the approximately 69,000-participant C8 Health Project survey, a distinction commonly elided.

Mastrantonio, M. et al. Drinking water contamination from perfluoroalkyl substances (PFAS): an ecological mortality study in the Veneto Region, Italy. European Journal of Public Health 28(1):180-185, 2018, with correction 2023.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10710324/

Source for the Veneto findings in chapter 17: statistically significant higher relative risks in both sexes for all-cause mortality, diabetes, cerebrovascular disease, myocardial infarction and Alzheimer's disease, over 1980-2013. Note the published correction to Table 1.

All-cause, circulatory disease and cancer mortality in the population of a large Italian area contaminated by perfluoroalkyl and polyfluoroalkyl substances (1980-2018). medRxiv, 2023.

https://www.medrxiv.org/content/10.1101/2023.12.09.23299764v1.full

Source for the scale and geography of the Veneto contamination: thirty municipalities, about 150,000 people, discovered in 2013.

Facchinello, L. The Effect of Long-run PFAS Exposure on Mortality. Collegio Carlo Alberto working paper no. 750, 2025.

https://www.carloalberto.org/wp-content/uploads/2025/10/no.750.pdf

Source for the statement that roughly 140,000 people were exposed from the mid-1980s and that exposure stopped in 2013, with age-standardised mortality covering 1982-2021. This is the paper that makes clear Veneto is a cessation cohort.

Investigating Molecular Mechanisms of Immunotoxicity and the Utility of ToxCast for Immunotoxicity Screening of Chemicals Added to Food.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8036665/

The central reference for chapter 18's first failure. States plainly that in epidemiological and toxicological studies these substances suppress the immune system and decrease vaccination response, that most of them show weak or no activity in immune-related ToxCast assays, and that the lack of concordance indicates the current limitations of in vitro screening for immunotoxicity.

EFSA CONTAM Panel. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal, 17 September 2020.

https://www.efsa.europa.eu/en/topics/per-and-polyfluoroalkyl-substances-pfas

The opinion that establishes the group tolerable weekly intake of 4.4 ng/kg body weight per week for the sum of PFOA, PFNA, PFOS and PFHxS, based on immunotoxicity — specifically reduced antibody response after vaccination in children.

Hoogenboom, R. EFSA 2020: Risk Assessment and Related Challenges. Presentation, BfR Academy.

https://www.bfr-akademie.de/media/wysiwyg/2025/PFAS2025/efsa-2020-risk-assessment-and-related-challenges.pdf

The most valuable single source for chapter 18's third failure, because it sets out the derivation chain on one page: BMDL10 of 17.5 ng/mL serum in infants, critical milk level 0.133 ng/mL, maternal

daily intake 0.63 ng/kg, times seven equals 4.4 ng/kg per week, with critical maternal serum level 6.9 ng/mL. A reader who wants to verify that the time axis is discarded should look here.

Abraham, K. et al. (2020), the critical study, as discussed in

https://www.bfr.bund.de/cm/349/pfas-in-food-bfr-confirms-critical-exposure-to-industrial-chemicals.pdf

and in the RIVM Front Office assessment at

https://www.rivm.nl/sites/default/files/2022-04/FO_Revised%20GenX%20and%20PFOA%20in%20food%20Part1_20210901_Def%20anon.pdf

Source for the design of the critical study: 101 one-year-old infants, plasma concentrations against antibody titres, with diphtheria antibody the modelled endpoint. *Consulted indirectly; the primary paper was not read.*

UK Committee on Toxicity, statements on the EFSA TWI.

https://cot.food.gov.uk/Uncertainties%20in%20the%20critical%20effects%2C%20dose-response%20assessment%20and%20derivation%20of%20an%20HBG

Source for the assessment that Grandjean 2012 and Abraham 2020 were the only studies suitable for benchmark dose modelling and only one provided a usable BMDL, for the observation that the Abraham cohort was relatively small, and for EFSA's own judgement that the impact of the uncertainties on this assessment is high. Included so that the reader can see this book is not alone in thinking the foundation narrower than the edifice.

Substantial decrease of per- and polyfluoroalkyl substances with anion exchange resin treatment: a randomised crossover trial. Environment International, 2024.

https://www.sciencedirect.com/science/article/pii/S0160412024000837

The Danish cholestyramine trial: forty-five participants, three times four grams daily, twelve weeks, sixty-three per cent reduction in serum PFOS. The central interventional evidence of chapter 16 and the baseline for the trial proposed in chapter 20.

Effect of Plasma and Blood Donations on Levels of Perfluoroalkyl and Polyfluoroalkyl Substances in Firefighters in Australia: A Randomized Clinical Trial. JAMA Network Open, 2022.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8994130/

285 participants; approximately thirty per cent reduction over twelve months with six-weekly plasma donation. Read in this book as the creation of an additional external exit rather than the blockade of a return limb.

Perfluoroalkyl substance excretion: effects of organic anion-inhibiting and resin-binding drugs in a community setting.

https://pfascentral.org/science/perfluoroalkyl-substance-excretion-effects-of-organic-anion-inhibiting-and-resin-binding-drugs-in-a-community-setting

The observational analysis of twenty-three probenecid and thirty-six cholestyramine users among 56,175 participants, in which the sequestrant lowered serum levels significantly and the transport inhibitor produced modest non-significant increases. Also confirmation that the two agents were analysed separately and never in combination, which is the gap chapter 20 proposes to close.

Third Report of the Independent PFAS Scientific Advisory Panel for Jersey. Government of Jersey, 2025.

https://www.gov.je/SiteCollectionDocuments/Health%20and%20wellbeing/Draft%20Third%20Report%20of%20the%20Independent%20PFAS%20Scientific%20Advisory%20Panel%20for%20Jersey.pdf

The current clinical position: bile acid sequestrants first line, phlebotomy second, transport inhibitors advised against. Chapter 20 proposes that the last of these be tested rather than accepted as settled.

Context: the regulatory state of play

European Court of Auditors, Special report 31/2026: The EU approach to PFAS ("forever chemicals") — Slow progress in a race against time. Adopted 8 October 2026, published 18 November 2026.

https://www.eca.europa.eu/en/publications/SR-2026-31

Source for the registration arithmetic referred to in chapter 20: approximately ten thousand substances in the family, more than fourteen hundred produced or imported at scale, and 231 registrations covering 119 substances in the European Chemicals Agency database, with polymers exempt from information requirements. Also for the average durations — six years for hazard assessment, five for a restriction, twenty-six months for Commission decision-making — and for the finding that the principle of no data, no market remains largely theoretical for this family. *The full report could not be retrieved directly by the author; the figures were obtained from the indexed text of the report and from press accounts of its presentation.*

Corporate Europe Observatory, How PFAS polluters muddy EU policy waters. 17 November 2026.

https://corporateeurope.org/en/2026/11/pfas-muddy-waters

Source for the lobbying context: seven companies and their trade association securing at least thirty-seven meetings in which these substances were discussed during the second von der Leyen Commission, and their demand for a full exemption for fluoropolymers. Included for completeness; this book's argument does not depend on it.

A note on the framework

The compression used in chapter 19 is set out by the present author in the Vacuum.Net foundational papers and in the derivations published at constable.blog, including the treatment of the closed strand, depth, and the direction carried at a crossing. The reading of harm as a closure deficit was first applied to host-pathogen pairs and then to a chemical guest in the essay *The Guest That Cannot Finish: PFAS retention, harm and cancer read as a closure problem*, version 1.0, 18 November 2026, of which Part V of this book is an expansion.

The reader should note that chapters 1 to 18 do not depend on the framework. They depend on the historical record and on the published experimental results cited above. The framework explains why those results have the shape they have. A reader who rejects the framework entirely is still left with Haber's unexplained exponent, Druckrey and Küpfmüller's reinforcement factor, a kinetic model that returns 4.4 hours when the transporters are switched off, a food standard derived from a return measurement and expressed as an amount, and a cell assay that cannot see the best-established effect in the literature.

Those five facts are the argument. The framework is the account of them.