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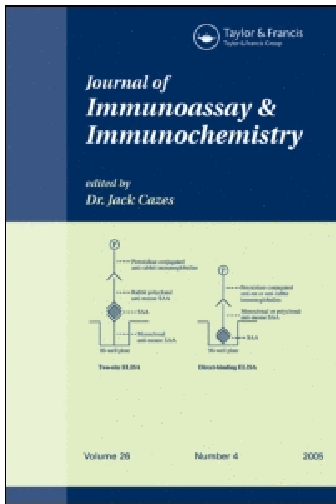

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Protein Radioiodination in a Radioassay Laboratory: Evaluation of Commercial $Na^{125}I$ Reagents and Related Biohazards

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PROTEIN RADIOIODINATION IN A RADIOASSAY LABORATORY: Evaluation of Commercial $Na^{125}I$ Reagents and Related Biohazards

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ABSTRACT

Three commercial $Na^{125}I$ solutions (Amersham, New England Nuclear, and Union Carbide) have been examined with respect to multiple parameters affecting their use in the radioiodination of three representative peptides (insulin, growth hormone, and gastrin): % of radioiodine incorporation in protein; immunoreactivity and non-specific binding properties of the radiolabeled proteins; pH, volatility, and radionuclidic purity of radioiodine solutions; and vial construction with respect to multidose use. All three commercial $Na^{125}I$ produced radioiodinated proteins of good quality for use in radioligand assays. The radioiodines differed with respect to the amount of iodine released during initial vial opening as a consequence of different pH levels: 15 nCi/mCi (pH 12.5) to 1.0 µCi/mCi (pH 7.5). Two of the three products were shipped in vials with poor construction with respect to multidose use. Selection of a radioiodine was therefore reduced to the secondary considerations of iodine volatility and vial construction. The volatilized radioiodine observed during the spill of millicuries quantities of unbuffered pH 7.5 $Na^{125}I$ was 14 microcuries per millicurie within the first 30 minutes. One thickness of rubber gloves reduced potential skin contamination from an accidental spill to insignificant levels: 20-30 picocuries per microcurie. Common good housekeeping procedures: i.e. rubber gloves, laboratory coat and a fume hood were found to be sufficient protection to eliminate most radioiodine volatility and contamination hazards associated with protein radiolabeling procedures.

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INTRODUCTION

As commercial kits become increasingly expensive, more radioligand assay laboratories are preparing their own reagents. Protein radioiodination is therefore becoming a more common procedure in many laboratories that previously purchased their reagents from commercial sources. Once the investigator selects a protein radiolabeling procedure (1-4), he must become knowledgeable about other issues involving selection criteria and related biohazards associated with the $Na^{125}I$ solution. This communication addresses the problem of commercial radioiodine selection by examining three products with respect to the efficiency of radioiodine incorporation in protein, the binding properties of the purified ^{125}I-peptides, the volatility of the radioiodine and the vial construction. The degree of protection afforded by standard protection devices is ultimately assessed under accidental spill conditions.

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MATERIALS

Radioiodines

The $Na^{125}I$ utilized in this study was obtained from three commercial sources: Amersham, Arlington Heights, IL (IMS-30), New England Nuclear (NEN), Boston, MA (NEZ-003), and Union Carbide, Tuxedo, NY (P-2).

Charcoal Air Samplers

Charcoal filter equipped air-sampling equipment (Atomic Products, Center Moriches, NY, # 086-004 and 199-244) was used to measure ^{125}I released from unbuffered radioiodine vials during initial opening. The air flow though the charcoal filter was maintained constant throughout all experiments at 21.1 feet^3 per minute as assessed by a volometer.

pH Determinations

Measurements of pH in unbuffered $Na^{125}I$ solutions were performed with BDH indicator solution: Ranges 1-14, 6.6-7.6, 7.7-8.5, 9.0-11, (Gallard Schlesinger Chemical Mnf. Co., Carle Place, NY).

METHODS

Evaluation of Commercial Radioiodines for RIA Reagent Preparation.

All three $Na^{125}I$ (100 mCi/ml) products were used to individually radioiodinate three peptides (gastrin, growth hormone, and insulin) using a modification of the chloramine T method (5). Radioiodine solutions were buffered with 0.25 M phosphate buffer at pH 7.4 prior to the iodination. Following the iodination, each radiolabeled protein was purified from unreacted iodine by starch gel electrophoresis as previously described (6). Portions of the radioiodinated protein were examined on paper electrophoresis to assess the % of the total radioiodine incorporated into the peptide and the amount of damaged ^{125}I-peptide resulting from chloramine T oxidation. The immunoreactivity of all ^{125}I-peptides was ultimately assessed by direct binding to their respective antisera using standard RIA conditions.

Measurement of Volatilized ^{125}I

Volatilized ^{125}I studies were performed within 2 days following the receipt of each radioiodine. Prior to opening, ^{125}I activity was quantitated by direct measurement in an ionization chamber dose calibrator (Capintec, Montvale, N.J.) using correction factors to adjust for the absorption of I-125 X-rays in the glass vial. The I-125 radioactivities were within $\pm$ 15% of the stated amount. The air sampler-^{125}I vial geometry used throughout all volatility studies is displayed in Figure 1. The vial was carefully opened under the air sampler to avoid droplet-spray contamination (7). Following the 2 minute sampling period, the vial cap was replaced and the residual radioactivity was assayed in the dose calibrator. Immediately thereafter, fifty microliters of I-125 solution were removed from the vial and added to a tube containing 2.5 microliters of BDH indicator solution to determine the pH. The pH was not assessed in the vials used for iodination to avoid interference of the indicator solution in the chloramine T reaction.

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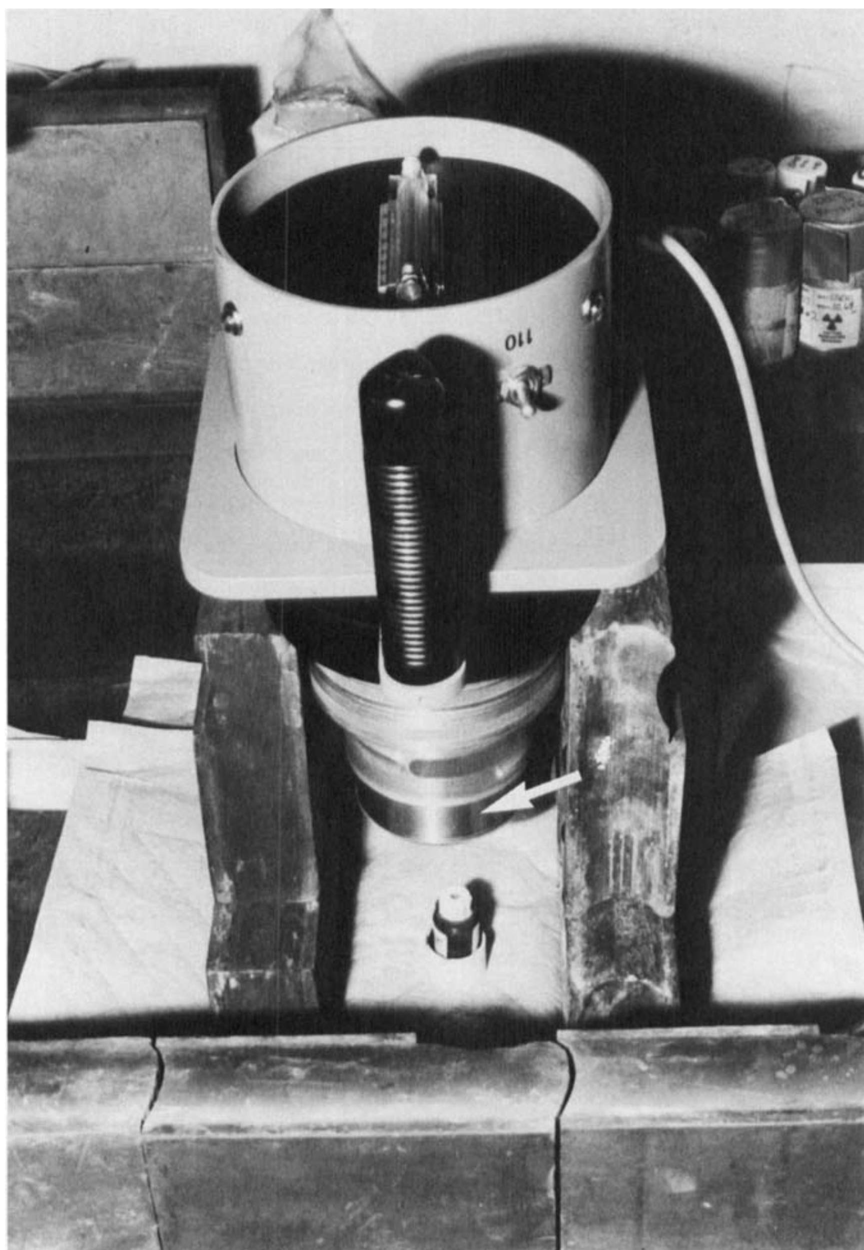

Figure 1. The TEDA charcoal filter equipped air sampler was maintained in a fixed geometry 5 cm above the top surface of each commercial vial containing Na-^{125}I during the volatilized iodine measurements. The air flow though the charcoal filter located in the metal housing of the sampler (arrow) was constant at 21.1 feet^3 per minute as assessed by a volometer.

$^{125}I_2$ vaporizing during a 6 hour period following the recapping of the radioiodine vial was assessed in the manner described above. The effect of acid pH conditions (pH 2-3) on the ^{125}I volatility was also examined by adding dilute acetate buffer to the radioiodine vial and assessing the amount of volatilized ^{125}I following a 24 hour period at room temperature.

^{125}I Measurement in Charcoal Filters

The ^{125}I activity present in each charcoal filter was assessed immediately following each volatility study using a previously described method (8) which corrects the measured activity for detector efficiency and charcoal self-absorption. Briefly, the charcoal filter was removed from the air sampler and placed in a fixed geometry below a NaI scintillation detector as displayed in Figure 2. Two measurements were performed with approximately 1% counting error statistics (i.e. >10,000 counts). The radioactivity in the filter was then calculated using the back surface counting rate which was corrected for detector efficiency and charcoal absorption using the formula $A_o = (C)(E)(A)$, where

A_o = the actual I-125 activity in the charcoal filter
C = net cpm: filter's inner face directed at the detector
E = efficiency of the detector = (μCi of ^{125}I)/cpm
A = charcoal absorption correction factor for ^{125}I photons = 2.09.

$Na^{125}I$ Spill Studies

The potential volatility hazard associated a millicurie spill was assessed by means of by air sampling techniques. Unbuffered $Na^{125}I$ (4.6 mCi, pH 7.5) was deposited in a confined area of an adsorbant pad and the charcoal filter equipped air sampler was positioned 5 cm above the area to assess the ^{125}I released into the air. The potential contamination hazard associated with a microcurie level spill on a rubber glove protected hand was assessed in a second series of experiments. Unbuffered $Na^{125}I$ (235 μCi, pH 7.5) was spilled on a rubber glove surface which had been stretched tightly over a piece of moist gauze. Wipe tests of the bottom of the rubber

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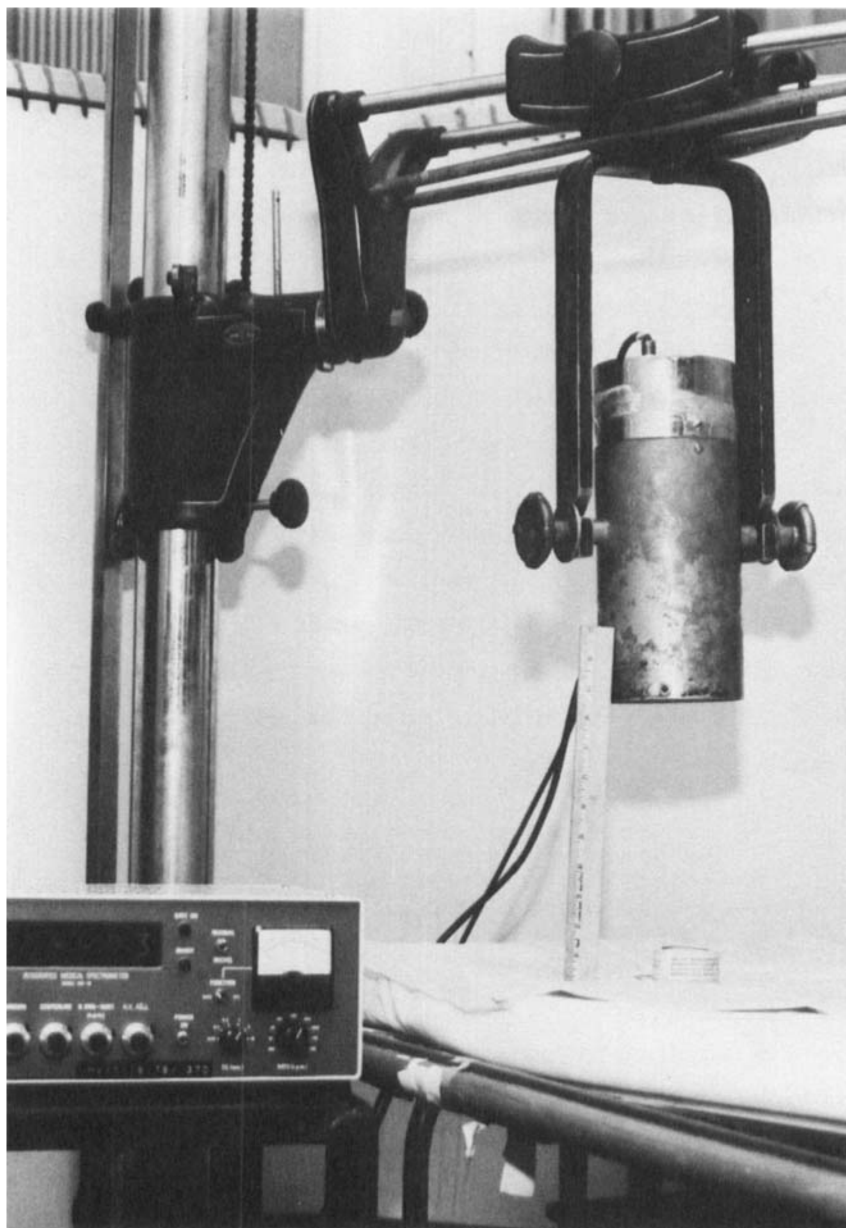

Figure 2. The ^{125}I trapped in each TEDA charcoal filter used in the air sampler (Figure 1) was assessed by placing the filter in a fixed geometry 25 cm below a NaI(tl)-gamma scintillation detection system. The single channel analyzer was calibrated for I-125 X-rays and cpm were read from the scaler in the lower left corner of the figure.

surface 30 minutes following the spill were performed to quantitate the penetration of radioiodine through the single layer of rubber.

RESULTS

Commercial Radioiodine Study

The % of total radioiodine incorporation in protein, non-specific binding (NSB) levels and the immunoreactivity properties of the three ^{125}I labeled peptides which had been prepared with the three commercial radioiodines are displayed in Table 1. All three radioiodines demonstrated equivalent percents of ^{125}I incorporation in a given peptide: insulin: 73 $\pm$ 2%; growth hormone 78 $\pm$ 2%; gastrin 58 $\pm$ 3% (mean $\pm$ 1SD, n=3). The NSB and immunoreactivity properties of each radioiodinated peptide were independent of the radioiodine used for its preparation: insulin: NSB: 7 $\pm$ 1%, B/F = 2.1 $\pm$ 0.2; growth hormone: NSB = 20 $\pm$ 1% (prior to complete purification),

TABLE 1

IMMUNOREACTIVITY OF RADIOIODINATED PROTEINS

	Insulin		Growth Hormone		Gastrin	
	NSB	B/F	NSB	B/F	NSB	B/F
Amersham	7%	2.1	19%	2.2	7%	1.3
Union Carbide	8%	2.0	20%	1.7	7%	1.0
New England Nuclear	7%	2.3	21%	1.9	7%	1.2

NSB = Non-specific binding tube with no antibody

B/F = bound - NSB/ Free

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B/F = 1.9 ± 0.3; gastrin: NSB = 7%, B/F = 1.2 ± 0.2 (mean ± 1SD, n=3). Table 2 summarizes other commercial radioiodine selection criteria including the detectable quantities of radioiodine released during initial vial opening, the radionuclidic purity of the radioiodine and the user acceptability of the vial construction.

Volatilized ^{125}I as a Function of pH

In Figure 6, the amount of vaporized ^{125}I measured upon initial opening of the vial is plotted as a function of pH. The ^{125}I collected in the charcoal filter ranged from 15 nCi/mCi (pH = 13) to 43.7 μCi/mCi (pH of 2.5). One μCi of ^{125}I was released per mCi at an unbuffered pH of 7.5. Six hours following recapping of the vial, the volatilized ^{125}I had doubled with respect to initial venting releases (data not shown). The addition of a pH 7.4 phosphate buffer immediately following the initial opening of the vial reduced this second venting release from 2 μCi/mCi to 10 nCi/ml (data not shown).

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TABLE 2

COMMERCIAL I-125 PREPARATIONS
SELECTION CRITERIA

	Amersham	Union Carbide	NEN
Radioligand Quality			
1. Immunoreactivity (B/F-insulin)	2.1	2.0	2.3
2. % Iodine Incorporation	60-80%	59-76%	54-80%
Volatility Properties			
2. Diluent	0.001 N NaOH	0.01N NaOH	0.1N NaOH
3. pH	7-8.5	9-10.5	11-13
4. Volatility nCi/mCi*	1050 ± 255	70 ± 27	15 ± 4
5. % of contents votalizing*	0.11	0.007	0.0015
Radionuclidic Purity			
6. I-126 contamination	0.22%	<0.05%	< 0.01%
Construction of Vial			
7. User acceptability	+	-	-
8. Multidose use	+	-	-

*evaluated at time of initial venting (n=3)

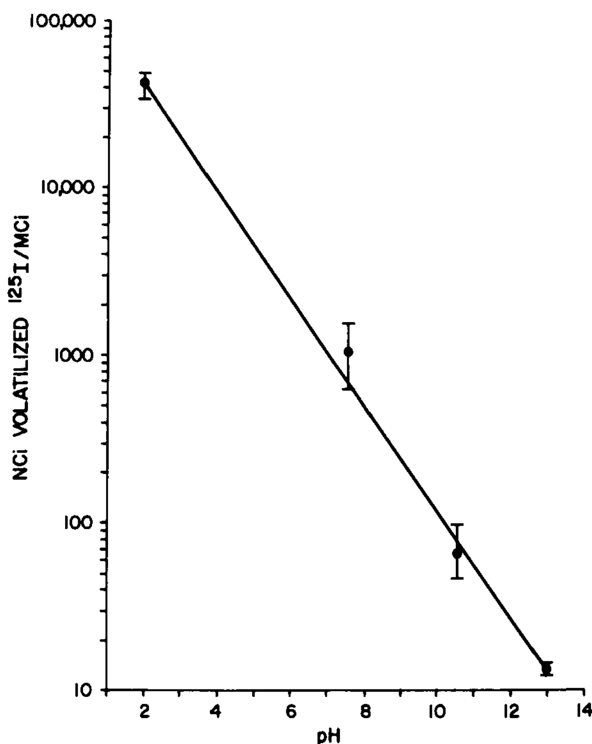

Figure 3. The nanocuries of ^{125}I volatilized per millicurie of radioiodine per vial are plotted as a function of pH. All measurements displayed were performed on unbuffered solutions ranging in pH from 13 to 2.5. The pH of each vial was determined with BDH indicator solution following the volatility measurement. Each point represents a mean $\pm$ 1 S.D. of 3 independent measurements.

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Na^{125}I Spill Studies

Volatilized radioiodine results obtained from the ^{125}I spill studies are presented in Table 3. During the first 30 minutes following the ^{125}I spill, 1.4% of the radioiodine contents (14 μCi/mCi) was collected in the charcoal filter-air sampler above the spill area. This amount was reduced

TABLE 3
I-125 VOLATILIZATION FOLLOWING AN ACCIDENTAL SPILL

A. SPILL ON ABSORBENT PAPER

time post	Paper condition	I-125 Volatilized*	% total
0-30 min	wet paper	14 uCi/mCi	1.4%
30-60 min	dry paper	1 uCi/mCi	0.1%

4.6 mCi of pH 7.5 I-125 were spilled on plastic backed adsorbant paper.
* determined by a charcoal filter equipped air sampler

B. SPILL ON RUBBER GLOVE

time post	lower surface of glove**	surface underneath glove**
30 min	14.4 nCi (0.003%)	5 nCi (0.002%)

235 µCi of ^{125}I (pH 7.5) were spilled on a rubber glove surface
** determined by a wipe test.

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to 0.1% (1 µCi/mCi) during the second 30 minutes. The rubber glove contamination studies demonstrated that 14.4 nCi or 0.003% of the ^{125}I spilled on the glove penetrated the rubber surface and that 5 nCi (0.002%) was found on a wet gauze located below the glove.

DISCUSSION

Selection of a commercial Na^{125}I solution for protein radioiodination requires the evaluation of multiple factors. Primary concerns generally focus on the radioiodine's ability to incorporate into protein and the quality of the final product with respect to non-specific binding and immunoreactivity. In the present study, the quality of the radioligands appeared to be independent of the radioiodine used in their preparation. This lead to the examination of secondary factors affecting the safety and ease of handling the different commercial radioiodines.

Previous reports describe multiple factors including pH, CO_2, O_2, Cl^- and temperature which affect the quantity of radioiodine volatilizing from an aqueous unbuffered solution (9-10). A decrease in pH resulting from the absorption of increasing amounts of CO_2 in the radioiodine is of particular concern with respect to radioiodine volatility issue: [$CO_2 + H_2O \rightleftharpoons H_2(CO_3)$]. The iodide ion is an anion of a strong acid which fully ionizes in water and readily oxidizes to iodine in acid solutions containing oxygen: $4I^- + O_2 + 4H^+ \rightleftharpoons 2I_2 + 2\,H_2O$. The volatility of a radioiodine solution can be minimized by several means, the first involving the manufacturer and the second involving the user. During the manufacturing process, NaOH is added to radioiodine. The use of 0.1N instead of 0.001-0.01N NaOH assures a high pH upon arrival (pH 12-13) which minimizes the $^{125}I_2$ released upon initial opening of the vial. Lower NaOH concentrations result in pHs of 7-10 upon arrival which can lead the release of microcuries of radioiodine per millicurie during the period of initial venting. Concern has been expressed that the presence of 0.1N NaOH in the radioiodine solution prevents the adjustment of the radioiodine to the proper pH for radioiodination. Contrary to this concept, the addition of 0.5M phosphate buffer will adjust the pH of microliter quantities of radioiodine in 0.1 N NaOH to 7-8 which is the reported optimal pH for most chloramine T and lactoperoxidase reactions (1). Immediately following receipt, the user can add 0.25-0.5M phosphate buffer to the radioiodine vial to maintain the vial pH at neutral levels. This reduces the possiblity of a decreasing pH condition near the lip of the vial where the radioiodine is exposed the most to CO_2 and thus it is subject to lower pHs and greater volatility.

The construction of the vial is also considered an important issue in the selection of a commercial radioiodine. The vial must be readily sealed for proper storage and multidose use. Currently, only one of the commercial products is dispensed into a vial which permits rapid secure recapping. The

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other designs require parafilm for securing the seal and the application of parafilm results in increased radiation exposure to the investigator.

Finally, radionuclidic purity of the radioiodine (i.e. the proportion of the radioactivity present as the stated radionuclide) should be examined when selecting a ^{125}I product for protein radioiodination. In perspective, radionuclidic purity is less important in the selection process that the other factors previously mentioned. All I-125 preparations contain some I-126 impurity as a by-product of the neutron irradiation process, i.e. ^{124}Xe (n,gamma)^{125}Xe → (EC) → ^{125}I. Initial criticism of the I-126 impurity emanates from a concern about the possible increased radiation exposure to the investigator as a result of the I-126 high energy gamma rays (386 and 667 keV) which readily penetrate lead shielding designed for ^{125}I. Second, these high energy photons are readily counted in the I-131 (364 keV) window making double I-125 and I-131 labeling studies difficult to perform in an accurate manner. Third, tellurium x-rays emitted from the I-126 are counted in the I-125 window which tends to overestimate the amount of I-125 present. These problems are considered to be minor because 1) handling of the radioiodine can be minimized by remote handling devices, 2) dual isotope (I-125, I-131) studies are not commonly performed and 3) I-126 Te x-rays spillover into the I-125 window will be uniform throughout the standard and unknown portions of the radioassay thus minimizing any effect on the interpolated results. Due the reasons above and the relatively low I-126 content in most of the commercial reagents (0.02-0.22%), the presence of this radionuclidic impurity is not considered to be a major criteria by which radioiodines should be selected.

Despite reports to the contrary (11-12), we have found that standard good housekeeping practices such as the use of a fume hood, rubber gloves and a laboratory coat are adequate for preventing microcurie thyroid burdens of ^{125}I from inhalation or direct skin contamination. Most fume hoods are

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generally adequate for evacuating the microcurie levels of radioiodine volatilized during a millicurie spill. If their is a accident involving a gloved hand, one thickness of rubber will reduce a hundred microcurie spill to picocuries of ^{125}I skin contamination. Based on these observations, closed systems such as glove boxes designed for radioiodination facilities are not considered a necessity for safe radioligand preparation. The use of these glove boxes equipped with charcoal filters can however be helpful in assuring that there is no ^{125}I released from areas where expired radioiodines are stored for decay-disposal.

In conclusion, the major selection criteria for commercial radioiodines include the binding properties of the purified ^{125}I-peptides, the radioiodine volatility properties and the ease of handling and resealing the vial. All three commercial Na^{125}I solutions appear to radioiodinate selected peptides in an manner which results in products with good binding properties. Radioiodine selection therefore focuses on secondary considerations involving radioiodine volatility and vial construction. Finally, precautions involving standard good housekeeping practices can successfully protect investigators performing protein radioiodinations from the potential biohazards associated with $^{125}I_2$ thyroid uptakes resulting from radioiodine inhalation and skin contamination.

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Requests for reprints should be addressed to Robert G. Hamilton, Ph.D., The Johns Hopkins School of Medicine at Good Samaritan Hospital, 3N O'Neill Laboratories, 5601 Loch Raven Blvd., Baltimore, Maryland 21239.

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