*Jimmie C. Oxley, Ph.D.; James L. Smith, Ph.D.; Louis J. Kirschenbaum, Ph.D.;
and Suvarna Marimganti, Ph.D.*

Accumulation of Explosives in Hair—Part II: Factors Affecting Sorption*

ABSTRACT: This study examines the sorption of eight explosives (2,4,6-trinitrotoluene [TNT]; pentaerythritol tetranitrate [PETN]; hexahydro-1,3,5-trinitro-s-triazine [RDX]; diacetone diperoxide [DADP]; triacetone triperoxide [TATP]; ethylene glycol [EGDN], nitroglycerin [NG]; and 2,4-dinitrotoluene [DNT]) to human hair. The study uses only cut hair, which is exposed to explosive vapor. The vapor transfer studies reported herein indicated that hair did not reach saturation even after 2.5 years of exposure to TNT. While previous studies showed black hair sorbed more explosive than blond or brown, this study reports that red hair sorption is similar to black, while grey hairs, exposed along with black hair from the same individual, sorbed significantly less explosive than the same individual's black hairs. In a study using only black hair, a slight racial bias was observed with sorption greater for Mongoloid hair as compared to Caucasian or Negroid. Only for Mongoloid hairs were enough samples studied to examine for a gender bias, but one was not observed. There was much variability in results in all categories (hair color, race, and gender) that trends were established only in general terms. Hair at different ages was tested for a few individuals. Detailed studies focused on the sorption of TATP and TNT as these appear to be sorbed most differently—TATP mainly on the hair surface and TNT both on the surface and in the cortex. The uptake of high vapor pressure explosives (e.g., TATP) and moderate vapor pressure explosives (e.g., TNT) by hair was rapid and could be detected within about 1 h of exposure. Both explosives were readily sorbed by pure melanin.

KEYWORDS: forensic science, TNT, RDX, PETN, TATP, EGDN, hair, explosive sorption, explosive vapor

Since the 1950s researchers have used hair of laboratory animals and humans to provide evidence of chemical exposure (1–3), but illicit drug detection has generated the most interest and controversy. The Society for Forensic Toxicology has accepted drug analysis of hair as a confirmatory technique, and the Substance Abuse and Mental Health Administration reviewed various factors pertinent to use of this technique as legal evidence (4,5). Hair testing has the advantages of being noninvasive, having the ability to provide a historical record of exposure, being resistant to countermeasures, and frequently offering a wider window of detection than analysis of body fluids (6). Despite the advantages, there remains much controversy in using hair as evidence of drug use. Drugs may be incorporated into hair by passive exposure of hair to drugs rather than by illicit use (6), and there is also evidence of color (7–9) (possibly racial) (10) bias.

Most studies on the sorption of chemicals by hair have focused on the uptake of ingested drugs. Atmospheric sorption of the drugs was a source of error in those studies. In examining the sorption of explosives by hair, only atmospheric uptake was considered. A number of studies have attempted to elucidate the sorption processes associated with the binding of drugs to hair. There is convincing evidence that drug-binding sites are associated with melanin granules, but the importance of other factors, such as hair lipid content, is a matter of some debate. One hypothesis is that hydrophobic interactions involving lipids bind nonpolar organic substrates to hair play an important role (11–15). The goal of the present study was to understand the factors affecting the binding of explosives to hair.

Department of Chemistry, University of Rhode Island, Kingston, RI 02881.

We had previously shown that human hair sorbs five explosives: 2,4,6-trinitrotoluene (TNT), pentaerythritol tetranitrate (PETN), hexahydro-1,3,5-trinitro-s-triazine (RDX), ethylene glycol (EGDN), and triacetone triperoxide (TATP) (16,17). We now add three more to the list, nitroglycerin (NG), diacetone diperoxide (DADP), and 2,4-dinitrotoluene (DNT). In these studies, sorption of explosive by hair was through vapor transfer only and was directly related to the vapor pressure of the explosive (Table 1). Figure 1 relates logarithms of explosive sorption to that of vapor pressure (i.e., EGDN > TATP >>> TNT >> PETN > RDX) at two different time intervals (48 and 144 h) Thus, for explosives with extremely low vapor pressure, such as PETN and RDX, it was necessary to hold the hair over the explosive for days so that there would be sufficient explosive sorbed to the hair for detection. For TNT, sufficient explosive was sorbed within an hour so that detection by gas chromatograph–electron capture detector (GC–ECD) was possible. However, because the standard deviation in results was large, longer times, typically 48, 144, or 920 h were routinely used.

Like uptake of explosives on hair, persistence of the explosive on hair also depended on the vapor pressure of the explosive. Explosives with high vapor pressure, e.g., EGDN, TATP, were lost after standing a few days outside the confines of the sealed containers. Explosives with low vapor pressure could persist on the hair for days and remain even after subject to repeated washings with 2% sodium dodecyl sulfate (16). It appears that hair might become a useful indicator of explosive exposure∕handling even in the absence of particulate deposition.

In addition to extending the number of compounds, this study was aimed at elucidating mechanisms by which explosives sorb to hair. Mechanistic studies were performed on two explosives—a highly volatile one, TATP, and a moderately volatile one, TNT. Work with poorly volatile explosives, such as RDX or PETN, would be impractical. Evidence considered in evaluating sorption sites included:

TABLE 1—*Vapor pressure of explosives.**

Explosives	Vapor pressure torr at 25°C
DADP	1.3E–01
EGDN	7.0E–02
TATP	5.2E–02
DNT	2.0E–03
NG	2.3E–04
TNT	5E–06
PETN	3E–08
RDX	2E–09

DADP, diacetone diperoxide; EGDN, ethylene glycol; TATP, triacetone triperoxide; DNT, 2,4-dinitrotoluene; NG, nitroglycerin; TNT, 2,4,6-trinitrotoluene; PETN, pentaerythritol tetranitrate; RDX, hexahydro-1,3,5-trinitro-s-triazine.
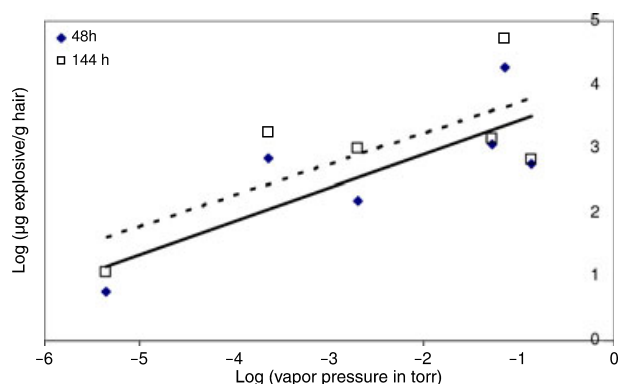
*From reference (8).

FIG. 1—*Log plot of explosive sorption versus vapor pressure for various explosives given in Table 2 (black hair).*

- Limitations on explosive sorption.
- Differences in sorption as a function of hair color.
- Differences in sorption as a function of race, gender, and age.
- Differential sorption on hair versus on melanin, cotton or glass fibers.
- Differential removal of the explosive from hair.
- Microscopic examination.

Experimental Section

Materials

Head hair was obtained by donation or purchased from Demeo Brothers Co., New York, NY. The hair was rinsed repeatedly with 2% sodium dodecyl sulfate, dried, and stored in Ziploc® bags. TATP, DADP, hexamethylene triperoxide diamine (HMTD), and EGDN were prepared in this laboratory. Nitroglycerine was purchased as 50% acetone solution from Copperhead Chemical (Tamaqua, PA). TNT, DNT, PETN, and RDX were acquired from military sources. Melanin used in experiments was either purchased from Sigma-Aldrich (Milwaukee, WI) or extracted from brown hair (18).

Protocol

Amber glass wide-mouth screw-cap jars (10.5 × 8.5 cm) were washed with soap and water, rinsed with acetone, oven-dried, and cooled in a desiccator. Explosive (c. 0.5 g) was placed in the bottom of the jar. Hair was cut into 1.5–2.5 cm lengths and about 100 strands (c. 0.3 g) were weighed into an aluminum foil basket. Three of these baskets were hung vertically so that they stacked in the wide-mouth jar one above the other. Care was taken that there was no physical contact between the explosive and hair. The lid of the jar was screwed in place, and the jar was allowed to sit undisturbed until the end of test interval. Generally, black hair was in the top basket; brown hair in the middle; and blond hair in the bottom, but a separate study showed the positioning was not significant (16).

Quantification of Explosives Sorption

Six explosives were pure powders: TNT; PETN; RDX; 2,4-DNT; DADP; and TATP; two (EGDN and NG) were liquids. At the completion of the exposure time, each hair specimen was divided into three portions of about 0.05 g (the melanin was c. 0.01 g) and weighed into an amber, 16 mL, screw-cap bottle. Acetonitrile (5 mL) was added, and the samples were sonicated for 20 min before they were placed on a shaker (speed 86 shakes/min). After shaking overnight, the acetonitrile was removed from the hair using a Pasteur pipette, and about 1 mL of this solution was put in a 2-mL septum screw-cap vial. All acetonitrile extracts were analyzed on a Hewlett Packard (HP) 5890 GC or an Agilent 6890N GC (Agilent Santa Clara, CA) using an ECD or micro-ECD, respectively. The column used was a J&W Scientific DB-5MS column (J&W Scientific, Folsom, CA) (8 m × 0.53 mm [megabore], film 1.5 um; HP) or a HP-5 (20 m × 0.25 mm, capillary column; Agilent) or DB-5MS (25 m × 0.25 mm, capillary column; Agilent) except EGDN. A summary of GC conditions for various explosives is given in Table 2. EGDN was analyzed and quantified at 214 nm wavelength with HP 1100 series Liquid Chromatography with photodiode array detector. Standard calibration curves were constructed (using 5 points between 0.01 and 1.0 ppm in acetonitrile) based on peak height and peak area. The squared correlation coefficients for the standard curves were better than 0.99. Concentrations of explosives in hair extracts were determined by extrapolations of peak area and height responses to concentrations from the standard curves. Given the concentrations and volumes of the extracts, the mass absorbed was obtained. Both the peak height and area data gave comparable results.

Recovery Experiment

Approximately 0.05 g of Asian female black hair was weighed and carefully transferred to the bottom of the 16 mL amber colored vials. Fifty microliter of known concentrations of TNT or TATP (5 ppm TNT, 1000 ppm TATP) in acetonitrile was spiked onto the hair by taking care that drops fell onto the hair. The vials were kept in the hood at room temperature. After evaporation of solvent, the first set was sonicated three times in the case of TNT and two times in the case of TATP each with 5 mL acetonitrile for 1 min. The extracts (15 mL of TNT and 10 mL TATP) were transferred into another vial. TNT extracts were concentrated by evaporating the solvent under N_2 gas purging to 5 mL, while TATP extracts were directly analyzed. After the initial washings, the hair was dried and extracted overnight as follows. Acetonitrile (5 mL) was added, and the samples were sonicated for 20 min before they were placed on a shaker (speed 86 shakes/min). After shaking overnight, the acetonitrile was removed from the hair using a Pasteur pipette, and the solution (c. 1 mL) was put in a 2-mL septum screw-cap vial. Blanks and controls were also prepared and analyzed.

TABLE 2—*Chromatographic conditions for analysis.*

Sample	Split/splitless	Chromatographic analysis conditions						
		Injector temperature	Detector temperature	Intial oven hold time (sec)	Ramp rate (degree/min)	Final temperature (°C)	Final hold time (min)	Retention time (min)
PETN hair	Split 5:1	175	250	60	20	200	5	7.7
RDX hair	Split 12.5:1	195	320	60	10–200, 20	250	5	15.5
TATP hair	Split 125:1	165	300	120	20	220	0.5	5.5
DADP hair	Split 125:1	165	300	120	20	220	0.5	3.1
TNT hair	Split 10:1	165	300	120	20	280	2	9.6
DNT hair	Split 25:1	165	300	120	20	280	2	8.6
TNT hair*	Splitless	175	325	30	15	200	10	7
NG	Split 10:1	175	300	60	20	200	2	6.7
NG*	Splitless	175	300	60	10–200, 20	250	5	2.2
EGDN, 40% methanol in water, flow rate 0.5 mL/min, Hypersil BDS column, 5 μL injection								

Oven temperature 50°C (for NG* 100°C), injection volume 1 μL. For GC, carrier gas is He except for NG* which is H_2 and make up gas is N_2.

PETN, pentaerythritol tetranitrate; RDX, hexahydro-1,3,5-trinitro-s-triazine; TATP, triacetone triperoxide; DADP, diacetone diperoxide; TNT, 2,4,6-trinitrotoluene; DNT, 2,4-dinitrotoluene; NG, nitroglycerin.

*Analyses performed with HP 5890 GC with DB-5MS, all other used Agilent 6890N with HP-5 and DB-5MS column.
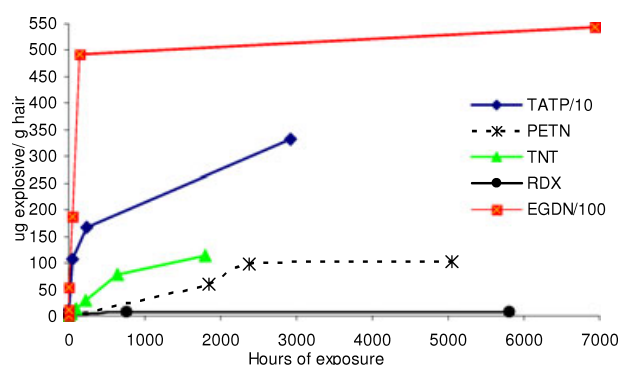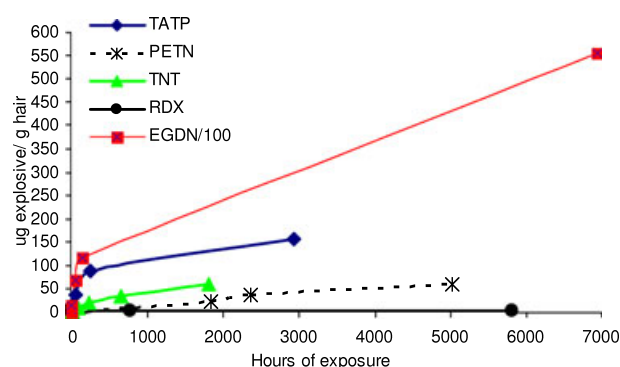

FIG. 2—*Sorption by black hair.*

FIG. 3—*Sorption by brown hair.*

Microscope

Hair strands, both clean and explosive-exposed, were pasted on microscope slides (75 × 50 × 1 mm) and observed under Nikon polarizing optical microscope (ECILIPSE E400 POL) (Nikon, Melville, NY), which was attached to the digital camera and a computer to store images. Images were taken at 200× (10×, eye piece and 20×, object) magnification.

Results and Discussion

Limitations on Explosive Sorption

Uptake of many chemicals by soil or other matrices is limited due to a limited number of binding sites. Figures 2–4 illustrate the amount of explosives sorbed when hair, black, brown and blond, respectively, is exposed to explosive vapor for lengths of time up to 7–9 months. The plots appear to suggest that after a period of time the hair became saturated with a particular explosive. For example, For TNT, after 2.5 months, the hair appeared to be reaching saturation (Figs. 2–4). Therefore, one set of hair was exposed to TNT for 2.5 years (Fig. 5). Figure 5 indicates saturation of the hair with TNT was not achieved after 2.5 months or even 2.5 year. This suggests that the leveling off of explosive sorption observed in Figs. 2–4 is a result of two different rates of explosive sorption—an initial very rapid one and a slower long-term one.
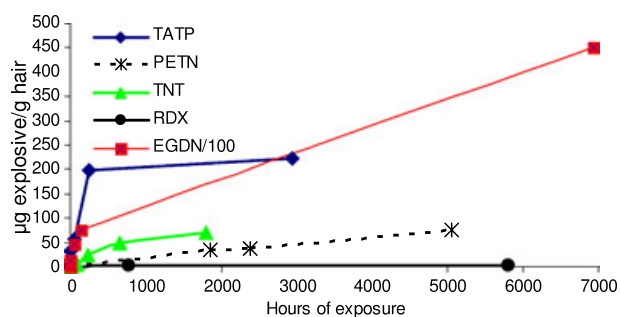

FIG. 4—*Sorption by blond hair. TATP, triacetone triperoxide; PETN, pentaerythritol tetranitrate; TNT, 2,4,6-trinitrotoluene; RDX, hexahydro-1,3,5-trinitro-s-triazine; EGDN, ethylene glycol.*

Differences in Sorption as a Function of Hair Color and Age

The data showed that, in general, black hair sorbed all explosives better than brown or blond (Figs. 2–5). A few samples of red hair were also tested; some sorbed as well as black hair but one, much more poorly (Fig. 6). To examine the effects of hair color, some oriental black hair was bleached. We found that the bleached black hair sorbed very little explosives (Table 3). Furthermore, if black hair and white hair from the same person's beard were exposed to explosive, the black hair preferentially sorbed the explosive (Table 3).
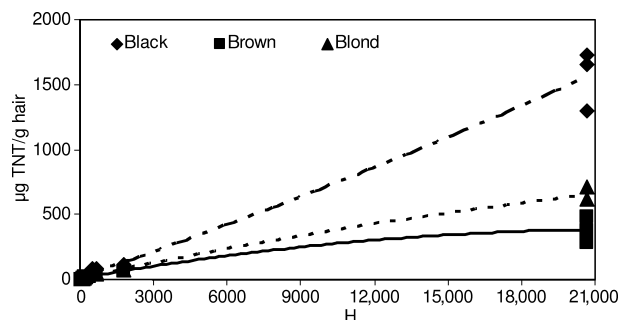
FIG. 5—*2,4,6-Trinitrotoluene sorption by hair of various colors. TNT, 2,4,6-trinitrotoluene.*
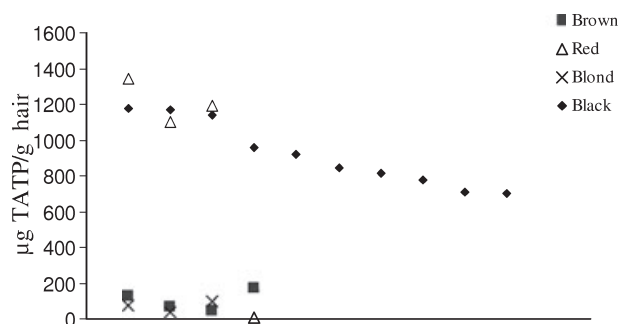

FIG. 6—*Sorption of triacetone triperoxide in 48 h. Each point is hair from a different individual. TATP, triacetone triperoxide.*

TABLE 3—*Sorption by black versus white or bleached hair.*

Exposed to hair type	TATP 48 h (µg/g hair)	SD	TNT 940 h (Hg/g hair)	SD
Unbleached black	1258	70	2 14	20
H_2O_2 bleached black	394	66	50	8
NaOH, H_2O_2 bleached black	625	3 1	73	12
Black hair	645		3 17	14
White hair	467	26	169	24

TATP, triacetone triperoxide; TNT, 2,4,6-trinitrotoluene.

Sorption of explosive by hair was not simply related to color; within each color category there were wide variations. Using black hair from 25 different sources and five different explosives, it was found that the uptake of explosives was a highly individual characteristic. For some hair sources, especially purchased hair, which had been treated by the vendor with naphthalene to prevent bacterial growth, sorption of explosive vapor was as poor as brown or blond hair. What was clear was that a hair source that sorbed one explosive well tended to sorb all explosives well. Furthermore, a number of people donated hair samples repeatedly and it was determined that the tendency to sorb explosives remained constant over the time of this study.

Because the ability to sorb hair appeared to be an individual characteristic, to examine the affects of aging hair, it was deemed necessary to compare hair from the same individual. In three trials using the childhood, brown hair of one female versus her hair as an adult, essentially no difference in amount sorbed was observed. For 48-h exposure to TATP sorption was 1467 ± 18 µg/g child hair versus 1071 ± 7 µg/g adult hair; for 6-day (144 h) exposure to TNT results were 36 ± 4 µg/g child hair versus 32 ± 8 µg/g adult hair.
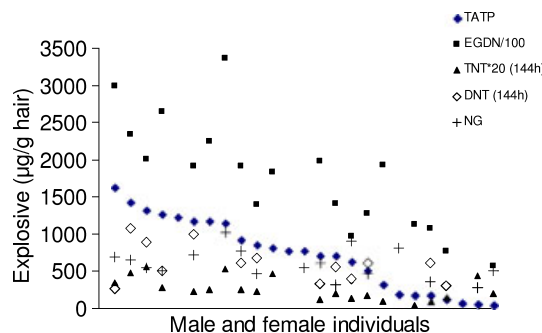

FIG. 7—*Explosive sorption by black hair in 48 h. Each point is average of three tests; each column is a person. TATP, triacetone triperoxide; EGDN, ethylene glycol; TNT, 2,4,6-trinitrotoluene; DNT, 2,4-dinitrotoluene; NG, nitroglycerin.*
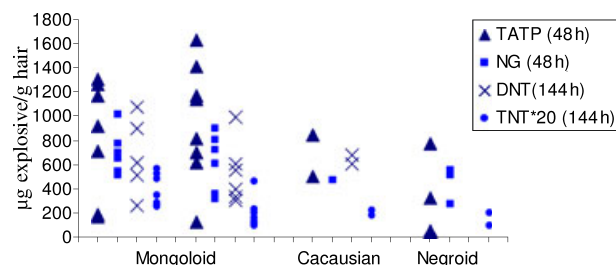

FIG. 8—*Sorption of explosive versus race for black hair; each point is average of three samples from a person. TATP, triacetone triperoxide; NG, nitroglycerin; DNT, 2,4-dinitrotoluene; TNT, 2,4,6-trinitrotoluene.*
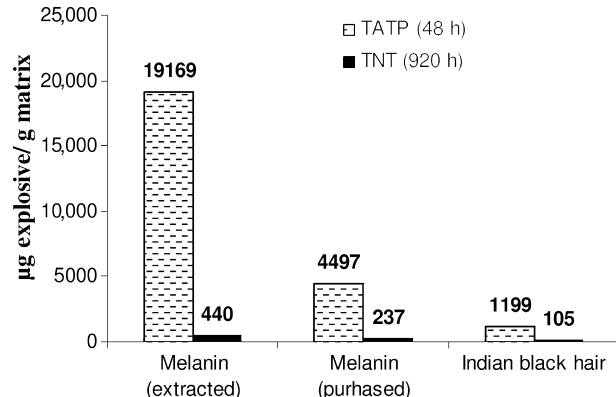

FIG. 9—*Comparison of the sorption of explosive by hair versus melanin. TATP, triacetone triperoxide; TNT, 2,4,6-trinitrotoluene.*

Differences in Sorption as a Function of Race and Gender

To test for potential racial bias, the black hair was segregated into three grouping—Mongoloid, Caucasian, and Negroid (Fig. 8). Only in the Mongoloid grouping was there female hair. In that grouping there appeared no gender bias. Similarly, no strong racial bias was obvious. Mongoloid hair may have had a slightly higher tendency to sorb explosive than the other two hair types, but the individual to individual variation was large.

Differential Sorption of Hair Versus Other Media

The observation that black, and possibly red, hair sorbed more explosive than brown and blond hair suggested that melanin might
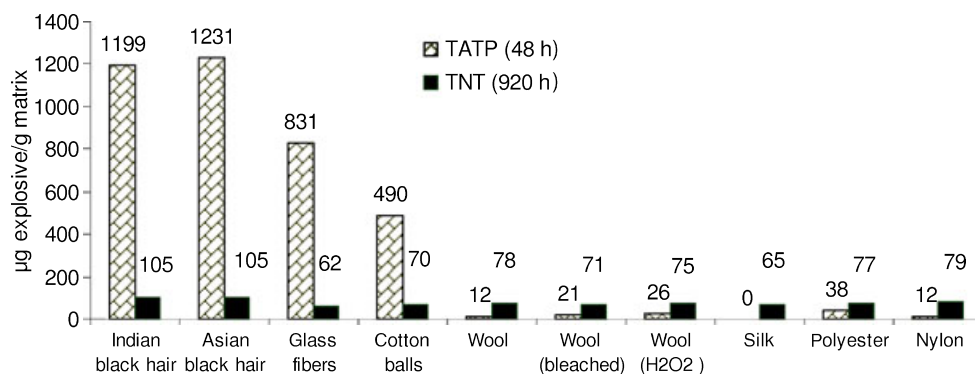
FIG. 10—*Relative sorption of triacetone triperoxide (TATP) and 2,4,6-trinitrotoluene (TNT) by various substrates.*
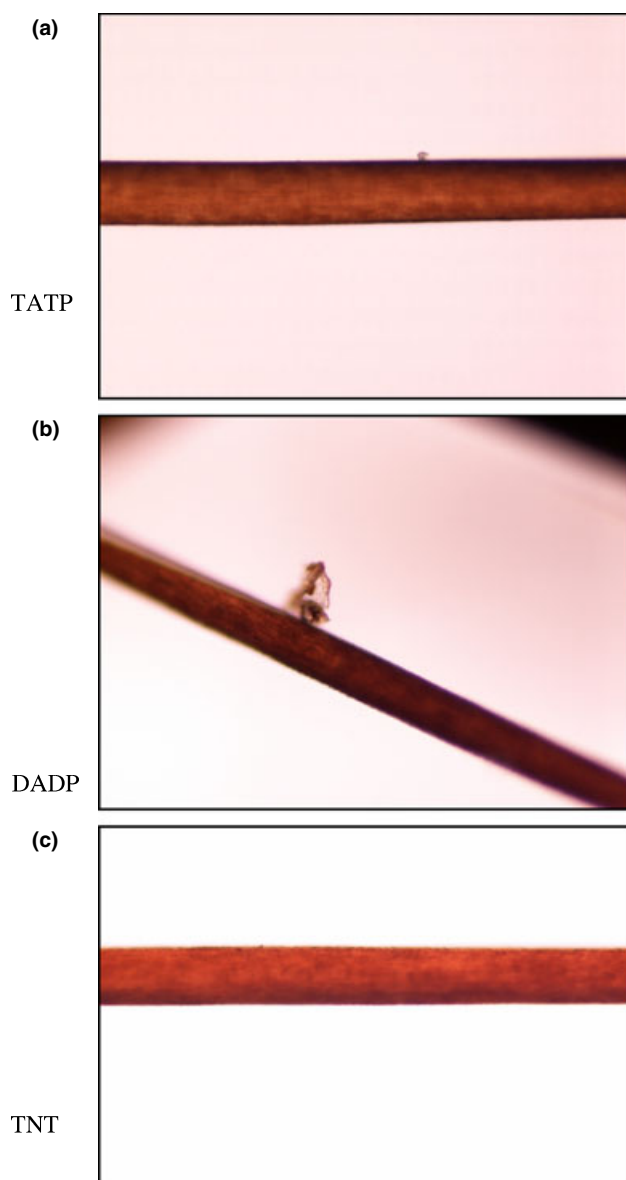

(a) TATP

(b) DADP

(c) TNT

FIG. 11—*Microscopic view of black hair exposed to vapor of triacetone triperoxide (TATP) or diacetone diperoxide (DADP) for 48 h or 2,4,6-trinitrotoluene (TNT) for 2880 h.*
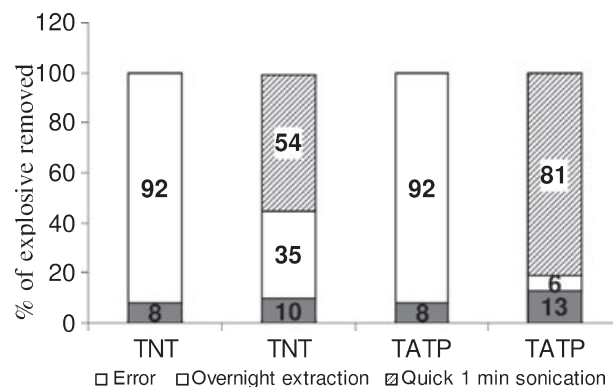

FIG. 12—*Comparison of removal efficiency—the usual overnight extraction versus quick wash with sonication followed by acetonitrile extraction. TNT, 2,4,6-trinitrotoluene; TATP, triacetone triperoxide.*

(Fig. 9). Although these differences probably reflect large differences in available surface areas, the low melanin content of hair (2%), and other factors, it is clear that there is substantial melanin/explosive interaction.

In another set of experiments, wool, silk, and other fibrous matrices, along with samples of black hair, were exposed to explosive vapor. Wool and silk were chosen because, like hair, they are protein, but unlike hair they possess no melanin. Most of these fibrous materials sorbed explosive more poorly than hair (Fig. 10). However, cotton balls and glass fibers, sorbed a large amount of TATP but not nearly as much as the black hair. The success of these fibers in sorbing TATP suggests that at least part of the uptake is related to sublimation of the explosive vapor onto the high surface area fiber. We found that over a 2-year period, sorption of TNT to black hair continued to increase (Fig. 5). Similarly, TATP sorption increased with time (Fig. 3); this was not true for TATP on cotton balls. After about 3 h, no further sorption of TATP on cotton balls was observed.

A Nikon optical microscope at 200× magnification was used to examine hair exposed to the vapors of TATP, of DADP and of TNT. The hair shafts shown in Fig. 11 are between 70 and 90 μm. Within 48 h, the explosives with high vapor pressure, TATP and DADP, could be observed as crystals on the hair strand; in that amount of time, analysis showed that a gram of hair picked up about 1100 μg of TATP. As sorption of TNT was dramatically slower, hair was examined after 6 days (144 h) and after 4 months (2880 h) of exposure to TNT. Typically, these times would result in about 30 and 200 μg, respectively, of TNT being sorbed onto a gram of hair. Under microscopic examination of the TNT exposed hair showed no easily recognizable individual crystals. These

play a role in the sorption of explosives. Melanin, exposed to the vapor of two very different explosives TATP and TNT, sorbed significantly more of each explosive than the best sorbing black hair

observations suggest that high sorption values for TATP may be a result of rapid crystal growth, and they suggest a difference in the way hair picks up TATP versus TNT.

Differential Removal of Explosive from Hair

A set of experiments was designed in an attempt to determine whether explosive uptake by hair was simply a matter of providing a surface for nucleation, in which case most of the explosive would be on the hair surface, or whether binding to a site on the hair was involved. In all the experiments described above, the explosive sorbed to hair was removed by agitating the hair overnight in aceto-nitrile. Using hair samples prepared by spiking hair with explosive-containing solution, the efficiency of overnight extraction was compared with a procedure of initial rinsing followed by extraction. Figure 12 shows that for TATP most of the explosive was removed in the initial wash, whereas with TNT, only about half of explosive was removed without overnight extraction.

Conclusions

The initial rate at which the explosive is sorbed to hair is relative to the vapor pressure of the explosive (Fig. 1) (16). While initial uptake was rapid, much more gradual uptake continued. For TNT, uptake continued even after 2 years. In a similar experiment performed over a four-month period, TATP sorption continued to increase with time (Figs. 2–4). Microscopy suggested that hair provided nucleation sites for TATP crystal grow on the surface of the hair. However, this was not the case when the matrix was cotton balls rather than hair; after about 3 h sorption leveled off.

While there is a general trend for dark hair to sorb explosive more readily than light hair, sorption is a highly individual characteristic, i.e., a person whose hair sorbs TATP well will also sorb TNT well. Once sorbed, the ease with which explosive is removed from the hair depends on the type of explosive. TNT is not lost simply by standing in the open and mild extraction, it requires vigorous washing; while TATP, an explosive with high vapor pressure, is readily lost upon standing and mild extraction. These observations as well as experiments characterized in Fig. 12 suggest that TNT and TATP are sorbed by hair by different mechanisms.

The preferential sorption of explosives by black hair over blond hair and brown hair, the preferential sorption of unbleached hair over bleached, and the preferential sorption of black hair over white hair from the same person suggest melanin is important in explosive sorption (Fig. 6, Table 2). This appears to be supported by studies showing the strong sorption of explosives to melanin (Fig. 9). Figures 11 and 12 clearly show that, at least for TATP, surface (cuticle) nucleation is very important. Furthermore, wide variation in ability to sorb explosives was observed among black hair from different individuals (Figs. 7 and 8). Therefore, it appears

that sorption of explosive to hair is not strictly a matter of binding to melanin.

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Additional information and reprint requests:
Jimmie C. Oxley, Ph.D.
Chemistry Department
University of Rhode Island
Kingston, RI 02881
E-mail: Joxley@chm.uri.edu