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Metal contamination and its effect on primary production measurements[1]

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Abstract

Minute amounts of trace elements can affect phytoplankton metabolic processes. Consequently, ^{14}C primary productivity measurements may be adversely affected by contaminant metals inadvertently added during sampling and handling procedures. We analyzed various components used in the standard ^{14}C method for metals; significant amounts of contaminants were found in many of the stock solutions used in this procedure and in associated containers. ^{14}C uptake rates were lower under contaminated conditions than those with clean techniques. The latter are described in detail; their adoption is urged for more accurate and precise estimates of this fundamentally important process.

In recent years, marine chemists have developed noncontaminating sampling and analytical procedures (Patterson and Settle 1976; Bruland et al. 1979) for the measurement of dissolved trace metals in ocean waters. As a result, many elements are now known to be present at order-of-magnitude lower levels than previously believed. Perhaps this is best exemplified by Zn, an element that has open-ocean surface concentrations of about 5–10 ng·liter^{-1} (Bruland et al. 1978; Bruland 1980). These values are three orders of magnitude lower than those published before 1976 (e.g. *see* Brewer 1975).

It is also becoming apparent that phytoplankton are affected by small amounts of metal ions. For example, Sunda and Guillard (1976) showed that calculated cupric ion "activities" in the range of 10^{-9} M (64 ng·liter^{-1}) inhibited growth rates of *Thalassiosira pseudonana*. Anderson and Morel (1978) reported that cells of *Gonyaulax tamarensis* were rendered completely nonmotile at calculated cupric ion activities of 10^{-9} M. On the other hand, low Zn ion activities (10^{-11} M) can limit growth in coastal diatoms (Anderson et al. 1978).

Thus it is becoming increasingly apparent that phytoplankton normally live in waters with very low levels of trace elements and that the addition of minute amounts of certain elements can positively or negatively affect various metabolic processes. It is also logical to suspect that phytoplankton rate measurements, such as primary productivity, have been adversely affected by heavy metals added inadvertently during various sampling and analytical procedures, despite the warnings of Steemann Nielsen and Wium-Anderson (1970) regarding Cu contamination.

Here we report on potential sources of metal contamination associated with the commonly used ^{14}C method (Steemann Nielsen 1952; outlined in Strickland and Parsons 1972) as well as clean techniques that we have developed to reduce substantially or to eliminate metal contamination during productivity measurements. We also address problems that may be encountered during both field and laboratory sample manipulation, and argue that noncontaminating procedures must be implemented before measuring plankton rate processes.

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Methods

Sample collection and manipulation—We recommend the use of Teflon-coated PVC Go-Flo ball-valve samplers (General Oceanics) fitted with silicone seals and Teflon stopcocks for the collection of noncontaminated water samples. All

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metal parts associated with these bottles (e.g. copper Nico press fittings) should be replaced with plastic fasteners or coated with plastic spray sealer (Flecto, Inc.). These samplers enter the water closed and are subsequently opened by a pressure release mechanism at about 10 m, thereby avoiding any surface contamination at the air–sea interface.

Before use, the external surface of the bottles should be well scrubbed with Micro (a commercially available laboratory detergent). Interior surfaces should be cleaned by sequential fillings (1-week soaks) with Micro, 0.5 N redistilled HNO_3 ($RHNO_3$; G. F. Smith redistilled HNO_3 or equivalent), and 0.25 N redistilled, quartz-distilled HNO_3 ($RQHNO_3$). Bottles should be well rinsed with Milli-Q type water between treatments. The above cleaning procedure need not be performed between each hydrocast. For short cruises (<2 weeks), rinses in 0.25 N $RQHNO_3$ followed by rinses in Milli-Q water every few days should be sufficient.

If Go-Flo type bottles are unavailable, standard Niskin bottles can be modified to minimize contamination problems. The black rubber O-rings can be replaced with silicone O-rings and the internal rubber closure tubing can be encased in a double layer of polyethylene plastic sheathing in accordianlike fashion to conform to the stretching of the tubing. This procedure has worked reasonably well for us, although the sheathing sometimes tears.

The potential contamination related to metal hydrowire can be avoided by using a synthetic hydroline such as Phillystrand (Philadelphia Resins) spooled on an epoxy-coated or plastic-wrapped hydrodrum. Two hundred meters of the synthetic line should be enough for sampling the euphotic zone. Metal messengers, which often contain associated metal particles and grease, are not recommended when such synthetic hydroline is used. Alternatives for messengers are solid PVC or Teflon (manufactured on a standard wood lathe), both of which we have used successfully.

If possible, samplers should be taken to a clean area before samples are removed in order to eliminate contamination by airborne particles (e.g. from the ship's stacks and air-conditioning system). We avoid such contaminants by using a self-contained, positive-pressure portable laboratory. Positive pressure is provided by blowing outside air into the Portalab through a Superflow HEPA filter (Flanders Filters, Inc.). The inside components of the Portalab are constructed for the most part with wood or plastic. Samplers are mounted on the exterior wall of the lab with sample water passed into it via acid-cleaned Bev-a-Line tubing (recommended because it can withstand strong acid cleaning) passing through the wall. All sample manipulations are done inside the Portalab. If a Portalab is unavailable, a small area of the ship should be isolated using plastic sheeting; walls, ceilings, and floors should be covered.

Experimental containers—The type of vessel used to maintain plankton populations may affect rate measurements in either a positive or negative sense by removal or addition of inorganic or organic materials via adsorption or desorption from container walls. For example, trace metals such as Fe can be rapidly lost from seawater by adsorption onto glass surfaces (Lewin and Chen 1973; Paasche 1977). In addition, glass has many trace elements, whereas plastics (e.g. conventional polyethylene) are relatively pure (Robertson 1968). Thus, in terms of trace metals, transparent plastic incubation containers such as polycarbonate are preferable.

This can be demonstrated when wall characteristics of polycarbonate plastic containers are compared with glass using the biologically active metals Cu, Mn, Fe, and Zn as the desorbed/sorbed species. Both plastic and glass bottles, all of which had been used previously for productivity work, were thoroughly cleaned as follows. The bottles were rinsed with deionized water (DIW), filled with warm Micro, and soaked for 5 days. After another rinsing with DIW,

they were filled with DIW and soaked overnight, emptied, rinsed with Milli-Q grade water, filled with 0.25 N $RQHNO_3$, and soaked for an additional 3 days. (Treatment of polycarbonate with strong acids will cause damage to wall surfaces which may alter adsorptive/desorptive characteristics.) The bottles were then emptied and thoroughly rinsed in Milli-Q water. (Rinsing after the HNO_3 step is required to ensure that no residual nitrate remains in the container.)

For Cu, eight polycarbonate and eight glass containers were filled with pH-adjusted (pH 8) Milli-Q water. (pH was adjusted by passing anhydrous NH_3 gas through an EDTA scrubber and then into Milli-Q water.) The adjusted Milli-Q water solutions were allowed to incubate 4 h and then analyzed by direct injection into a Perkin-Elmer HGA 500 graphite furnace interfaced with a Perkin-Elmer 603 atomic absorption spectrophotometer. After the analysis for desorbed Cu, the solutions were spiked with ionic Cu and allowed to stand for 18 h. The containers were then emptied, rinsed well with pH adjusted water, and oven-dried. The walls were rinsed with 5 ml of 0.25 N $RQHNO_3$ and analyzed for adsorbed Cu as above. Salt water was not used, to facilitate analysis.

To test the adsorptive characteristics of polycarbonate for Mn, Fe, and Zn, we added the following radionuclides to 200 ml of filtered seawater in acid-washed vessels: ^{54}Mn, ^{65}Zn, ^{55}Fe. Carrier-free solutions were used to keep additions within ambient levels. Standard glass bottles cleaned in the same manner were again used for comparision. The different energy spectra for the isotopes ^{54}Mn and ^{65}Zn allowed these experiments to be combined, but the ^{55}Fe experiment was conducted separately. Over time, 4-ml aliquots of treatment set 1 were counted on a Spectrum Sciences 1064 channel gamma-counting system. Five milliliters of treatment set 2 were placed in scintillation vials containing 10 ml of Aquasol scintillation fluor and subsequently counted on a Beckman LSC-100 liquid scintillation counter. The radioisotope of Cu was not used because of its short half-life (12.75 h).

^{14}C preparation and use—The ^{14}C primary productivity method described by Strickland and Parsons (1972) calls for the dilution of a concentrated [^{14}C]bicarbonate stock with a solution containing sodium chloride, sodium carbonate, and sodium hydroxide. One or two milliliters of this diluted solution are then sealed in soft glass ampoules until used.

Potential metal contamination for the Na compounds mentioned above was determined by analyzing replicates prepared from two new bottles each of reagent-grade Fisher and Baker salts. Reagents were prepared in a positive-pressure, filtered-air laboratory using acid-washed plasticware. Replicates were analyzed for Cu, Fe, Mn, Ni, Pb, and Zn by the standard additions method using a Perkin-Elmer 603 atomic absorption spectrophotometer coupled with a 2100 graphite furnace and AS-40 auto sampler. A concentrated sodium carbonate solution without ^{14}C from Amersham (Cat. No. CFA 481) was analyzed for metals.

If the ^{14}C stock solution is stored in glass ampoules as suggested by Strickland and Parsons, additional contamination may occur. We randomly selected five new glass ampoules and rinsed the walls with 0.25 N $RQHNO_3$. The wall rinses were analyzed for Cu, Fe, Mn, and Zn by direct injection into the graphite furnace.

To reduce metal contamination associated with ^{14}C solutions, we omitted all of the reagents except sodium carbonate (0.3 $g \cdot liter^{-1}$). The use of this reagent alone is sufficient to maintain a pH of about 9.7. These solutions have been stored for up to 6 months without any loss of ^{14}C. To reduce contamination problems from the manufacturer's concentrated ^{14}C stocks, we dilute high activity stocks (i.e. 10 $mCi \cdot 5\ ml^{-1}$) to 100 ml with the sodium carbonate solution. To avoid metal contamination from glass ampoules, we store the solution as a single

Table 1. Desorption/adsorption of copper from walls of 250-ml polycarbonate/glass incubation flasks cleaned according to text. "Less than" values indicate instrument sensitivity.

Cu added (ng)	Polycarbonate	Glass
	Cu desorbed (ng)	
	<50	322
	<50	90
	<50	160
	<50	<50*
	<50	260
	<50	177
	<50	198
	<50	232
	$\bar{x}$ <50	206
	Cu adsorbed (ng)	
125	1.5	4.3
	1.7	61.0
500	2.6	90.0
	2.4	3.0
1,250	5.4	212.0
	4.3	147.0

* Not included in calculation of $\bar{x}$.

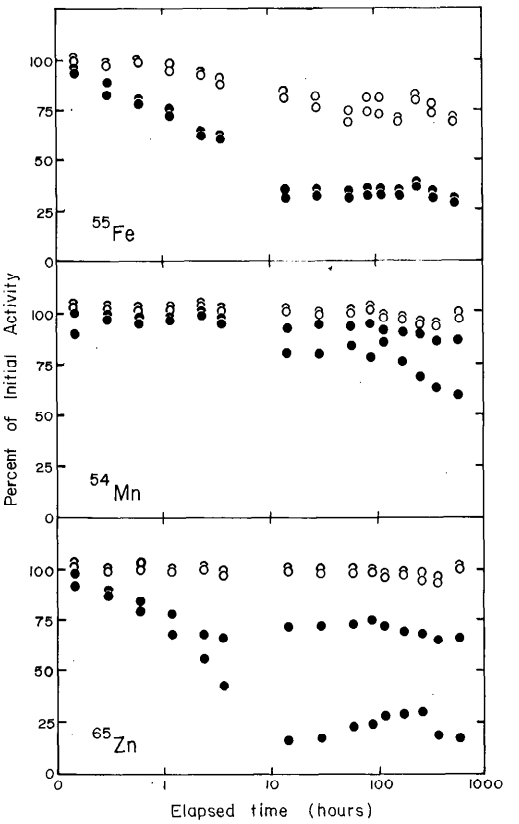

Fig. 1. Wall adsorption characteristics of polycarbonate (o) and glass (•) using ^{55}Fe, ^{54}Mn, and ^{65}Zn. Points indicate percent of initial activity remaining in solution over time (0.16 ng Fe·ml^{-1}; 0.022 ng Mn·ml^{-1}; 0.112 ng Zn·ml^{-1}).

stock in Teflon bottles (Nalge Company) which have been acid-cleaned as previously described for the Go-Flo samplers.

Samples are inoculated with Eppendorf micropipettes fitted with disposable polypropylene plastic tips. Plastic tips, which can contain metal contaminants, are acid-cleaned before inoculation by pipetting 2 N RQIINO$_3$ three times through the tip, followed by six rinses with Milli-Q water. Microliter inoculation volumes (we normally adjust the ^{14}C stocks so that no more than 250 μl are injected) are pipetted from the Teflon bottle to the sample, with care to avoid contact of the pipette tip with the mouth of either bottle or the sample water. By varying the inoculation volumes, a range of ^{14}C concentrations can be quickly and precisely delivered from one stock. Disposable plastic gloves and labcoats are worn when injecting samples.

Results and discussion

Sample collection and manipulation—The possibility of introducing toxic metal or organic contaminants via sample collection has been recognized for some time. Strickland (1960) recommended the use of plastic collectors, stating that (p. 39) ". . . the use of non-metallic sampling equipment is essential when studying the metabolism of phytoplankton . . . ." However, the severe metal contamination problems encountered by chemical oceanographers imply that the simple use of plastic samplers is not sufficient for obtaining noncontaminated samples and that further precautions are necessary. We strongly recommend that the water sampling procedures outlined in the preceding section be used when collecting water for primary production measurements.

Experimental containers—After col-

Table 2. Primary productivity comparisons (in dpm) using clean ^{14}C vs. standard ^{14}C methods in glass and polycarbonate containers.

	Clean ^{14}C polycarbonate	Clean ^{14}C glass	Standard ^{14}C polycarbonate
	Open ocean, North Pacific gyre		
	4,230	2,750	1,140
	4,150	2,740	2,110
	4,230	2,820	975
	4,780	2,710	1,050
$\bar{x}$	4,350	2,760	1,320
C.V.	7	2	40
	Coastal samples*		
	17,700	14,700	7,240
	18,800	10,400	6,140
	16,100	18,900	5,170
	16,800	13,400	7,530
$\bar{x}$	17,400	13,300	6,520
C.V.	7	15	17

* Modified from Carpenter and Lively (1980).

lecting clean water and its resident phytoplankton, one must use clean containers for the primary productivity measurements. Results of desorption experiments (Table 1) indicate that, even after rigorous cleaning, significant and variable amounts of Cu (i.e. $\bar{x}$ = 206 ng Cu·250 ml^{-1} container = 820 ng·liter^{-1}, which is 20× higher than open-ocean surface Cu levels) continue to be released from glass bottles, while no released Cu was detected when using polycarbonate containers. On the other hand, essential trace metals may also be removed via adsorption onto container surfaces. Glass again appears to adsorb larger quantities of Cu than polycarbonate. We realize that in actual measurements, Cu adsorption/desorption reactions occur simultaneously and thus the adsorption experiment represents a net result. The point is that both processes can occur with glass surfaces, although their magnitude when seawater is used is not known.

Polycarbonate vessels also adsorbed very little Mn and Zn over the period studied (1,000 h). However, there were significant losses of Zn to glass productivity bottles (Fig. 1). Such adsorption was also variable between replicates, which could lead to imprecision if Zn were to become limiting (*see* Anderson et al. 1978) during a particular experiment. Added levels of the nanonutrient Fe also showed severe adsorption loss in glass containers (Fig. 1). Solutions contained in polycarbonate also began to show losses of Fe after about 6 h. Thus, during short term incubations, polycarbonate is superior to glass, although for extended incubations (>6 h), Fe may become limiting using either material.

The use of polycarbonate containers is also suggested by our experiments in open-ocean waters and by those of Carpenter and Lively (1980) with coastal populations (Table 2). The use of "clean" ^{14}C in glass productivity bottles cleaned by old procedures (e.g. chromic acid: Strickland and Parsons 1972) showed that productivity results derived from glass bottle incubations are lowered by 25–35% relative to values obtained with clean ^{14}C and acid-cleaned polycarbonate productivity bottles.

As a result, we recommend the use of polycarbonate incubation flasks thoroughly cleaned as described above. These cleaning procedures are absolutely necessary for new bottles in order to remove not only potentially toxic metals but also residues of the organic catalyst used in the manufacture of polycarbonate (Corning p.c.).

When such rigorous cleaning procedures are not feasible, as on extended cruises with numerous samplings, a shorter procedure (after the initial cleaning) can be used. The bottles should be rinsed in reagent-grade 0.25 N HCl to remove residual [^{14}C]bicarbonate, followed by consecutive rinses with Milli-Q water, 0.25 N RQHNO$_3$, and another thorough rinse in Milli-Q water.

^{14}C preparation and use—It is obvious that the 5% NaCl solution contains larger quantities of all metals analyzed than the solutions of Na_2CO_3 or NaOH, with the exception of Pb which was below our detection limits in all cases (Table 3). The Mn levels in the two brands of NaCl were quite different: 560 pg·ml^{-1} for Fisher and 38,500 for Baker, which could lead to variable results. The same potential for

Table 3. Trace metal concentrations ($pg \cdot ml^{-1}$) in ^{14}C reagents prepared from procedure outlined in Strickland and Parsons (1972) and the final effective concentrations when introduced into 250-ml productivity bottles. Final effective metal concentrations using Moss Landing Marine Laboratories' procedure, together with ambient open-ocean metal levels, are included for comparative purposes. "Less than" values indicate instrument detection limits.

	Reagent*	Mn	Zn	Cu	Ni	Pb	Fe
NaCl (5% wt/vol)	F	560	1,410	5,080	107,000	<300	5,070
	B	38,500	1,360	4,090	101,000	<300	4,590
Na_2CO_3 (0.3 $g \cdot liter^{-1}$)	F	<100	<100	<200	550	<300	130
	B	<100	<100	<200	400	<300	650
NaOH (0.2 $g \cdot liter^{-1}$)	F	<100	<100	<200	400	<300	<100
	B	<100	<100	<200	500	<300	<100
^{14}C solution		15,000	61,000	86,000	230,000	25,000	260,000
Final effective concn†							
Strickland and Parsons	F	10	25	60	970	5	90
	B	300	25	50	840	5	90
Moss Landing Marine Labs		1	2	3	16	1	12
Open-ocean surface‡		45[1]	1–15[2]	40[2]	160[2]	5[3]	15[4]

* F and B indicate Fisher and Baker reagent-grade salts.
† Explanation given in text.
‡ Key to superscripts: 1–Moss Landing Marine Laboratories Chelex 100 ion exchange; 2—from Bruland (1980) Pacific open ocean; 3—R. Flegal and C. Patterson pers. comm.; 4—Moss Landing Marine Laboratories organic extraction, after Bruland.

contamination probably exists for all reagent-grade salts, and therefore some precautions should be taken in nutrient enrichment or similar studies.

In terms of the concentrated ^{14}C solution (we only analyzed one batch, although we have spot-checked others in the past and found similar contamination), it is interesting that not only are there large quantities of Cu present (Steemann Nielsen and Wium-Anderson 1970), but also Mn, Pb, Zn, and particularly Fe and Ni (Table 3). There can be significant addition of metals from these components to the 250-ml productivity bottle used in the Strickland and Parsons procedure (i.e. a 25 $\mu Ci \cdot 2\ ml^{-1}$ inoculation made by diluting a $0.5\ mCi \cdot ml^{-1}$ initial ^{14}C stock with the three salt components), particularly if productivity is measured in open-ocean waters where metal levels are extremely low. For example, Cu levels can be increased by 60 or 50 $pg \cdot ml^{-1}$, depending on the choice of reagents; this more than doubles the ambient Cu level (compare with open-ocean values: Table 3). We must also stress that these levels probably represent the least amount of possible contamination because we prepared the reagents from new bottles using clean techniques.

Concentrations in the ampoule rinses were elevated for all metals, with Fe values the highest (Table 4). Variability among ampoules was also pronounced, especially for Fe and Zn. Again, such additions may result in enhancement or suppression of productivity. The effect of improperly prepared ^{14}C is illustrated by the data in Table 2, column 3. Besides considerable reduction in productivity relative to controls (i.e. clean ^{14}C, poly-

Table 4. Concentrations ($ng \cdot liter^{-1}$) of Cu, Fe, Mn, and Zn washed from standard soft glass ampoules with 2 ml of 0.25 N $RQHNO_3$. Values in parentheses are final effective concentrations if 2 ml are injected into 250-ml productivity bottles.

	Ampoule				
	1	2	3	4	5
Cu	700 (6)	1,800 (14)	600 (5)	600 (5)	700 (6)
Fe	66,000 (528)	22,000 (176)	40,000 (320)	7,200 (58)	9,900 (79)
Mn	800 (6)	1,700 (14)	1,000 (8)	500 (4)	500 (4)
Zn	4,800 (38)	1,100 (9)	5,200 (42)	300 (2)	500 (4)

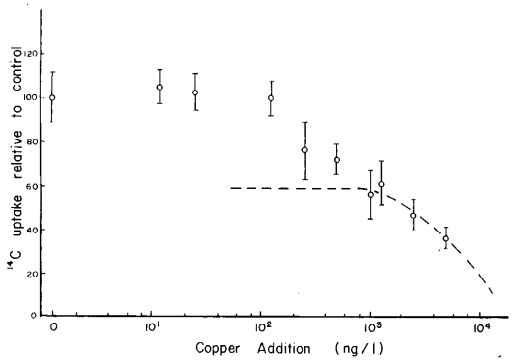

Fig. 2. Effects of copper ion additions on ^{14}C uptake relative to controls. O—Mean (±1 SD, n = 20) of a series of 6-h bioassays done over a 5-day period. Dashed line shows possible results if controls were contaminated with up to 1,000 ng Cu·liter^{-1}.

carbonate), the coefficients of variation were even greater when standard ^{14}C solutions and glass ampoules were used. Thus, differential contamination implies less precision as well as less accuracy. Another aspect of the procedure described by Strickland and Parsons, which may lead to further contamination, is the use of glass or metal syringes equipped with metal cannulae. We did not try to assess contamination levels, but we assume that significant additions do occur since the method requires immersion of the metal cannula in the sample.

Obviously, all of these potential sources of contamination can contribute significant quantities of metals to a sample. Perhaps this is why so many previous trace metal bioassays failed to show effects at low metal levels, as illustrated in Fig. 2. Here we have treated open-ocean phytoplankton with varying amounts of Cu, ranging from 0 (controls) to 5,000 ng·liter^{-1}, using clean techniques (Fitzwater et al. in prep.). If standard sampling and ^{14}C methods are used, it is quite possible that as much as 1,000 ng Cu·liter^{-1} can be added to each sample, as suggested by the above component analysis. In this case, controls would already be inhibited as much as 40% (indicated by the dashed line). Thus, any effects of lower additions would not be resolvable and the erroneous conclusion would be made that inhibition does not occur until levels of 2,500 ng·liter^{-1} are reached. This is a potential order-of-magnitude error, which could result in serious underestimation of the effects of Cu or other pollutants.

Conclusions

Although the ^{14}C primary productivity measurement technique has been used extensively since first described by Steemann Nielsen (1952), it has been found at times to be highly variable. There are several factors which contribute to this variability that we have not discussed here. For example, much work remains to be done in the application of dark bottles, optimal sample volume (for incubation and filtering purposes), extent of incubation time, and self-absorption problems associated with counting techniques. We report only two sets of experiments, but they show that trace metal contamination can seriously affect both the accuracy and precision of the technique so that evaluation of these other problems becomes difficult, if not impossible.

Contamination-related errors can be significant. For example, such errors may contribute to the apparent discrepancies in estimating phytoplankton growth rates, as described by Eppley (1980). When the standard ^{14}C method is used as a bioassay tool for estimating potential effects of pollutants, serious errors can result (*see Fig.* 2).

As we have indicated here, the three most serious sources of trace metal contamination are dirty sampling procedures, reagents of questionable purity, and the use of glass incubation bottles and storage ampoules. The use of clean sampling procedures and polycarbonate incubation flasks, the omission of all reagents used in preparing the ^{14}C stock except reagent-grade sodium carbonate, the storage of stock solutions in Teflon bottles, and inoculations with plastic tips will significantly reduce contaminant metals. We believe that it is imperative to incorporate such clean techniques in productivity methodology. If the proce-

dures outlined here are adopted, we believe that the problems of metal contamination can essentially be eliminated. The result will be more accurate and precise rate estimates for this fundamentally important process.

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