nated R&D program. Consequently in 2005, NPA built research facilities for dogs adjacent to APOPO's rat facilities and in early 2006, NPA moved its dogs and equipment there from Lubango. McLean and Sargisson had recently resigned from GICHD, but Dr. Max Jones had been hired to direct the dog program. Jones established an Advisory Committee (comprising chemists, psychologists and dog-training specialists) and lobbied for a new approach to REST R&D; namely, one that relied less on experts' opinions and more on findings with the animals. He stressed the importance of carefully controlled research using the methods characteristic of a field known as **behavior analysis**. 11 This approach is described in the following section.

Behavior Analysis and REST

The field of behavior analysis favors within-subject experimental designs, which involve comparing repeated measurements of an animal's performance across different conditions of interest, in order to obtain meaningful information with a small number of animals. Jones' team tried to replicate procedures precisely across training sessions, and to control all those variables that could affect the dogs' detection accuracy, so that the variation in accuracies across sessions was minimized and the effect (if any) of a single procedural change could be assessed.

To this end, all procedures were carefully documented, staff received regular training and reliability checks were conducted. The overall plan was to add complexity to the training and testing procedures gradually and

stimulus control by the intended target odor (the odor of landmines) and instead allowed some other odor difference between positive and negative samples to serve as the basis for the rats' discrimination. Apparently, because all of the positive training samples came from APOPO's minefield, the rats learned to respond to odors unique to that location, and not to odors unique to landmines. NPA's REST researchers in Angola had recently observed similar inappropriate stimulus control in dogs. Unfortunately, in both cases it was impossible to establish exactly what odor fea-

tures were functioning as cues.

The Angola research did, however, yield some interesting results. For example, a recently published study by Sargisson and McLean,¹⁰ who were then employed by GICHD, shows that for six dogs in the Angola program the hit rate was higher and the false alarm rate lower when the rate of reinforcement was medium or high than when it was low. That is, the dog's performance was poorer when a low percentage of correct responses was reinforced (i.e., rewarded) than when the percentage was substantially higher. The results of the filter tests in Angola and the reinforcement experiments clearly illustrate the value of applying signal-detection analysis to the performance of explosives-detection animals and the importance of reinforcement (reward) scheduling in

influencing that performance.

Although APOPO's and NPA's test results were viewed as setbacks, in hindsight they were useful because their similarity highlighted the need for greater cooperation among NPA, APOPO and GICHD, and for a coordi-

system appeared feasible for two reasons. First, Mechem was already using such a system.5 Second, the animals in REST perform a detection task fundamentally similar to that faced by ERW-detection animals in minefields, which had had reported success.

O Photo courtesy of Jessie Poling. We are the past decade, there has been considerable interest in the was used to detect explosives and small arms in vehicles and transport detection accuracies for operational REST sy with explosive remnants of war using a system known as Remote Explosive Scent Tracing. Since 2005, research has been underway in Morogoro to develop an operational REST system using dogs and giant African pouched rats as detection animals. The typical procedure is summarized as follows. A team uses a suction pump to vacuum the air over a road section, typically 100 or 200-m long and about 5-m wide. The air is sucked through a filter, and careful records are kept of the road section that each filter represents. The filters are transferred to a laboratory where they are presented to trained detectors (usually dogs or rats) using a standard methodology, such as on the arms of a carousel or in a line of stands.

In February 2002, the first REST workshop was held in Morogoro. Attendees were generally enthusiastic about the value of REST, particularly for minefield detection. Nonetheless, the challenges posed by this application were also discussed at length (as by Bach and McLean¹) and attendees endorsed the need for a substantial program of research to optimize a REST system. Despite the complexity of the issues they faced, the researchers were confident that operational REST for ERW would soon be developed.

was used to detect explosives and small arms in vehicles and transport containers during the South African Border War.⁴ When applied in humanitarian demining, it was reported to be fast, inexpensive and able to detect ERW that were missed by prior searches.⁵

That confidence is evident in the 2003 publication of *International Mine Action Standards 09.43: Remote Explosives Scent Tracing.8* This document, and the revision that followed in 2005, provided guidelines and minimum detection accuracies for operational REST systems. Similarly, in 2004 McLean and Dr. Rebecca Sargisson noted that REST "is likely to be implemented for road clearance in Sudan and Angola by the end of 2004."9 Furthermore, this confidence was matched by increased resourcing of research and development. GICHD employed Sargisson to work on the project, NPA and GICHD shared the costs of a research facility in Lubango, Angola, and APOPO joined the fold by allocating staff and resources for REST research. In addition, GICHD contracted the analytical chemist, Dr. Kai-Uwe Goss, to investigate filter materials and sampling techniques, and assisted APOPO in establishing a chemistry laboratory.

Early Findings from Morogoro and Elsewhere

While chemists were studying filter materials and sampling methods, polyvinyl-chloride gauze filters developed by Mechem were used to construct training samples for NPA's dogs and APOPO's rats. Unlike Fjellanger *et al*.'s method, however, positive samples were created by drawing air from immediately above a buried landmine through a filter, whereas negative samples were created by drawing air above ground distant from mines.

Preliminary results appeared quite promising in that pouched rats reliably indicated filters (by scratching at them) from APOPO's training minefield, but they did not indicate filters from other locations where mines were not present. When tested with positive samples from other locations where mines were present, however, the rats failed to indicate (missed) most of them. These results suggested that their training had failed to establish

by Brent "Max" Jones [University of Massachusetts Medical School], Rune Fiellanger [Fiellanger Dog Training Academy], Christophe Cox [APOPO] and Alan Poling [Western Michigan University]

A pouched rat identifies a positive sample by pausing and scratching at a hole above an aluminum pot containing soil to which mine water has been added.

REST refers to a method for identifying areas of land that contain chemical residues of explosive remnants of war (landmines, unexploded ordnance, etc.). REST involves collecting samples of air or dust from defined locations and presenting those samples to mechanical or animate detectors in a remote location. Areas producing samples judged to be positive by the detectors are then either searched more thoroughly by other methods, such as direct-detection animals or humans with metal detectors, or cleared by machines. Areas producing negative samples are exempt from further inspection except for quality control.

From 2002–10 the Geneva International Centre for Humanitarian Demining joined other agencies to support a series of REST workshops and a REST research project centered in Morogoro, Tanzania. The research project recently came to an end and its findings, along with the status of REST in general, were the topics of a GICHD workshop held in Geneva from 23–25 November 2010, and a forthcoming GICHD publication that will appear in early 2011, titled *Remote Scent Tracing (REST) of Landmines: 1990–2010*. A few peer-reviewed publications directly relevant to REST for ERW have been published,^{1,2,3} but no systematic overviews are available. The GICHD publication, therefore, will be of value as a reference work and the present manuscript may interest a general audience.

Early REST Activities

REST for ERW is not new. In the early 1990s, a South African company, Mechem Consultants, began using REST with dogs to search for ERW in Mozambique and Angola. Their system evolved from one that

Given the apparent success of Mechem's system, Norwegian People's Aid contracted Mechem in the late 1990s to supply dogs, equipment and personnel when they sought a REST program to support their demining activities in Angola. Several months into this contract, NPA asked GICHD to evaluate the accuracy of their system, as well as the general methods used in it. The first major study of REST was subsequently conducted in 2000. Unfortunately, in situations where their handlers did not know the number and position of positive samples, the accuracy with which dogs indicated (sat in front of) samples taken from mine-contaminated areas was less than satisfactory. NPA subsequently suspended their planned operational use of the system in favor of conducting research and development, and forged a relationship with GICHD for that purpose. GICHD supplied the services of Dr. Ian McLean (an environmental biologist) and Mr. Rune Fjellanger (a dog-training specialist) to develop more effective procedures for NPA's system.

Fjellanger, McLean and Espen Kruger Anderson (who works at the Fjellanger Dog Training Academy)² focused on the preparation of dogs as the detectors in a REST system. They demonstrated that with about six months' training, each of the four dogs learned to indicate filters through which air had been vacuumed over 2-4-6 TNT, the explosive found in many ERW. The functional utility of Fjellanger et al.'s system² was then assessed in a subsequent pilot study³ using the same dogs but filters from test minefields in Bosnia. Positive filters were created by drawing air through filters in the near vicinity of landmines, whereas negative filters were created in the same manner but in mine-free areas. Overall, the dogs indicated 60 of 88 of the positive filters (a hit rate of 68%), suggesting that the system had potential but that further work was needed to improve its accuracy.

Land Release and REST

By 2000, many operators in humanitarian demining had embraced the principles of land release^{6,7} in order to maximize returns from limited resources. The promising results reported by Fjellanger *et al*. combined with the appeal of REST for land-release applications generated considerable interest in the technique. In addition, an operational REST

Remote Explosives Scent Tracing of Explosive Remnants of War: A Perspective from the 2010 Morogoro Workshop

In March 2010, a workshop was held in Morogoro, Tanzania, to consider the past, present and future status of the Remote Explosive Scent Tracing system for explosive-remnants-of-war detection. This article summarizes the workshop's discussions and explains lessons learned from the REST research project in Morogoro.

field samples and to use this information to create training samples, to train animals with these samples, and finally to test them with samples obtained from mined areas (in Mozambique and Angola) and nearby areas with no mines. Unfortunately, the analytical chemistry proved more difficult than expected, and this work was in its early stages when the 2010 REST workshop convened.

The Current Status of REST

Since it began, the primary goal of R&D into REST was to develop an operational, empirically validated system for ERW detection that comprised procedures sufficiently documented to allow others to replicate the system. When the project started, the future of

> REST looked promising: NPA had real need for such a system, Mechem was winning United Nations contracts with its REST system, and GICHD wanted REST technology to be documented and in the public domain.

> Unfortunately, an operational REST for ERW system has not yet resulted from the Morogoro project. Moreover, the need for REST has been reduced by the application of long-leash minedetection dogs for clearing suspected hazardous areas during Technical Survey. Mr. Terje Berntsen, manager of NPA's Global Training Centre for Mine Detection Dogs, described this system at the 2010 workshop. Not unexpectedly, NPA and GICHD representatives revealed at that workshop that neither organization had funds available to spon-

sor further REST R&D. Put simply, the project had failed to produce an operational system by the agreed deadline and would not be continued. Therefore, the dog-training facility closed quickly and APOPO reduced its R&D of REST for ERW.

Although many of the researchers involved with the Morogoro project aspired to a progressive, scientific approach, the actual work often involved attempts to produce an operational system as quickly as possible. Therefore, procedures sometimes were changed radically and without adequate investigation of the relevant behavioral phenomena when problems were identified. Throughout the project, changes in sponsorship and high staff turnover reduced research productivity and quality. Moreover, the absence of consis-

selected to appear in samples according to specific rules. In addition, each animal's hit and false-alarm rates were analyzed for each contaminant and soil type separately. Although results were often promising, consistent detection of TNT solution did not occur across all soil types and contaminants, and the animals were not tested with samples taken from areas that actually contained landmines.

Disarray and Brief Renewal

NPA sponsored the dog program until April 2007, when it was replaced by the Swedish Rescue Services Agency. In addition, Jones resigned from GICHD in August 2007, leaving Dunn to direct the dog project. Another Morogoro REST workshop occurred in October that same year. Although significant progress was reported, the Advisory Committee and the Moro-

goro researchers drafted a plan for evaluating procedural changes that moved the system closer to being operationally viable, and for investigating a subset of the behavioral phenomena that had been discovered.

Unfortunately, several months after the 2007 workshop, Dunn resigned from the dog project, SRSA terminated its sponsorship, and research activities rapidly deteriorated. In fact, plans were laid to terminate employment of local staff, give the dogs to another organization and donate the infrastructure to Sokoine University of Agriculture in Tanzania. Members of the advisory

committee argued successfully for the project's worth, however, and after several months without adequate project supervision NPA resumed control of it in April 2009. At that time, GICHD recontracted Fjellanger to direct the project and hired Dr. Adee Schoon, an expert in establishing and evaluating scent detection by animals, to consult on the project. They were given to the end of the calendar year to establish an operational system and quickly planned training and testing steps for the dogs and APOPO's rats, and some chemistry studies for APOPO's laboratory. In August 2009, Dr. Alan Poling was appointed as APOPO's Scientific Advisor and assisted with REST R&D.

As Fjellanger and Schoon were implementing their plans, chemists from APOPO and other labs learned that soil around landmines contained much higher concentrations of TNT-breakdown products (2,4-DNT; 2-A-DNT; 4-A-DNT) than of TNT itself. These findings caused the REST researchers to question whether TNT in solution was in fact the best training stimulus. Despite knowing little about the relative salience of various chemicals as odor cues for rats and dogs, participants as another REST workshop in January 2009 concluded that presenting the whole "bouquet" of a mine's odor in positive training samples should result in a more accurate REST system.

After tests demonstrated that rats trained on only TNT did not reliably identify field samples collected over mines, in the summer of 2009, Fjellanger and Schoon began to

train rats and dogs to detect a mine's bouquet. To accomplish this, all of the dogs and some of the rats began receiving positive samples where so-called "mine water" was added to a measured amount of soil, and negative samples where only water was added to soil. Mine water was produced by soaking a given type of landmine for several weeks in tap water that covered the mine completely.

Dogs and rats readily learned to indicate mine-water samples, but their performance was strongly affected by the type of soil presented and preliminary tests indicated that soil samples collected near mines in APOPO's minefield were not reliably detected. Plans were made to use a headspace analyzer and mass spectrometer recently purchased by NPA to analyze potential odor cues in training and

Despite these struggles, much has been learned from the Morogoro research. This knowledge has valuable applications in the training and testing of animals in a range of odor-detection roles (including direct detection of ERW), and efforts, perhaps to be supported by GICHD, are underway to publish some of those findings.

systematically (by making single procedure changes), eventually developing operationally viable techniques. Jones and his assistant, Yolande Dunn, also devised a brief test methodology to regularly assess whether irrelevant odor features of positive training samples had acquired stimulus control.

Two general procedural changes were made in the APOPO and NPA programs at this time. First, both ceased using filters and began presenting measured amounts of sieved soil in aluminum canisters as samples. This change was in anticipation of collecting loose surface dust in operational REST samples because Goss had discovered that such dust yielded higher and more consistent levels of explosive compounds from mines/ UXO than air samples. In response to this finding, the Morogoro team also began developing a prototype device for collecting dust particles.

Second, rather than using operationally viable devices and methods to collect training samples from field sites, researchers began manufacturing samples under laboratory conditions. Specifically, a measured amount of TNT in solution appeared on positive samples to mimic the soil above a mine or UXO. Unlike using field samples, constructing samples in the lab potentially offered precise control of target and other odors presented to animals. Moreover, as previous findings illustrated, positive field samples needed to come from a large number of contaminated locations, including actual minefields that were discovered with minimal application of detection technologies and thus left virtually undisturbed, as well as negative field samples from minimally inspected

locations that were previously considered hazardous but actually free of ERW. Collecting a large quantity of field samples posed insurmountable logistical and safety challenges.

The first attempts to train dogs and rats to indicate the presence of TNT in solution involved making a uniform set of positive samples and a uniform set of negative samples with the only difference between sets being, in principle, that the positives contained TNT. Both NPA and APOPO researchers found that animals trained with these samples readily learned to indicate only positive samples, and that detection accuracies generally decreased as the amount of TNT in a sample decreased. This decrease suggested that the odor emanating from TNT in solution—and not some other odor common to all positives—was the stimulus controlling the indication responses.

However, several challenging phenomena were also revealed in this work. For example, both groups found that hit rates fell more rapidly wiht the decrease in TNT concentration when concentration was varied within a training session than when it was varied between sessions. They also found that detection accuracies usually declined on the first few sessions in which a different soil type was used in all samples.

This latter result illustrated the importance of varying the irrelevant odors on positive and negative samples, and both programs implemented systems (albeit different ones) for doing this. A library of around 600 contaminants (including foods, plants and inorganic materials) and about 20 soil types was established and the contaminants/soil types were

A dog sniffs a container with soil samples, some of which contain a small amount of TNT (positive sample) and others that are TNT-free (negative sample). The dog moves around the carousel, sniffing samples at the end of the arms and identifying positive samples by sitting beside them. *Photo courtesy of Max Jones.*

Christophe Cox leads APOPO as its Chief Executive Officer. Cox holds a Master of Science in product development and development sciences and has many years of management experience in East Africa. He created most of APOPO's training strategies and devices.

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Rune Fjellanger leads Fjellanger Dog Training Academy as its Chief Executive Officer. Rune holds a Master of Science in zoological and human physiology and has many years of practical experience in many kinds of detection dog training. He is especially interested in remote scent tracing by animals.

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tent daily supervision by senior researchers made it difficult to ensure that procedures were consistently implemented correctly by laboratory assistants and trainers. Finally, in part because procuring required equipment and supplies was slow and difficult, detailed chemical analysis of samples proved extremely challenging and failed to provide useful information for preparing training samples.

Despite these struggles, much has been learned from the Morogoro research. This knowledge has valuable applications in the training and testing of animals in a range of odor-detection roles (including direct detection of ERW), and efforts, supported by GICHD, are underway to publish some of those findings.

The Future for REST

M ost of the current methods for an-
alyzing explosive contaminants involve chemical extraction of explosives from collected soil samples. The complexity of these techniques typically requires that the samples be moved off-site. In addition to requiring extensive handling, expensive equipment and highly skilled workers, these methods involve transferring soil samples to a laboratory and using extraction techniques according to U.S. Environmental Protection Agency Method 8330.1

Developing an operational REST system for ERW detection is an extremely complex interdisciplinary undertaking that poses significant challenges for engineers, analytical chemists and behavioral scientists. In the end it may be impossible to overcome those challenges and develop a workable system. It is, however, premature to assume that this is the case. In our opinion, the best way forward is to focus generally on the variables that affect odor detection by animals while endeavoring to develop a variety of useful operational Remote Scent Tracing applications, including ERW detection. Doing so affords opportunity for obtaining funds outside humanitarian demining and enlisting the services of experts in a range of industries. APOPO presently is taking this tack with its R&D.

see endnotes page 83

Alan Poling has a Ph.D. in psychology and is a Professor at Western Michigan University. He is an experienced researcher with articles in 40 different journals and more than 250 publications. Poling has particular expertise in within-subject research designs and behavior analysis. He recently joined APOPO to further its scientific activities.

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Using Encapsulated Fluorescent Bioprobes to Detect Explosive Materials in Soil

This article examines the methods involved in using fluorescent bioprobes to detect explosive devices within soil. By genetically modifying plants through the injection of certain chemicals, visible responses indicate the presence and placement of explosive material, aiding demining agents in the process of mapping and removing various landmines or other explosive remnants of war. The adoption of these tools proves useful for stand-off detection of low TNT concentrations in the laboratory and controlled microcosm studies.

by Clint B. Smith [U.S. Army ERDC] and Joel S. Tabb [Agave BioSystems]

Over the past decade, novel efforts for detecting landmines in field environments included using genetically modified plants, which have been one of the focuses for biosensors. The idea involves plants that have been genetically modified to consume trace explosive materials and aid in landmine detection via a fluorescence or visual response when interrogated with an external light source. Plant leaves glow a brighter green when consuming the trace explosive material. These genomic analyses of plants may one day provide a range of bio- and nanotechnologies for development to look for trinitrotoluene (TNT) based materials using fluorescent or bright tags such as green fluorescent protein.2 These plant alterations will need to withstand the natural constraints of the environmental conditions, i.e., changes in soil pH. In addition to using plants as biosensors, genetically modified microorganisms have been investigated for their potential to detect various chemicals, namely TNT.3,4,5,6 While GFP may serve as a useful bioreporter in the laboratory setting, recent reports suggest that this reporter may not be suitable for soil-contaminant detection. Smith et al. demonstrated that expressed

GFP produced high fluorescence levels at pH 7.0, but at more acidic or alkaline pH levels, such as those likely encountered in potentially contaminated soil, fluorescence output was diminished, rendering the "ON switch" unreadable for a potential end user or operator.7 With support from the United States Army Engineer Research and Development Center, via the U.S. Army Small Business Technology Transfer program, Agave BioSystems is developing a novel fluorescent system capable of de-

tecting explosive materials present in surface

soils. The research initiative involves the proof of concept and experimentation on TNT detection in select soils using solution-based bioprobe slurries.

The bioprobes, or "dust" material, use fluorescent-labeled biological components called antibodies (known as the "ON switch") and fluorescent quencher analogs (known as the "OFF switch") to detect the presence of specific explosive residues like TNT. To provide environmental stability to the dust material's

functional part, the bioprobe was encapsulated to protect and preserve the ON/OFF switch's functionality. When free TNT is present in the soil, the soil containing the TNT turns the dust ON, causing an increase in fluorescence and a brighter soil area when illuminated, indicating that a landmine is present beneath the soil's surface. Using the "dust" material, TNT concentrations from low levels (0.02 ppm) to higher levels (200 ppm) were readily detected

at room temperature by spiking soil samples with TNT within our laboratory experimentation microcosm. Future efforts will focus on scale-up of materials for attempting experiments at larger ranges and keeping the bioprobe at the soil's surface to adapt for stand-off detection in field conditions and testing in various soil types and conditions (wet/dry, hot/cold, low pH/high pH, low salinity/high salinity). This research focuses on the technical clearance stages and non-daylight exercises to

Figure 1: Measurement of silica microspheres comprised of fluorescent antibody-based bioprobes at a concentration range of 0.2 ppm TNT in solution to 100 ppm TNT in soil samples. Y-axis clarification: Tecan fluorescence is a measure of relative fluorescence. *Graphic courtesy of Joel Tabb/CISR.*

