Forensic Science: The Promise and Perils of Using Science in the Courtroom

An ACS-e! Discovery Report that examines the history, latest technologies and challenges of forensic science and the law

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About The Author

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I. INTRODUCTION

A Brief History of Forensic Science

Using scientific evidence to solve crimes is nearly as old as courtrooms themselves. When the ancient Greek scientist Archimedes was asked to find out whether a goldsmith had swapped silver for gold when crafting a crown, he turned to water for a solution. Using specific weights of the two metals, he calculated how much water each would displace, to provide the king with evidence of the craftsman's dishonesty.

Advances in scientific methods – ranging from microscopy to study hairs and fibers, to chemical analyses of poison or paint, and better ways to dissect DNA – have since informed the practice of law. Even prior to Archimedes' tests, historical records suggest that individuals attempted to use fingerprints or tested inks and dyes to study documents.

The first U.S. crime laboratory was established in Los Angeles in 1924, followed by the Federal Bureau of Investigation (FBI) crime lab in 1932. Since then, forensic applications of science have kept pace with new discoveries across disciplines.[1] Investigators often draw on fields such as toxicology or analytical chemistry to sift through data. Techniques such as mass spectrometry and Raman spectroscopy can help analyze trace evidence such as hairs, gunshot residue, ink, or drugs and poisons.

As science has progressed, these methods have been extended beyond human crimes. Wildlife forensic scientists use similar means to solve mysterious animal deaths or track illegal materials such as poached animal parts or wood from endangered forests. Similarly, environmental forensic cases track down the source of pollutants, or fingerprint nuclear fuels for better security. Over the last decades, forensic approaches have expanded from human crimes to also encompass environmental law, oversee international weapons treaties, and inform global health measures for epidemic infections.

Challenges Facing Modern Forensics

The increasing use of forensic science, however, has also highlighted the pitfalls of such data. In recent years, a growing body of evidence has suggested that science in the courtroom—particularly evidence involving human forensics—has been riddled with poor analysis and should have been held to more rigorous standards. A 2009 report on the state of forensic science from the National Research Council, the research arm of the National Academies, highlights the need for quantitative methods with clear statistical confidence[2]. In a *Chemical & Engineering News (C&EN)* article titled "Questionable Crime Scene Science," Rochelle Bohaty writes that the report found forensic science "suffers from a lack of standards, insufficient oversight, and flaws in interpretations." Bohaty writes: "The problems with forensic science have many roots, the report states. 'Most forensic techniques have evolved piecemeal and vary between labs and jurisdictions,' explained Constantine A. Gatsonis, report committee co-chair, at a press conference. Gatsonis, a professor of biostatistics at Brown University, added that 'these techniques are often administered by law enforcement agencies, which can introduce bias.'[3]

Although the science underlying the analysis of evidence such as fibers, paints, explosives, or DNA is often adequate, variable interpretations of data and a lack of quantifiable standards diminish the value of forensic evidence. In 2016, a Presidential panel found that many forensic techniques (such as bite mark or tire tread analysis) fell short of scientific standards, and recommended ongoing evaluations of forensic techniques by the National Institute of Standards and Technology (NIST).[4]

A large part of the problem is a poor understanding of statistics. In a 2012 *C&EN* article quoting Gatsonis, Andrea Widener writes about the continued need for a better system: 'Scientific results always come with some kind of error bars,' Gatsonis says, but, except for DNA analysis, the studies to determine error ranges just haven't been done. 'It is surprising how many people would stand up and defend' the current practice, he notes. 'There is a lot here that needs to be improved, and part of it is people who are the key contributors in the system need to get more understanding of the scientific process.'[5]

Reform has been slow in the making. But in 2014, the Department of Justice and NIST teamed up to create an oversight organization, known as the National Commission on Forensic Science, that aims to turn the 2009 recommendations into concrete action[6]. Some of these recommendations include ensuring that forensic labs have proper accreditation, resources, and administration, and that they use more precise wording; the term "match" could mean different things in different labs, for example. Better education—for scientists, lawyers, and crime investigators—will be key to this progress.[7]

Training New Researchers

The recognition of science's importance to the practice of law—coupled with the popularity of crime shows on television—has led to a booming interest in forensic science degrees. The burgeoning number of aspiring forensic scientists has driven at least 55 U.S. academic institutions to offer undergraduate concentrations in forensics, and several to offer master's degrees or programs specializing in forensics. In 2003, the American Association of Forensic Sciences created the Forensic Science Education Programs Accreditation Commission (FEPAC) to ensure that training for students met standards set by the National Institute of Justice. In a *C&EN* story about academic training in forensic sciences, Victoria Gilman writes: "The accreditation standards allow for variations in how a program is administered, but require a stronger base in the sciences—especially chemistry—than traditional forensics curricula have incorporated."[8]

Even so, specialized degrees are not the only route to a career in the field. Forensic scientists come from widely varied backgrounds, but typically have a strong undergraduate-level science degree. Many study in analytical chemistry, molecular biology, or toxicology. Internships analyzing common forensic data types also offer valuable opportunities for training. A career in forensic analysis could involve anything from working in a crime lab, to being out on a boat analyzing marine waters, or understanding how materials are engineered to minimize construction accidents. In a 2008 feature for *inChemistry*, Allison Byrum Proffitt writes: "Jimmie Oxley, a professor of chemistry at the University of Rhode Island and the head of its forensics program, encourages students to think outside the box, and consider the many possibilities beyond crime labs. 'They often think that doing forensics means only one thing: working in a crime lab. Students need to know that forensic scientists also work in many other settings.'[9]

II. CRIMINALISTICS AND HUMAN EVIDENCE

DNA Evidence

It begins with a plastic cup or a strand of hair stashed in a little baggie. After a brief stint in the lab, a white coat-clad scientist holds a translucent film up, lit by luminous blue stripes of varying sizes. The minute the team sees it, they know: they've nailed their killer. While on-screen crime dramas often play out in this manner, real-world forensic analysis rarely does. Nonetheless, the potential wealth of information that DNA carries has captured our collective fascination with forensics—and with good reason. Over the last 30 years, DNA evidence has been used in thousands of cases to nab criminals, solve cold cases, exonerate the innocent, and identify victims of disasters or war casualties.

Unlike ballistics, blood splatter, or other types of trace evidence that have drawn criticism for faulty methods or poor data standards, DNA has remained a strong basis for investigations. In part, this is because other kinds of forensic data were in use for years before modern scientific standards were established. In a 2012 *C&EN* cover story titled "Forensic Science And The Innocence Project," Carmen Drahl writes: "'Traditional forensic science got grandfathered into the justice system,' says Carrie Leonetti, an attorney who has served on the American Bar Association's Task Force on Biological Evidence. 'It's a lot harder to ask a court to exclude evidence that has been admitted for a hundred years than to exclude evidence it's never seen before. Because DNA was so new, it went through incredible vetting.'[10]

Early attempts used a classical technique to fingerprint DNA: A sample recovered from a crime scene is chopped into fragments using enzymes that cut at specific sites within the genetic sequence; the pieces are then labeled with probes that can bind to a sub-set of DNA sequences that share a repeating genetic sequence that varies in length between individuals, but tends to be similar among related people. If the profile of a crime scene sample and one taken from a suspect resemble each other—that is, the same-sized fragments from both individuals are labeled by the probes—they're considered a match.[11]

In 1985, Alec Jeffreys of the University of Leicester and his colleagues used this method for the first time in a court case. A young boy returned to his mother in the UK after visiting his father in Ghana, but evidence suggested that the woman might have been his aunt, or unrelated to the child. Using the DNA fingerprinting method to compare the child's samples to those of the woman and her other children, Jeffreys and his team confirmed that they were, in fact, mother and child.[12] A few years later, the researchers used the technique to eliminate a suspect and help nab a serial killer who had raped and murdered two girls. This method, however, needed large amounts of a high quality, undamaged DNA sample. In a 2013 review of DNA forensics, Lutz Roewer, associate professor for forensic genetics at the Humboldt-University Berlin in Germany, writes: "What was needed was a DNA code, which could ideally be generated even from a single nucleated cell and from highly degraded DNA, a code which could be rapidly generated, numerically encrypted, automatically compared, and easily supported in court."[13]

With the advent of better technologies such as polymerase chain reaction (PCR) and improved analytical tools, researchers can amplify tiny amounts of samples and study them with greater precision. Over time, the database of known markers in the human genome has expanded to include population-specific sequences as well. Now, forensic researchers rely on a specific set of short, tandem repeat (STR) markers combined with PCR and other methods to analyze DNA samples.

Criminal databases in Europe currently use a standard set of 12 STR markers; the U.S. CODIS system uses 13. With these markers, the chances of two people randomly having all 13 identical STR markers in their DNA are vanishingly small. "If a DNA match occurs between an accused individual and a crime scene stain, the correct courtroom expression would be that the probability of a match if the crime-scene sample came from someone other than the suspect (considering the random, not closely-related man) is at most one in a billion," Roewer writes.[13] American lawyers Barry Scheck and Peter Neufeld founded the Innocence Project, in 1992, which seeks to use DNA testing to help people who might have been wrongfully convicted of crimes. As of 2013, approximately 300 people had been exonerated based on DNA evidence.[14]

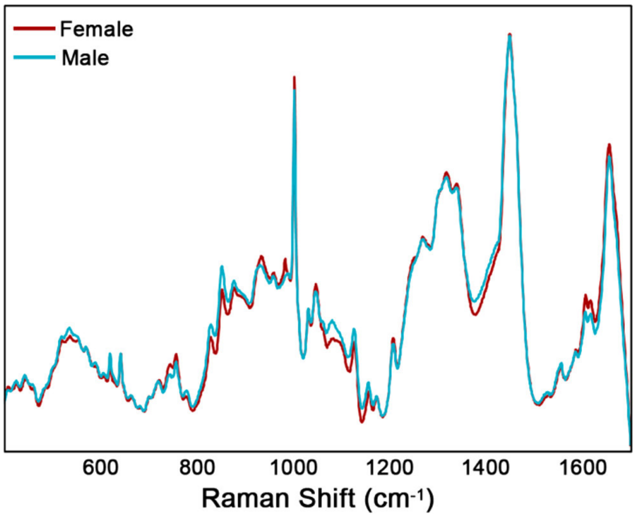
Now, researchers have also gleaned markers from the Y-chromosome, which passes from father to son, and from mitochondrial DNA, which children inherit only from their mothers; many of these markers can be used to identify a person's race, gender, or relatedness to another person from their DNA alone. These markers have proved especially useful in establishing ancestry or identifying disaster victims.

As genetic studies have advanced, so has the field of forensic DNA analysis. The Human Genome Project and other studies have revealed many additional markers that could be used in different situations to characterize samples.[15] Next-generation sequencing and automated processing have led to speedy, less expensive ways to analyze samples, although newer sequencing technologies may not currently meet established forensic standards.[16]

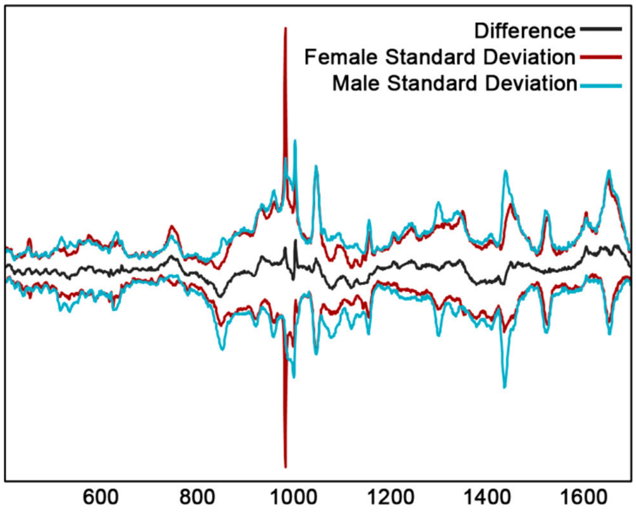
When a sample does not yield a match, researchers can still use immuno-assays or catalytic tests to identify features such as race or other phenotypes. A recent study turned to Raman spectroscopy—a common chemistry method that yields a "vibrational fingerprint" based on chemical structure—to determine the sex of donor DNA from a saliva sample.

Unlike other biochemical tests, Raman spectroscopy does not damage the evidence, so samples can be preserved for later studies.[17] In the future, researchers will continue to enhance the information they can gather from DNA, even from minuscule amounts or degraded samples.

The greatest challenge facing DNA forensics is not technological, however, but an ethical one. As it has become easier to collect, store, and analyze DNA, databases of genetic evidence maintained by law enforcement agencies have expanded, raising concerns that individual civil liberties and privacy could be violated. The UK National DNA Database (NDNAD) had stored nearly 6 million STR profiles,[18] and the



Mean Raman spectra of female (red) and male (blue) saliva donors. The experimental spectra have been preprocessed by baseline correction and normalization.



Summary spectra from male and female saliva donors, illustrating the Differences between and within the two groups. Difference between female and male mean spectra (black), and standard deviation of male donors (blue) and female donors (red).

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U.S. National DNA Index (NDIS) contained more than 12 million profiles as of 2016.[19] A controversial Supreme Court ruling in 2013 enabled Maryland officers to take DNA samples from suspects in custody before an arrest had been made, even without a warrant. William Schulz writes: "Although the case—Maryland v. King—centered on constitutional issues of privacy, experts say the decision will likely fuel an enormous expansion of the federal government's DNA database for crime solving. That could increase DNA-matching error rates, create more chances for contamination of evidence and reference samples, and force the criminal justice system to rethink best practices for DNA collection and evidence processing."[20]

In recent years, a method known as familial DNA searching has also been used in some criminal cases. When a crime scene sample fails to exactly match any known suspects, specialized algorithms can help investigators find a partial match to people who may be related to the DNA sample—typically male siblings or first cousins.[21] Familial searching can yield investigative leads and has resulted in many high-profile convictions; perhaps most famous is the Grim Sleeper case, where serial killer Lonnie David Franklin Jr. was sentenced to death for committing 10 murders. Investigators closed in on him based on DNA gathered from a half-eaten slice of pizza discarded by his son.[22] Many scientists have raised concerns that the combination of larger databases and the familial search algorithm will result in innocent people being wrongly accused of crimes, or racial profiling because some ethnicities are disproportionately represented in suspect databases.[23,24]

Other ethical concerns center on aspects such as the use of discarded DNA, which could be collected without a warrant. For example, does your DNA still belong to you if you tossed it in the trash on a coffee cup? For now, authorities in the U.S. have established rules to prevent the indiscriminate use of familial searches or a "DNA dragnet" that scans hundreds of profiles. Meanwhile, investigators, policy makers, and researchers continue to assess ways to improve the science of DNA searches without violating individual privacy or liberty.

Trace Evidence

Almost any evidence that can be transferred between individuals, or between a person and their surroundings at a crime scene, could be considered trace evidence. From blood to knife-wound patterns, these samples were among the earliest forms of forensic materials used by investigators. In the early 19th century, chemist James Marsh developed a test for arsenic that was used to help solve a murder case. Scotland Yard investigators at the time also used a physical flaw in a bullet to trace it back to its mold, and thus to the person who had bought it.[25]

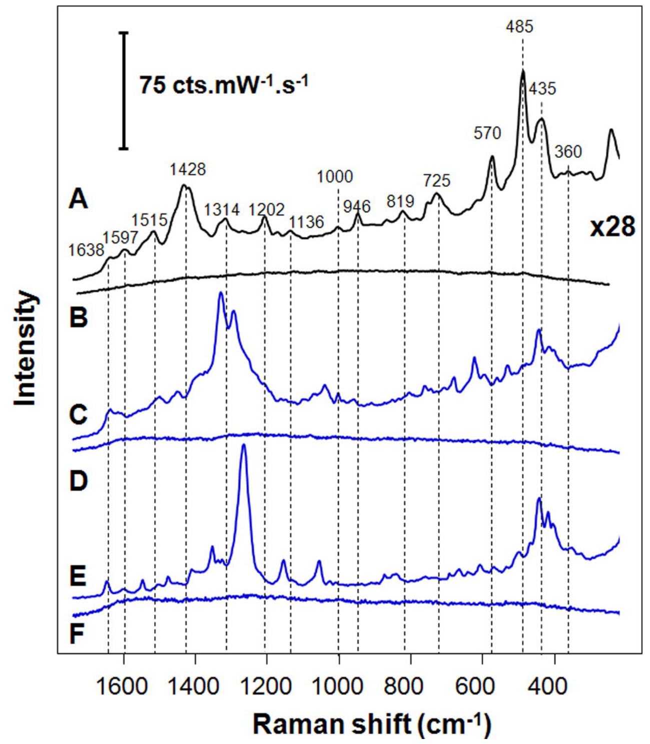
These tests were often developed and used by investigators rather than scientists. Nonetheless, scientific advances often found applications to solve crimes. Studies that revealed how unique human traits could be used as identifying marks were particularly useful; these included the discovery of blood types, handwriting analysis, or anthropometric measurements such as fingerprints or bite marks. Some were scientific, others are now considered pseudo-science.

Since many of these data types were grandfathered into the justice system, however, objective standards for their use in the courtroom are often lacking, and even experts on a subject may differ in the conclusions they reach about a given piece of evidence. For example, different labs in the U.S. can have different standards for what they consider a matching fingerprint. Andrea Widener writes: "[Fingerprints] are frequently used by law enforcement agencies to identify criminals and are commonly admitted as evidence in court. But there is no research that says what the probability is that everyone has a unique fingerprint or how many characteristics on each print would be needed to confirm the identity of a specific individual. […] In one often-cited example, the U.S. government settled with Oregon lawyer Brandon Mayfield for $2 million after it wrongly implicated him in the 2004 Madrid train bombings. Multiple analysts matched Mayfield's fingerprints to one found at the scene, but Mayfield was proven innocent when the real bomber was found."[5] Fueled by the need for better technology, researchers in many disciplines have begun to develop analytical tools that could help create objective, high-quality standards for trace forensic evidence. As of 2016, the National Institute of Justice had sponsored nearly $30 million in research grants to study and improve the quality of trace evidence analysis.[26]

Over the years, the variety of materials that constitute trace evidence has expanded; soil, pollen, inks, fabrics, and even a person's microbiome can now be studied for clues using advanced physical and chemical tests. Many newer tests for these materials are still being evaluated and validated in research labs. In the long run, they aim to improve the standards for forensic trace analysis. Following are a few examples of how testing techniques are being improved:

Fingerprints – The characteristic swirls of a fingerprint have been used by investigators for more than a century. But modern analytical chemists are looking beyond physical markings to the molecular composition of sweat in a fingerprint to identify its owner's gender, ethnicity, or how old the fingerprint is.[27,28] In one recent study, researchers found they could extract the amino acids present in a print to distinguish between male and female sweat.[29]

Microbiome – Every person carries a unique menagerie of microbes on their body, and different body parts have distinct sets of microbes. Just like DNA, these microbes can get deposited on objects a person touches. Even in instances where very little human DNA is left behind, a person's microbial signature may be detectable.[30] Researchers are now evaluating the possibility that these traces could serve as forensic evidence; recent studies have found that a person's microbiome can be identified from phones and shoes, for example, and a certain amount of matching is possible between a shoe and its wearer.[31,32] Others have found that the "necrobiome" of human remains can be used to accurately determine time of death.[33] Once these methods are refined and sufficiently validated, microbial signatures could be used much like other DNA evidence.



SER spectra of hair colored with permanent Ion Jet Black (trace A), semipermanent Ion Black (trace C), and Clairol Jet Black black colorants (trace E) and the corresponding normal Raman spectra (traces B, D, and F, respectively).

P = 1.5 mW (traces A and B) and 0.4 mW (traces C–F), λ = 785 nm.

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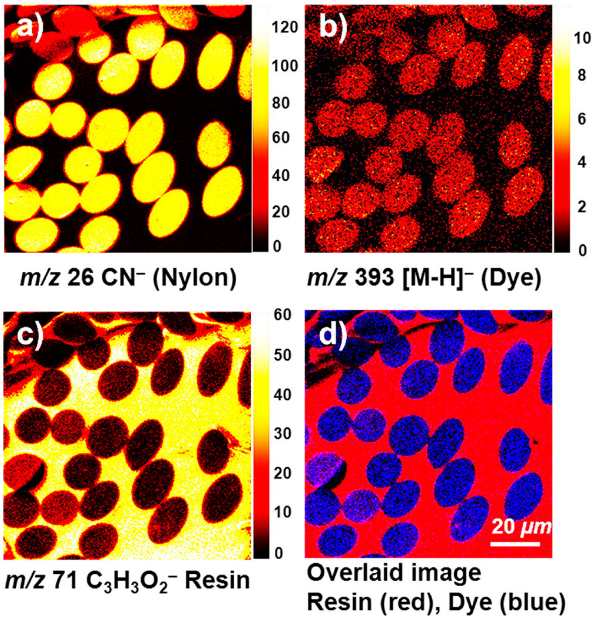
Hair – Typically, forensic analysis of human hair relies on a microscopic exam of its physical features—a method that lacks clear quantitative standards. Now, scientists are peering into hair's chemical make-up; one recent study found that surface enhanced Raman spectroscopy could distinguish different types and brands of hair dyes in a single strand of hair.[34]

And a team at Lawrence Livermore National Laboratory aims to create "hairprints." Ryan Cross writes: "The human hair shaft contains more than 300 different proteins, which gave the scientists an idea to create a system for turning hair samples into molecular fingerprints by examining single amino acid variations between individuals. 'It is a method that goes beyond the ambiguities of appearance,' says team member Deon S. Anex."[35]

Trace fibers and dyes – Most labs analyze stray strands of clothing or carpet fibers using microscopy followed by chromatography to study their dyes and chemistry. Newer methods now employ time-of-flight secondary ion mass spectrometry (TOF-SIMS), a tool that uses less of a sample and can simultaneously study the chemistry of the fiber, its dye, and any trace chemicals present.[36]

Other researchers are testing methods such as capillary electrophoresis-mass spectrometry, which requires minuscule amounts of samples to reveal clues about fiber chemistry.[37]



TOF-SIMS images (100 × 100 μm) of a 1% owf Acid Blue 25-dyed nylon fiber cross section showing the spatial distribution of (a) CN–, a characteristic ion of nylon, (b) the molecular ion of Acid Blue 25, (c) $C_3H_3O_2$–, a characteristic ion of the embedding resin, and (d) the overlaid image of resin (in red) and the Acid Blue 25 molecular ion (in blue).

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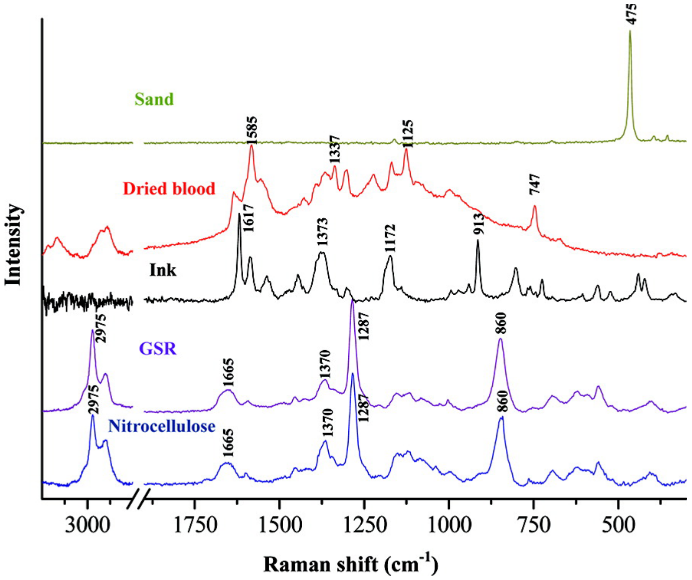
Blood and body fluids – Blood, semen, saliva, and other fluids are among the most valuable evidence collected at a crime scene. Since the discovery of blood types in 1901, the proteins present in these samples have offered up investigative leads. In addition to protein and DNA data, the quantity, splatter patterns, and other characteristics of these fluids can also hold clues. Even if DNA cannot be retrieved from a sample or doesn't yield a match, researchers can examine the age of the sample or how long ago it was left at the scene. Although these tests are usually performed in labs, researchers are now developing assays for a blood-based marker named alkaline phosphatase (ALP) that can reveal how old a person is and how long ago the blood was left at a scene. Similar tests for sweat and fingerprints are also in development.[38]

Guns and gunshot residue – Firing a gun creates shear marks on the cartridge; these striations have been used by forensic experts to match a firearm to cartridge remnants discovered at a crime scene. But this traditional "pattern matching" system is based heavily on an examiner's visual impressions and lacks external, quantifiable standards. In 2011, researchers turned to computational analysis instead. Rather than having individuals compare the markings, they extracted the information specialists typically use and entered it into a 3D model that could objectively assign associations and a confidence level to the likelihood of a match.[39]

Researchers are also turning to Raman spectroscopy—a method that can help identify chemicals based on how their molecular bonds scatter light of a specific wavelength—to improve studies of gunshot residue. Unlike older methods, which could not be applied to lead-free ammunition, Raman spectroscopy works as effectively with newer ammunition, is faster, and requires less of a sample. The technique can also be applied to distinguish sand, blood or ink from ammunition residues.[40]



Raman spectra of a sand particle, dried blood, ink from a black BIC crystal medium ballpoint pen, GSR, and nitrocellulose.

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Case Study: Better Science Overturns A Conviction

On a May evening in Mississippi in 1992, Kenneth Brewer was babysitting his girlfriend's three-year-old daughter, Christine Jackson, and their two other children. Some time that night, Christine was abducted, raped and murdered; her body was recovered from a creek near the family's home. Suspected of the crime, Brewer was arrested in 1992. When his trial began three years later, he was accused of having raped and killed Christine at home and then carrying her body to the creek. The key evidence: 19 marks on the child's body, which a forensic odonatologist examined and confirmed were "indeed and without a doubt" made by Brewer's teeth. This expert witness was at the time suspended from the American Board of Forensic Odontology, and a licensed dentist who served as a witness for the defense disagreed with his conclusions. Even so, Brewer was convicted of the murder and rape and sentenced to death. But a key piece of evidence—a semen sample recovered from Christine's body—was not tested at the time. In 2001, DNA testing on the semen revealed that it did not come from Brewer or any of his male relatives; Brewer was released in 2007. At the time, the Innocence Project was working with the Mississippi Attorney General's office on another case when they found that the 2001 DNA test matched another suspect, Justin Albert Johnson, who was also a suspect in another, identical case of rape and murder. Johnson confessed to both crimes. All charges were dropped, and Brewer was exonerated in 2008.[41]

III. ENVIRONMENTAL FORENSICS

Like fingerprints and stray hairs, human activity leaves traces of evidence everywhere. Growing needs for energy and materials have fueled the large-scale production and extraction of minerals, oil, nuclear fuels, and other materials. Over the last several decades, these activities have changed the air, water, and earth around us, affecting biodiversity, human health, and the planet's future. Laws to protect the environment were created as scientific advances revealed the extent of these effects. Regulatory agencies such as the U.S. Environmental Protection Agency (EPA) and the Nuclear Regulatory Commission (NRC) now work to ensure that companies adhere to these legislations, and those who breach environmental protection laws are prosecuted in a court of law.

The field of environment forensics blossomed as researchers provided the scientific evidence to validate claims of chemical contamination that caused harm to human health or the environment. Studies typically use a mix of field-based observations, data interpretation, and theoretical modeling to understand the long-term impacts of human activities.[42] For example, the 1989 Exxon Valdez oil spill in Alaska released 11 million gallons of crude oil into the environment. Twelve years later, researchers tracking identifying chemical components in the oil found that 78 of 91 randomly sampled beaches in the region still carried traces of the spilled oil. Analyzing one class of compounds known as terpanes showed that more than 90 percent of the surface oil and all the subsurface oils in the region were a result of the accident.[43] This and other analyses confirmed that the source of these oils was the spill, not natural seeps in the region.

Knowing the source of chemicals in the environment serves many purposes. First, it's helpful in the assessment of ongoing environmental crises, so that decisions can be made on the best course of mitigating potential harm. Second, understanding how a particular chemical affects an ecosystem over time can help inform policy-makers, so as to ensure better environmental protection through evidence-backed legal protections. Finally, tracing the source of a substance to its origins—whether it came accidentally from an oil spill, routine construction activities, or was intentionally released—can help investigators track down guilty parties, assess civil claims for damages, and identify strategies to mitigate long-term environmental damage.

Today, environmental forensics has evolved far beyond its early scope of studying chemicals alone. Advances in genetics and chemistry, as well as a greater emphasis on biodiversity and conservation, have led to progress in wildlife forensics and other areas. In a feature for *Environmental Science & Technology*, authors Anthony Capri and Jeffrey Mital write: "To reflect the present range of application, an alternative definition of environmental forensics is proposed—the scientific investigation of a criminal or civil offense against the environment. This broader explanation better reflects the diversity of the field by including chemical liability inquiries, as well as investigations into wildlife and plant poaching, illegal trade of protected organisms, arson in natural areas, and liability associated with biological pollutants."[44]

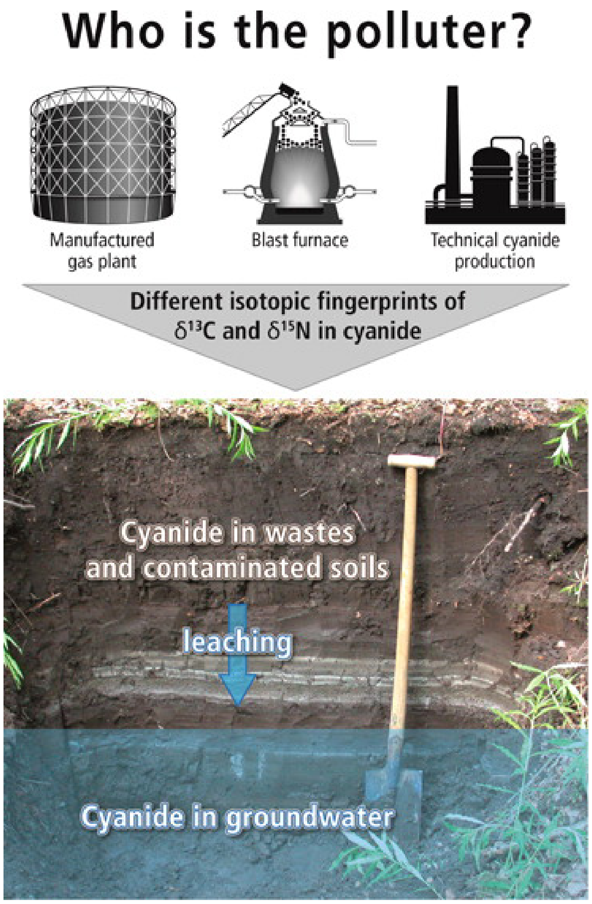
Environmental Chemicals

Even before the average worker reaches their desk on a typical workday, they've unleashed a potent cocktail of chemicals into the environment. Human activities —doing the dishes, shampooing a pet or carpet, driving to work, or taking an anti-inflammatory for everyday aches and pains—release minuscule amounts of man-made chemicals into the air and water.[45] Combined with large-scale activities like road construction, this anthropogenic runoff can build up to have a large-scale environmental effect.

Forensic researchers studying these issues scoop data out of odd spaces: septic systems, landfills, and open ocean water are all potential mines of information. Relying on physical or chemical methods as well as historical data sources, researchers aim to assess the impact of chemicals released by human activities on the environment. When faced with a lawsuit over environmental chemicals, a potential defendant's question is often: Am I truly responsible for the damage? Collectively, environmental forensics data help investigators arrive at an answer that can help prosecute violators. In the U.S., the EPA is responsible for a criminal enforcement program that helps protect people and the environment from illegal activities. Common violations include illegal disposal of hazardous wastes, importing restricted chemicals, oil spills, and money laundering related to environmental crimes.[46]

To prosecute such cases, the government has to prove beyond reasonable doubt that a person or company knowingly violated an environmental law containing criminal sanctions. Gathering scientific evidence toward this proof is where researchers collaborate with legal and investigative teams.[47] In a feature for *Environmental Science & Technology* titled "Environmental Forensics Unraveling Site Liability," a team of researchers describing the role of environmental forensics investigations wrote: "Successful collaborative investigations employ an integrated approach in which historical data (chemical, geological, modeling, and site-specific) are used to formulate a technically defensible opinion that can be easily understood by the non-expert."[48]

Chemical fingerprinting to identify the relative abundance of specific molecules in a sample often relies on methods such as high-resolution gas chromatography,



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stable isotope analysis, or gas chromatography combined with mass spectrometry (GC-MS). For example, cyanide compounds are released into soil and water from a wide range of anthropogenic sources, such as landfill wastes produced from gas or coke oven plants, aluminum manufacturing, gold mining, road salt and wildfire retardants that use Prussian blue. Any of these can cause a toxic buildup of cyanides in groundwater and soil. In one study, Tim Mansfeldt of the University of Koln in Germany and Patrick Höhener of Aix-Marseille University in France used stable isotope analysis to study cyanides from diverse sources and found this could help trace the precise origins of chemicals. "The requirement to discriminate among different sources of cyanide pollution is important from a legal perspective according to the 'polluter pays' principle," the authors wrote in their study.[49]

Other teams have turned to chemical fingerprinting to spot nitrate contaminants in groundwater from explosives used during construction versus those from agricultural runoff or wastewater.[50] But fingerprinting alone may not be sufficient in all cases, particularly when the source of contaminants is far removed—either in time or space—from the site of study. The gradually emerging discipline of "geoforensics" uses geological data to add layers of information to such situations. For example, knowing how soils are structured or different strata are organized in a region can help identify how contaminants might move through the earth. Similarly, understanding the direction and rate of groundwater flow can assist efforts to assess the source of a chemical and its destination.[48]

Historical records can also prove to be invaluable sources of evidence. In the same feature, the researchers provide an example of how a multitude of chemicals can exist in a region because of past industrial activity: "Contamination may have come from historic sources such as a dock fabricated from creosote-soaked planks in the late 1800s, replaced by a manufactured-gas plant built on fill material in the early 1900s, and finally replaced by an operating petroleum storage facility that was built on the site in the 1920s. Unraveling sources and ages of contamination at such sites would be difficult without any historical perspective."[48]

In addition to asbestos, cyanides, and myriad other chemicals known to cause harm, human activities release thousands of chemicals and their byproducts into the environment. Because their impact on human health and wildlife is still unclear, the EPA has dubbed them "chemicals of emerging concern."[51] As data emerge about their effects, environmental forensics and legal protections may expand to encompass a wider range of chemicals.

Oil Spills

Oil spills are a major source of chemical contaminants; as such, they have been widely studied for forensic investigations. Since the early 20th century, a rise in environmental awareness led to regulations such as the Federal Water Pollution Control Act of 1948, which was eventually amended and modernized as the Clean Water Act of 1972, and the Oil Pollution Act of 1990, which enables the EPA to prevent and respond to oil spills.

Despite the requirement that oil storage facilities and vessels provide plans to mitigate damage in the event of an accidental spill, when spills do occur they can still have disastrous, long-lasting environmental effects. Investigating such occurrences is much like analyzing crime scenes. Forensic researchers seek out chemical fingerprints that reveal the molecular composition of the oil, patterns of weathering to see how the chemicals degrade over time, and the effects of a spill on local ecology. Fingerprinting oil spills began in the 1970s, and has grown more sophisticated over the years with advanced analytical chemistry techniques and better data interpretation.[52]

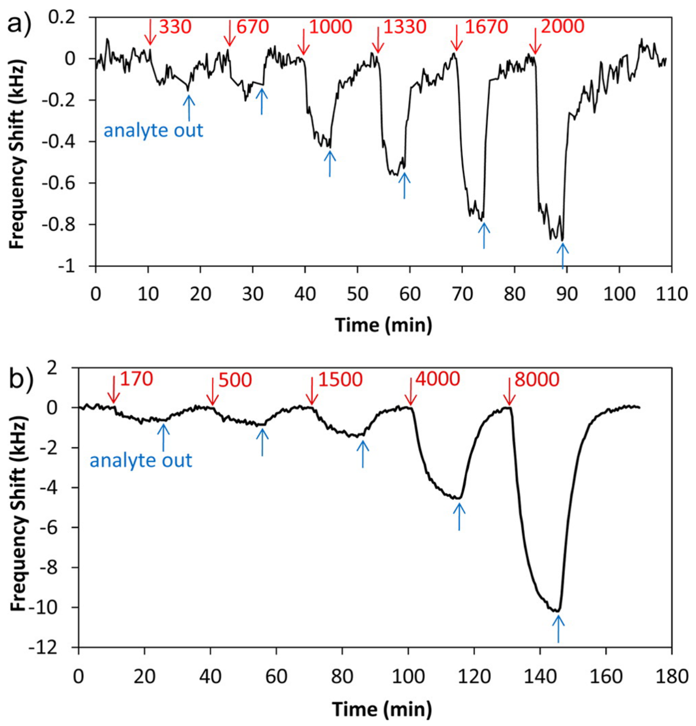
Chemical fingerprints have also become crucial to pinpointing sources of drinking water contamination from high-volume hydraulic fracturing. In one instance, natural gas and foam from the Marcellus shale gas wells in Pennsylvania were transported through several kilometers of rock, eventually finding their way into groundwater used by many households. Using comprehensive 2D gas chromatography and time of flight mass spectrometry, researchers from Pennsylvania State University and their colleagues correlated the compounds in drinking water with those present in the gas wells. "The organic contaminants—likely derived from drilling or HVHF fluids—were detected using instrumentation not available in most commercial laboratories," they wrote in their study.[53]

In addition to spills from human activities, natural seeps and fissures can also release hydrocarbons from deep sub-surface reservoirs. Since oil is lighter than water, it floats to the surface. These natural seeps can account for about half the oil in coastal regions—about five times as much as accidental spills—and offer an immense opportunity to understand the fate of oil in the ocean, according to coastal researcher Christopher M. Reddy of Woods Hole Oceanographic Institution in Massachusetts.[54] While out on a boat with a colleague, Reddy noticed freshly-surfacing samples from a natural seep near Santa Barbara, California. Using methods such as comprehensive 2D gas chromatography, Reddy and his team discovered that several molecules in oil were consumed by ocean microbes, and others were so volatile they evaporated within minutes of reaching the water surface. Some chemicals sank back to sub-surface sediments. These studies were the first to help quantify how much oil might be released from a natural seep—in the Santa Barbara instance, it was about 8 to 80 times the amount released by the Exxon Valdez incident.[55, 56] Understanding the fate of oil from natural seeps can help improve responses to accidental spills, and can also identify better ways to interpret chemical fingerprint data.

Oil samples are typically rich in saturated hydrocarbons, and a sample may also contain aromatic compounds, alkenes, and small proportions of metals and other chemicals depending on its source. Collectively, the unique proportions of these chemicals create a sample's distinctive molecular fingerprint, which can help reveal how the components of a spill are dissipated. The 2010 Deepwater Horizon rig explosion released nearly 5 million gallons of oil into the Gulf of Mexico, but 75% of that was neither recovered nor burned. Instead, it spread across the seafloor, dissolved, evaporated, or settled into coastal sediments. "Pinpointing the ultimate fate of the oil released in the disaster is crucial for understanding ecological effects of the spill, as well as for determining how best to respond to future spills to minimize environmental damage," Jyllian Kemsley wrote in an article for *C&EN*.[57] In order to figure out the ultimate fate of oil, one of the most crucial elements is a "ground zero" sample "of material from the wellhead that preserved both gas and oil before they mixed with seawater. That sample is the control against which all others are measured," Kemsley wrote in a 2013 *C&EN* cover story.[58]

Beyond getting the right samples, better analytical tools are also key. Standard gas chromatography tests used to fingerprint samples since the 1980s can miss about half the chemicals present in oil, according to a study of beach sand samples from the Deepwater Horizon spill conducted by Reddy and his colleagues.[59] Using solvent-based extraction coupled with gas chromatography detected many highly oxidized chemicals. “Reddy says overlooking these chemicals could hinder spill research in several ways, including thwarting scientists’ attempts to account for what happens to oil after a spill,” Mark Schrope wrote in a *C&EN* article about the study.[60] Now, more advanced methods aim to improve the detection of these hitherto-overlooked chemicals. In one study, researchers developed a compact device that relies on sensors to detect shear horizontal surface acoustic waves, which can be used to detect benzene and other aromatic hydrocarbons from oil present in water.[61]

Seven weeks after the Deepwater Horizon spill at the Macondo site, the U.S. National Oceanic & Atmospheric Administration (NOAA) conducted a field study sampling atmospheric chemicals over the site. Using levels of benzene, naphthalene, and other compounds, scientists could estimate the flow rate of oil at the well. Lessons from that study helped engineers decide what to do when the Elgin drilling platform in the North Sea sprang a leak in 2012. Based on the atmospheric chemicals detected 24 hours after the leak, researchers determined that the source was a low-pressure reservoir, not a high-pressure formation that would blow up, so workers could be allowed to return to the site and quickly fix the problem. Without the atmospheric data, the alternative would have been a months-long process of digging relief wells.



a) Response of a SH-SAW sensor coated with 1.0 μm PEA, successively exposed to various samples of benzene in water (concentrations are indicated in the graph in parts per billion). (b) Response of a SH-SAW sensor coated with 0.8 μm PIB to various samples of ethylbenzene in water (concentrations in parts per billion).

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Armed with these data, researchers now aim to prepare better for future accidents. Quoting NOAA research chemist Thomas Ryerson, Kemsley wrote in a 2013 *C&EN* story: “The lesson from Macondo and Elgin, Ryerson says, is that atmospheric measurements should be considered critical to spill response, because their quick turnaround time and accurate volume estimates can help mobilize responses of appropriate scale—such as how much containment boom or dispersant to use and what size recovery vessels are needed."[58] In addition, Ryerson told *C&EN* that if researchers could develop a specialized set of critical instruments to equip Coast Guard or other aircraft, they could then "provide key spill information within a few days of the start of a spill," allowing a quicker, more efficient response to accidents.[58]

Wildlife Forensics

Shortly after the discovery that human DNA could serve as a genetic fingerprint to help solve crimes, researchers began extending forensic methods from human crimes to those involving other species. But they quickly ran into a practical problem. Special agent Terry Grosz of the U.S. Fish and Wildlife Service (USFWS) Office of Law Enforcement, who had been assigned to investigate endangered species crimes around the world, found that there wasn't a place to actually examine evidence from wildlife. FBI crime labs did not handle animal data, and few others hired forensic scientists. Efforts to establish such a lab began in the late 1970s, and in 1988 the world's first laboratory dedicated to wildlife forensics was established.[62] The lab helps enforce federal laws that protect endangered plants and animals as well as those covered by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) treaty. Like other forensic labs studying wildlife, the USFWS facility handles a dizzying array of specimens from around the world, including processed parts and products from illegal logging or trade in endangered species. While one investigation may involve the entire carcass of a coyote poisoned by a farmer, others may require researchers to analyze hair or blood, furniture carved out of tropical rosewood, cosmetics, traditional medicine, or a threatened species rescued from the pet trade.

For wildlife forensic investigations, researchers employ techniques similar to those used for human specimens—ranging from visual exams, microscopy, and genetic analysis to chromatography and mass spectrometry—but the challenges researchers face may be quite different. For example, a lab must often begin by figuring out a victim's species. That's often an easier question than narrowing a sample down to a specific individual animal. Nonetheless, these scientific tools can help determine the origins of many samples, such as whether a trafficked product is made from the shell of an endangered turtle or dyed cow horns. In a *C&EN* story, USFWS lab director Ken Goddard told Melody Bomgardner: "Wildlife investigations present special complications. Not every killed animal represents a crime. It usually depends on the species and may depend on time of day or place of death because of hunting laws, for example. We could also have an animal as a perpetrator as well as a victim."[63]

In one recent case, USFWS had to warn hunters not to eat migrating snow geese caught near the Berkeley Pit, a former copper mine in Montana, because the geese had been in waters that were highly acidic and contained high levels of metals.[64]

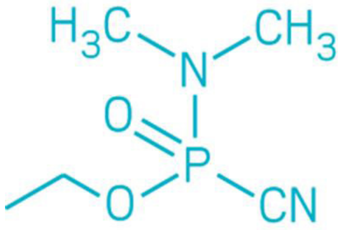
In addition to its efforts to map and help reduce the massive global burden of illegal wildlife trade, the USFWS lab also identifies ongoing challenges and priority areas for wildlife forensics research. Working with an international group called the Society for Wildlife Forensic Science, the team helps establish techniques, standards, and guidelines for forensic data, so that evidence is of a sufficiently high standard as to be admissible in court. These efforts have been particularly challenging for timber. In an article for The Conversation, timber forensics researcher Eleanor Dormontt of the University of Adelaide in Australia writes: "Timber is notoriously hard to identify, even for experts. By looking at the structure of the wood alone, it is usually only possible to identify it to the genus level, rather than the species itself. This is a problem because most timber laws protect individual species, and often only part of the range of that species. This means that law enforcement must rely on the paper trail that accompanies timber shipments, which is open to fraud."[65]

By combining genetic, chemical, and anatomy-based approaches, researchers around the world aim to improve the identification tools available. In a recent article, Andrew Lowe of the University of Adelaide and his colleagues emphasized the strong need for better timber identification technology. Although legislation to prosecute cases of illegal logging exists, actually doing so is difficult because of the lack of scientific evidence. The article's authors stress that investing in better forensic tools and building a database of reference samples should be a global priority.[66]

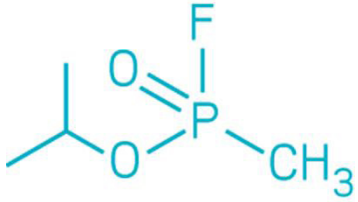
IV. FORENSICS FOR GLOBAL SECURITY

Chemical and biological agents used as a means of warfare have a long history. Ancient hints of chemical use date back to Greek myths about poison-tipped arrows used by Hercules and in the Trojan War[67], and a 14th century memoir suggests that biological warfare may have caused the spread of plague in Europe. In 1346, Mongol warriors in Crimea threw plague-infected corpses into the besieged city of Caffa, transmitting the disease into Europe. Although this is plausible, researchers doubt it was the main cause of the subsequent Black Death pandemic.[68]

During World War I, approximately 1.3 million deaths were a direct result of chemical warfare, where armies used chlorine, phosgene, and mustard gas. Nazi scientists at work during World War II discovered the lethal nerve gases tabun and sarin, which can kill within minutes of exposure.[69] In 1997, an arms control treaty known as the Chemical Weapons Convention came into effect; the agreement outlaws the stockpiling, production, or use of such weapons or their precursors and is mediated by an intergovernmental organization known as the Organisation for the Prohibition of Chemical Weapons (OPCW).[70]



Tabun



Sarin

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Biological weapons, such as toxins and poisons, pathogenic microbes, or pests that target a nation's agriculture, have been developed by many countries over the years. But these weapons were less effective to deploy, due to technical challenges. Their use was initially prohibited by the 1925 Geneva Protocol, and now the Biological Weapons Convention of 1972, accepted by most of the world, prohibits the production, storage, or transfer of these materials.

In addition to chemical and biological arms, nuclear materials may also pose a threat to global security. Nuclear forensics—defined as "the analysis of materials recovered either from the capture of unused materials or from the radioactive debris following a nuclear explosion"—can help trace the origins of these materials and the methods used to make them. Unlike nuclear forensics research during and after the Cold War, modern studies in the field require greater international cooperation among researchers and governments so as to identify source materials, and to share information via international databases and archives.[71]

Forensic studies of chemical, biological, and nuclear weapons were once spurred by the fear that these tools might be used by the governments of warring countries. With greater cooperation and peacekeeping efforts, the potential for government-sponsored use has declined. But these materials continue to pose security risks because of the potential for their use by terrorists or individuals with technical expertise or access to stock materials. The threat of global pandemics caused by pathogens such as SARS has heightened the need for microbial forensic studies to trace the origins of emerging infectious agents. Working with law enforcement and regulatory authorities, researchers now use varied forensic approaches to understand threats and improve global security.

Nuclear Materials

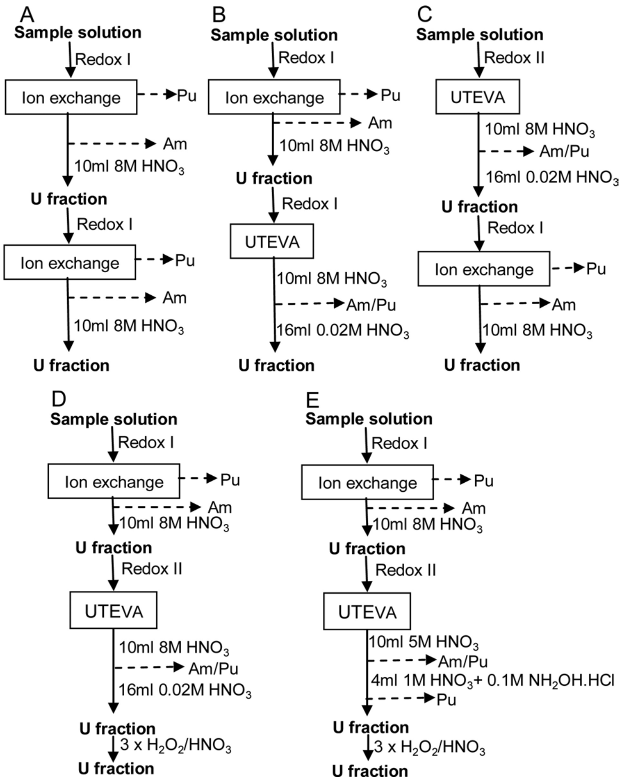
Nuclear materials have a much shorter history than chemical or biological weapons, but minuscule amounts of nuclear fuel carry the potential for large-scale destruction. At first, the field of nuclear forensics burgeoned in clandestine efforts by countries to track each other's nuclear weapons programs. The earliest reports of such efforts stem from 1944, when the U.S. Air Force attempted to detect atmospheric radioactive xenon gas—which is released during the production of plutonium from uranium—in an effort to monitor the German nuclear program.[72]

Since the 1990s, nuclear forensics studies are largely conducted in efforts to combat illegal trafficking and to support legal investigations of criminal acts involving nuclear materials. The International Atomic Energy Agency reported 1,340 confirmed global incidents of illicit trafficking and unauthorized activities involving nuclear and radiological materials between 1993 and 2007.[73] Many of these were materials smuggled in Europe in the early and mid-90s, according to a 2009 report from the U.S. Nuclear Regulatory Commission. In a *C&EN* story about nuclear forensics, Elizabeth K. Wilson writes: "These smugglers presumably hoped to sell the contraband to shadowy figures intent on building weapons. Paralleling drug-smuggling practices, the quantities are usually quite small, likely introductory samples for potential buyers. Frequently, the material is 'junk'-non-weapons-grade scraps—but sometimes plutonium and highly enriched uranium have been uncovered."[74]

In the present day, techniques developed to track such smuggling are being applied in other contexts. Analytical chemistry for radionuclide elements has helped solve historical mysteries; in one instance, analysis of a uranium cube recovered from physicist Werner Heisenberg's last experiment revealed that Nazi Germany did not have the means to produce a working nuclear weapon during World War II.[75] And perhaps most importantly, these studies provide the evidence to develop global standards for how nations can best respond when nuclear materials are found outside authorities' control.

Nuclear forensics researchers use a wide range of methods to glean data from unused fuels, known as pre-detonation analysis, or from spent material, in post-detonation forensic tests. Some studies, such as the analysis of trace elements, are only feasible with pre-detonation samples, since these chemicals are lost during use. Chemical tests unveil many physical and chemical properties of radioactive materials that can help identify their legal owners or place of origin.

Isotopic ratios – The isotopic composition of uranium and plutonium have been the most widely used chemical signatures used for forensic analysis. Uranium deposits typically contain varied proportions of three isotopes, namely U-235, U-234, and U-238. In natural fuels, the abundance of U-235 was initially considered a constant. But recent research has shown that tiny variations in the ratio of U-235 and U-238 can occur and may be used for forensic studies. One study, for example, found that low-temperature deposits are on average 0.4% heavier than uranium deposited at high temperatures, and these variations help trace the source of an ore.[76] The isotope ratios of plutonium, a man-made nuclear fuel, reveal the type of reactor used to produce it and traits of the uranium used as starting material—details which can provide clues to the source of unidentified plutonium samples.[77]



Schematics for separation procedures A to E tested for purification of the uranium fraction from aged plutonium samples for plutonium age determination.

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Age dating – Age dating, which tests the ratio of a parent radioactive material to its breakdown products, can help reveal the last time a material was purified. “This signature was found to be highly valuable in nuclear forensic investigations as being a predictive characteristic (i.e., no comparison sample or information is necessary) in comparison to the other parameters used for origin assessment,” according to Klaus Mayer of the Nuclear Forensics International Technical Working Group and colleagues.[78]

Chemical impurities – Trace elements or metals such as lead and strontium are sometimes added to fuels to impart specific properties. In other instances, these impurities are left over from original ore samples or processing. Patterns such as the presence of certain elements or their isotope ratios can be used to distinguish ores from different sources. In one study, Elizabeth Keegan of the Australian Nuclear Science and Technology Organisation and her colleagues could identify uranium ores from 19 different mines based on the signatures of trace elements present.[79]

Physical properties – Different manufacturing processes result in different physical properties, such as particle size, shape, porosity, or other features, all of which may serve as distinguishing features to identify a material's origin.

Although many of these forensic tests were developed as a way to track fuels for reactors and weapons, the same methods extend to radioactive materials that are widely used in industry and medicine. And they have been found to be useful when studying a traditional crime scene that also includes radiological evidence. So far, studies suggest that radioactive materials do not affect the data that can be gleaned from DNA, fingerprints, electronics, or other trace evidence. The outstanding question with these situations, however, is identifying the best way to safely study such evidence to glean the most information. In a feature for *Analytical Chemistry*, Keegan and her colleagues write: "Forensic laboratories are generally not equipped to handle radioactive materials in any quantity. The options are either to decontaminate the evidence prior to its analysis at a forensic laboratory or to examine the item with the contamination in situ at a laboratory equipped to handle radioactive material."[80]

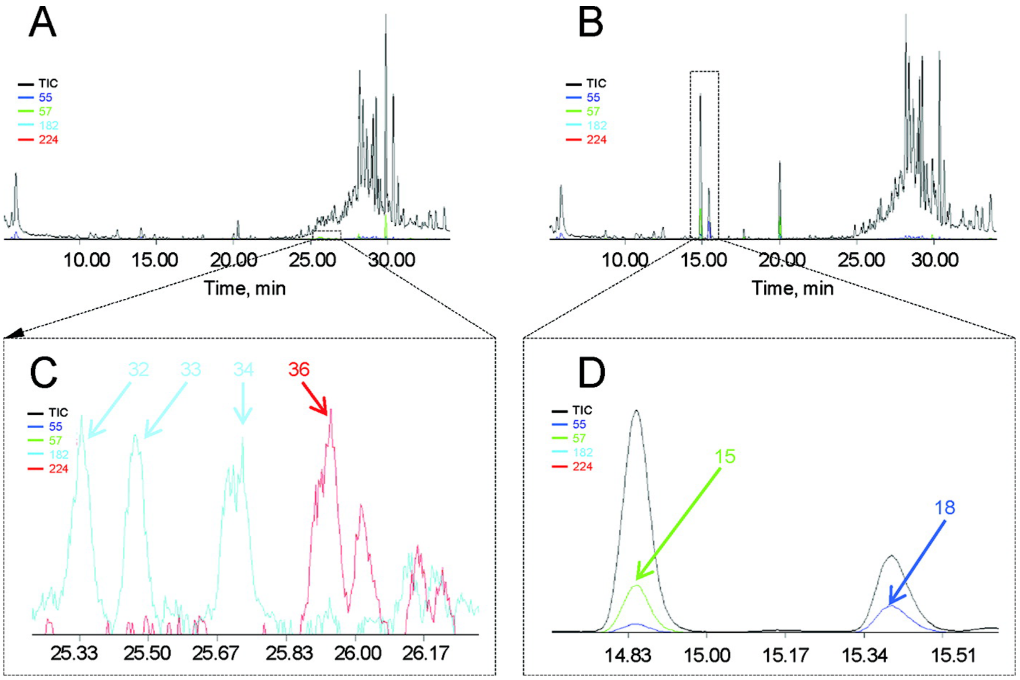
Despite many advances in technology and the expanding uses of nuclear forensics, certain hurdles remain. The 2009 NRC report noted that agencies handling such evidence face challenges in reducing the time needed to arrive at conclusions from testing, and were experiencing a shortage of investigators skilled in techniques such as radiochemistry. In a *C&EN* article about the report, David J. Hanson writes: "According to the report, there are too few people skilled in nuclear forensics, and the facilities and equipment used for most nuclear forensics work are old, outdated, and not built to modern environmental and safety standards. NRC recommends that the several agencies responsible for this security issue streamline their organizational structures and responsibilities, work to build a larger modern forensics workforce, and adapt their nuclear forensics efforts to the challenges of real situations."[81]

Chemical and Biological Weapons

While some chemicals slip into streams and soils from routine uses, others spill over accidentally. And still others are deliberately let loose with the intent to harm. State-sponsored use of chemical weapons is now prohibited; as of 2016, 90% of the world's stockpiled chemical agents have been destroyed.[82] Nonetheless, chemical weapons such as sarin, chlorine, or fentanyl gas continue to be used in terrorist attacks around the world; thus, it is crucial for authorities to have the means to track these chemicals to their sources in order to find the perpetrators.

Because molecules carry their own history within their elemental constituents, researchers can trace their origins using techniques such as impurity profiling, stable isotope analysis, or Raman spectroscopy. In the U.S., these studies are largely supported by the Department of Homeland Security's Chemical Forensics Program. One common technique, based on impurity profiling, relies on identifying a chemical attribution signature (CAS) for a particular compound. The CAS carries data on minuscule amounts of impurities in ingredients, side products, or materials formed when a chemical breaks down. Much like a fingerprint, "that signature can help reveal the route used to make a compound, the conditions a compound was prepared under, and even what specific batch of a precursor was used," writes Bethany Halford in a 2012 *C&EN* story.[83]

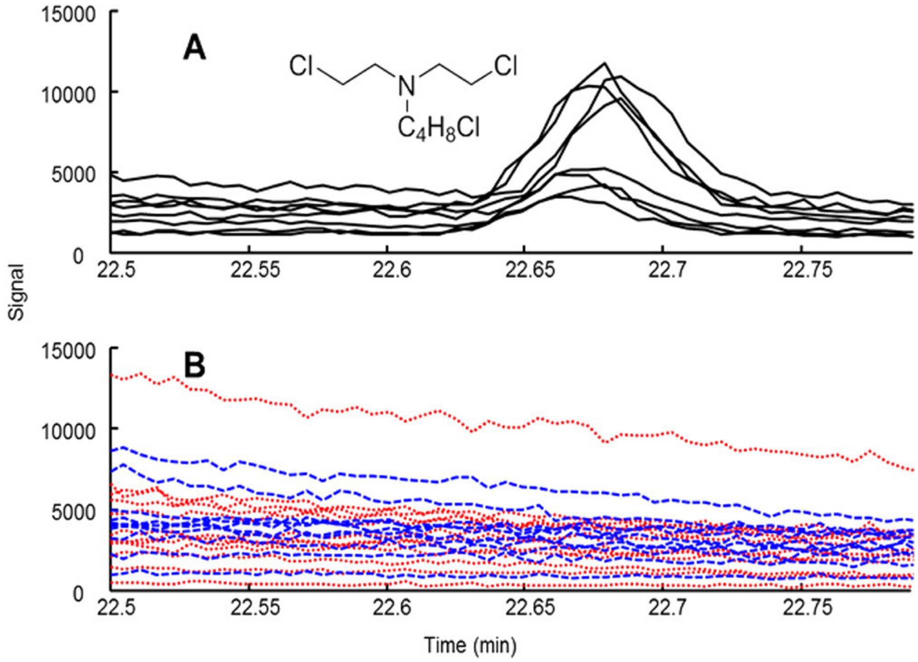
Carlos G. Fraga of Pacific Northwest National Laboratory in Richland, Washington, began his studies of chemical forensics after more than a decade as an officer of the U.S. Air Force, where he researched and dealt with chemical weapons-related materials.[84] In 2011, Fraga and his colleagues used gas chromatography-mass spectrometry (GC/MS) data to match lab-made samples of the nerve gas sarin to their precursor materials. By tracing impurities such as hexanone, pyridine, or benzyl alcohol present in five batches of stock material and six samples of sarin, the team found that 57-88% of impurities persisted through various stages of synthesis and could be used to match sample to source.[85]



Overlays of the TIC chromatogram and selected-ion chromatograms for *m/z* values of 55, 57, 182, and 224 for (A) G-DC-1-3 and (B) A-DC-1-3. Blow-up sections depict some of the 43 GC/MS peaks that are part of the impurity profiles for (C) G-DC-1-3 and (D) A-DC-1-3.

Reprinted in part from: Anal. Chem., 2011, 83 (24), pp 9564–9572
DOI: 10.1021/ac202340u

Similarly, these researchers produced 30 batches of tris(2-chloroethyl)amine (HN3), a kind of mustard gas, using different combinations of commercial stock reagents, and found they could identify distinct impurity profiles for each sample of mustard gas and stock reagents, and that these signatures could point to the origins of samples.[86]



(A) Overlaid extracted ion chromatograms (*m/z* 154) depicting component A present in all HN3 batches (n = 8) synthesized using TEA stock S. (B) Absence of signal for component A in HN3 batches (*n* = 22) made not using TEA stock S. Component A had an estimated concentration of 40 ppm in HN3 based on relative TIC peak areas.

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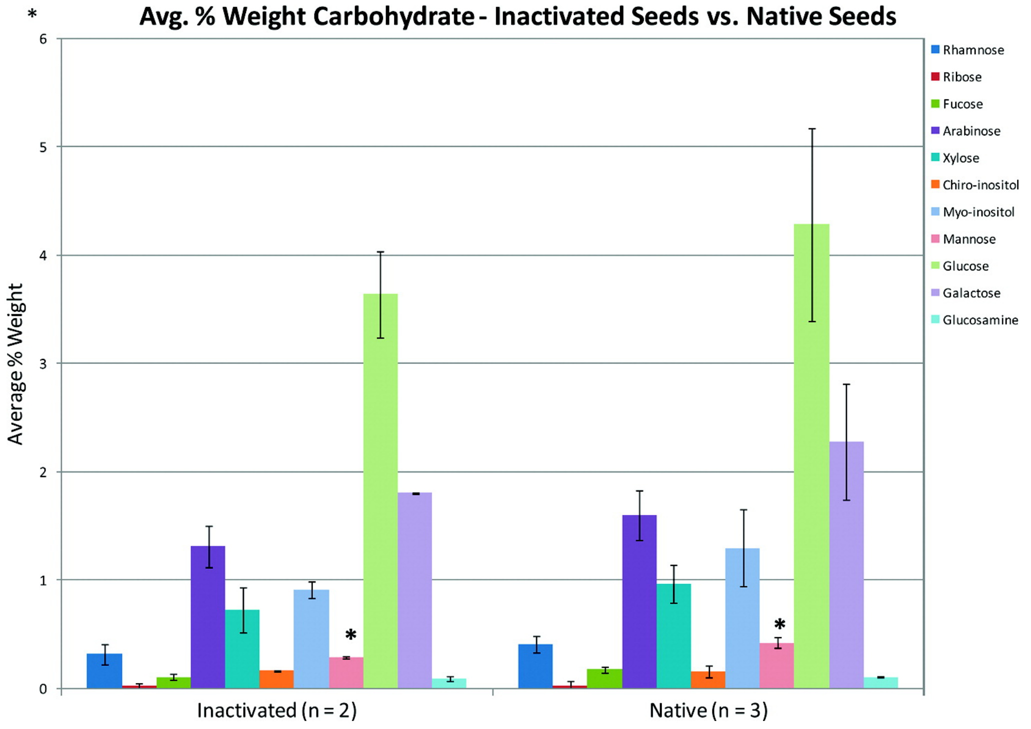
A second method, known as isotope ratio mass spectrometry (IRMS), relies on identifying the characteristic proportions of elemental isotopes of sulfur, hydrogen, oxygen and others that occur in different natural sources. Using the abundance of C-13 and N-15, for example, researchers can identify specific production batches of synthetic drugs, pinpoint their starting materials, and determine how the materials were made. Toxic cyanide gases, for example, were identified by the U.S. Centers for Disease Control and Prevention as one of the most likely agents to be used in a chemical terrorism attack. Helen Kreuzer of Pacific Northwest National Laboratory and her colleagues reported that, based on stable carbon and nitrogen isotope content, cyanide samples could be matched to their source correctly 95% of the time, and thus could serve as a useful forensic signature.[87] “The strength of using the stable isotopes is that you can tell the difference between substances with the same exact chemical composition, so it works for highly purified compounds where there may not be a signature from impurities,” Michael Singleton of Lawrence Livermore National Laboratory told Halford for *C&EN*.[83]

Another laboratory technique being adapted to the field for the detection of drugs and explosives is Raman spectroscopy. Modified Raman-based techniques are also being used to spot illicit or hazardous materials hidden within non-transparent packages.[88] Other laboratory methods gradually being adapted to these analyses include nuclear magnetic resonance (NMR), high-performance liquid chromatography/mass spectrometry (HPLC/MS), GC/MS, ion mobility spectroscopy, and more.

Biological Agents

Many of the techniques used to analyze chemical agents are also applied to toxins or materials of biological origin. The castor plant (*Ricinus communis*) is a common ornamental, and it is widely cultivated for castor oil production. Castor seeds are considered an agricultural product, but when split open they yield ricin, a lethal, fast-acting toxin with no antidote. Ricin has been used in bioterrorism attacks around the world, including a 2003 case where letters laden with the substance were recovered from a South Carolina post office. Ricin can be prepared using various "kitchen recipes" or laboratory procedures; the proportion of castor oil, proteins, and carbohydrates in a ricin sample varies depending on the method used to extract the toxin. Using GC/MS, researchers have found that they can identify how a sample was prepared based on the relative abundance of fats and sugars present.[89]



Comparison of castor seed mash prepared from heat inactivated and native castor seeds. Error bars represent 1 standard deviation. Asterisks indicate statistically significant differences between inactivated and native seeds.

Reprinted in part from: *Anal. Chem.*, 2010, 82 (14), pp 6040–6047 **DOI:** 10.1021/ac1006206

But applying forensic approaches to living organisms is more complex. Microbial pathogens—whether fungi, bacteria, or viruses—have the ability to replicate themselves, can evolve over time, and can be obtained from multiple sources, making it difficult to attribute an attack to a precise origin. With certain microbes, such as the food-borne pathogen *Salmonella*, it may even be difficult to ascertain whether an outbreak was accidental or deliberately caused.

Efforts at using microbiology to study bio-weapons began in the early 1990s, when scientists tried to use genetic sequencing to trace the spread of infections such as HIV/AIDS.[90] In an early application to security, researchers at Los Alamos National Laboratories analyzed tissue samples from victims of a 1979 anthrax outbreak in the former Soviet Union. They identified DNA sequences known as variable number tandem repeats (VNTR) present in different genes. These VNTRs differ in length depending on how many times a certain DNA sequence is repeated within the gene, and these lengths help scientists determine how closely related different strains of a given bacterium are.

Using this analysis, the team found that the 1979 victims had been infected by multiple strains—whereas a natural outbreak would typically be caused by one strain of the pathogen that was spread between individuals. The study validated other data suggesting that the outbreak had occurred due to an accidental release of spores from a Soviet biological weapons production facility.[91]

Since 2001, large advances have been made in the field of microbial forensics, fueled in part by the investigation of anthrax mailed to news offices and U.S. senators in September of that year. Aided by technological advances such as high-throughput, next-generation sequencing and the falling cost of whole genome analysis, researchers can now use a wide range of methods to characterize infectious agents with the potential to cause epidemics. In addition to DNA information, researchers rely on a suite of physical, chemical, and microbiological analysis such as electron microscopy, microbial cultures, chromatography, and more. Beyond the threat of bio-weapons, microbial forensics holds promise as a means to control emerging infectious diseases and improve global health.

Case Study: The Amerithrax Investigation

Spores of the bacterium *Bacillus anthracis* are invisible to the naked eye, but their effects become quickly apparent. Within days of exposure, a person risks disease that begins as local ulcers or lung infections and quickly develops into fever, pain, and shortness of breath. Even with modern treatments, anthrax infections are fatal in 28-45% of cases.[92]

In September 2001, letters containing a dry white powder—pure anthrax spores—were delivered to several media outlets and the offices of two U.S. senators. At least 22 people contracted infections from being exposed to these letters; five suffered fatal lung infections. Working with investigators and law enforcement officials on the case, known as Amerithrax, researchers turned to microbial forensics to track down the source of the spores. The formal investigation ended in 2010; in 2011, researchers published the first scientific paper based on their forensics analysis.[93] A press release linked to this publication states: "As one of the first and most high-profile investigations of its kind, Amerithrax has helped to shape the emerging field of microbial forensics."[94] The researchers grew samples of spores from the letters in the lab and identified four variant strains that formed yellow or yellowish-gray colonies with textures, shapes, and sizes that were distinctly different from the typical gray-white colonies formed by ancestral strains of *B. anthracis*. The researchers extracted DNA from these variant colonies and performed whole-genome sequencing, eventually identifying four unique mutations that were present in eight of the mailed samples but not in a standard lab strain of bacteria. All eight of these samples were traced back to one particular flask of microbes. Combined with other evidence, this scientific evidence eventually led investigators to now-deceased Army scientist Bruce Ivins.[95]

In the press release, study author David Rasko, assistant professor for microbiology and immunology at the University of Maryland School of Medicine and a research scientist at the Institute for Genome Sciences, said: "Before Amerithrax, no one appreciated the precision, accuracy and reliability that this type of genomics can offer as a microbial forensic technique. To this day, this is still the only case in which microbiology and genomics have been used in a criminal investigation. Microbial forensics would be a critical investigative tool if another bioterror attack were ever to strike the U.S."[94] In their research paper, the authors wrote that the accuracy and reliability of whole-genome sequencing as a microbial forensic technique was not apparent before Amerithrax. The investigation helped show how these data could serve much like a human genetic fingerprint to link microbial samples to their sources. Now, the team and officials are working to establish clear standards and validated techniques to produce microbial forensic evidence that can hold up in criminal court.[93] "It is a much higher standard than our own academic research," Rasko said. "Your results need to be completely foolproof and stand in a court of law. Those are the kinds of standards and guidelines we're developing now, so that microbial forensic scientists can be prepared in the event of another biological attack."

V. CONCLUSION

Across nearly every domain of human influence, people have found that scientific advances can be used to aid and inform the practice of the law. This highlights the promise and the many challenges that lie ahead for forensic science and its practitioners.

Following nearly a century of evolving as a field that served legal investigations rather than a conventional science, forensic science has now arrived at a critical inflection point.[96] The widespread use of forensic investigations—to solve crime, to accord responsibility for environmental damages, or to ensure that peacekeeping efforts are successful—make it near-impossible to envision a world where science and its methods are not available to legal investigations. Whether data stems from a human crime scene or an environmental one, it's obvious that chemists and the myriad quantifiable techniques they employ will play a key role in the future of forensic science. Yet the 2009 examination of forensics as a field by the NRC, followed by the 2016 evaluation by a Presidential panel, have revealed the many pitfalls of mixing science and law enforcement. The need for better training for forensic scientists and legal investigators is apparent, as is the need for better laboratory accreditation and clear, quantifiable standards for data presented in a courtroom.

Scientists, ethicists, and legal experts from various spheres have now begun conversations to improve standards for forensic evidence so that it remains relevant, accurate, and useful in modern society. The barriers are not just ones of technology or education. Future forensic scientists will need to gauge the ethics of situations such as the use of discarded DNA to track down suspects or the possibility of remotely accessing a person's electronic devices without their permission. Databases built for forensic science—which may hold anything from genetic information to nuclear signatures—could be misused if they fall into the wrong hands, or be used to bypass an individual's civil liberties. The future of forensic science hinges on successfully navigating both the technological and ethical landscapes.

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