

PRECAUTIONS IN THE USE OF ^{110m}Ag AS A TRACER OF SILVER METABOLISM IN ECOTOXICOLOGY: PREFERENTIAL BIOCONCENTRATION OF ^{109}Cd BY TROUT GILLS AFTER ^{110m}Ag EXPOSURE

HEINZ J.M. HANSEN,† MARTIN GROSELL,*‡ ULF JACOBSEN,† JESPER C. JØRGENSEN,† CHRISTER HOGSTRAND,§ and CHRISTOPHER M. WOOD||

†Risø National Laboratory, Nuclear Department, P.O. Box 49, Roskilde DK-4000, Denmark

‡Zoofysiological Laboratory, August Krogh Institute, University of Copenhagen, Universitetsparken 13, Copenhagen DK-2100, Denmark

§King's College London, School of Health and Life Science, Division of Life Science, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9WA, United Kingdom

||McMaster University, Department of Biology, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada

(Received 1 August 2001; Accepted 2 November 2001)

Abstract—An often overlooked problem in the use of radiotracers is the possibility of isotopic contamination. Commercially available silver ^{110m}Ag was used to study silver uptake and depuration in rainbow trout and European eel. Quality control by means of comparative γ and β counting brought our attention to a contamination of the ^{110m}Ag stock with ^{109}Cd , which could be seen only because the ^{109}Cd was markedly bioconcentrated by trout gills. The contamination could not be detected in eel gills or in other tissues of both species. The difference between trout and eel gill structure and function is the probable explanation for the marked difference in ^{109}Cd accumulation. This contamination was identified as ^{109}Cd by γ spectroscopy and its origin by transmutation of natural silver as a result of neutron activation is described. Failure to recognize this contamination problem would have resulted in serious misinterpretation of the data set. Guidance for avoiding this problem is given.

Keywords—Silver Cadium Rainbow trout European eel Subcellular distribution

INTRODUCTION

The use of radioactive isotopes as tracers in ecotoxicology is a powerful tool. It enables a dynamic study of metal metabolism in vivo with a lower detection limit than most, if not all, nonradioactive assays of metal concentrations. Furthermore, by counting the radioactivity we are able to follow the fate of just the metal we have added, independent of the metal that was there beforehand. The latter can also be achieved with stable isotopes, but in a much more tedious manner. All other assays regard total metal contents, so that any changes will be measured as arithmetic differences, with associated loss of precision.

An often overlooked disadvantage is possible radioactive impurities, because the assumption commonly is made that commercially available radioisotopes are chemically pure. When biology is involved we risk a bioconcentration of even small impurities, which can disturb the final result in a significant manner. These radioactive impurities can occur even after the neutron activation of chemically pure targets, as in the present study.

The present investigation concerns the accumulation of the radioactive silver isotope ^{110m}Ag in two freshwater fish species, the rainbow trout (*Oncorhynchus mykiss*) and the European eel (*Anguilla anguilla*). The ^{110m}Ag is commercially produced at RISØ National Nuclear Research Institute by the neutron activation of natural silver, which is a mixture of 52% ^{107}Ag and 48% ^{109}Ag . Through the remarkable differential bioconcentrative properties of trout gills, we were able to detect that the supposedly pure ^{110m}Ag stock contained minute amounts

of ^{109}Cd . We subsequently worked out how this ^{109}Cd resulted from the production procedure, and showed by theory that it was unavoidable, and furthermore undetectable, by the traditional quality-control counting procedures used by the manufacturer. What does this minute amount of ^{109}Cd mean to the application of commercially available ^{110m}Ag as a radiotracer in fish ecotoxicology?

In the following, we present the use of ^{110m}Ag in a study of ambient silver uptake and depuration, covering both the rainbow trout and the European eel. In spite of only 0.16% ^{109}Cd originally occurring in the ^{110m}Ag stock solution, results showed that trout gill tissue could contain up to seven times as much actual ^{109}Cd activity as ^{110m}Ag activity, and that the ^{109}Cd depuration kinetics were very different from those of ^{110m}Ag . In contrast, eel gill tissue showed no such bioconcentration. If we had not detected this ^{109}Cd in the ^{110m}Ag stock, thanks to the differential bioconcentrative properties of trout gills, we would have seriously misinterpreted the results.

MATERIALS AND METHODS

Silver of natural composition in the form of 3.96 mg silver wool (36.7 μmol) was irradiated in a flame-sealed quartz-glass ampule in the core center facility of the Danish Reactor 3 (DR3; RISØ Laboratory, Roskilde, Denmark). The nominal fluence densities in this facility are $f_{\text{th}} = 1.5 \times 10^{18}$ n/m²/s and $f_{\text{epi}} = 1.2 \times 10^{17}$ n/m²/s and the irradiation time was 92.83 d. After irradiation, the target ampoule was broken and the contents were dissolved in hot 4 M HNO_3 , the solution was evaporated to dryness, and the residue was measured in a calibrated ionization chamber (1383A, National Physics Laboratory, Teddington, Middlesex, UK) and then redissolved in 0.2 M HNO_3 to a radioactive concentration of approximately

* To whom correspondence may be addressed (mgrossell@aki.ku.dk).

Table 1. Beta and γ activity in exposure water and in trout and eel gills after 2 and 15 d of depuration. The γ activity was determined at 658 keV

Sample type	Treatment	<i>n</i> (samples)	β Activity (cps)	γ Activity (cps; 658 keV)	β : γ (mean \pm SEM) ^a
Water		3	72	3.79	19 \pm 1
Trout gills	Depuration, 2 d	10	54	0.89	73 \pm 16
Trout gills	Depuration, 15 d	10	45	0.40	135 \pm 29
Eel gills	Depuration, 2 d	10	32	2.08	15 \pm 1
Eel gills	Depuration, 15 d	10	15.5	0.85	19 \pm 1

^a SEM = standard error of the mean.

125 MBq/ml. A sample was taken for γ -spectrometric measurement in a calibrated germanium lithium (Ge-Li) multi-channel system to determine the content of ^{110m}Ag and to identify possible radioactive impurities. The γ -spectrum revealed only one impurity, ^{111}Ag ($t_{1/2} = 7.5$ d). The activity of ^{110m}Ag differed only by 2.8% from the activity measured in the ionization chamber. The total activity at the calibration date (1.774 GBq) was taken as the mean of these two determinations.

Fish studies

Eels (45–75 g, mean 60 g) were caught in fyke nets in Roskilde Fjord, Denmark. All eels were at the yellow stage (adult, nonmigrating). Rainbow trout (20–30 g, mean 25 g) were obtained from Reersø Fish Farm, Kalundborg, Denmark. The fish were acclimated to a synthetic softwater medium for 16 days. Water chemistry was as follows: $[\text{Na}^+] = 50 \mu\text{M}$, $[\text{Cl}^-] = 1 \text{ mM}$, $[\text{Ca}^{2+}] = 10 \mu\text{M}$, $[\text{Mg}^{2+}] < 1 \mu\text{M}$, pH 7.0. The fish were exposed to ^{110m}Ag in the adaptation medium for 24 h under static conditions at 170 KBq/L. During the following 67 d, the fish were depurated by exposure to running synthetic soft water, as during the adaptation process. Subcellular distribution of silver was followed by recording ^{110m}Ag radioactivity in cellular fractions separated by differential centrifugation [1].

Measurements of β and γ

The present results are part of a study [1] covering more than 5,000 tissue samples from trout and eel. Because ^{110m}Ag is both a β and γ emitter, the original protocol was based on a simple β counting of dried homogenized tissue. Tissue samples were counted on aluminium planchets (6-cm diameter, 10 ml) with a thin window ($< 1 \text{ mg/cm}^2$), low-background, five-unit Geiger–Müller multiscaler (RISOE) [2]. We elected to use β counting because of much higher efficiency than γ counting (e.g., β : γ ratio of 15–19 in the uncontaminated samples; Table 1).

The presence of ^{109}Cd was not listed in the assay report of the commercially prepared ^{110m}Ag , and therefore the influence of ^{109}Cd in these experiments was not expected. The β measurements were originally just occasionally compared to γ measurements in selected samples by γ counting at 658 keV. The β activities were counted from the top and the corresponding γ activities of the dried samples were counted from the bottom with a liquid nitrogen-cooled Ge-Li semiconductor. These γ measurements were further calibrated by also measuring three representative trout gill samples, dissolved in fuming HNO_3 , in the above mentioned absolute calibrated, Ge-Li multichannel system. When ^{109}Cd was revealed as an impurity by the bioselectivity of the trout gills, all the relevant samples

were recounted by γ counting at 658 keV (^{110m}Ag) and 88 keV (^{109}Cd).

RESULTS AND DISCUSSION

Bioconcentration of ^{109}Cd

Table 1 presents some of the unexpected discrepancies observed between β and γ measurements. Trout gill tissue routinely showed markedly enhanced β : γ ratios, relative to ambient water because of what turned out to be an extra registration by the Geiger–Müller multiscaler of significant low-energy ^{109}Cd γ activity at 88 keV. Remarkably, the same did not apply to any other tissue of trout nor to any tissue of the eels including the gills, all of which showed β : γ ratios equivalent to that of the water. Therefore, the phenomenon was limited exclusively to trout gill tissue. Both ^{110m}Ag and ^{109}Cd levels were below detection limits in nonexposed eel and trout, regardless of detection method.

Figure 1 illustrates the magnitude of the ^{109}Cd bioconcentration process by trout gills. Figure 1b shows a dominant ^{109}Cd peak in the γ spectrum of a trout gill sample (at day 8 of depuration). In clear contrast, Figure 1a presents a γ spectrum of the original ^{110m}Ag stock solution where no ^{109}Cd activity could be detected. In this sample the ^{109}Cd : ^{110m}Ag ratio was impossible to determine because the 88 keV emission of ^{109}Cd was too small and could not be completely resolved from emissions at other γ lines (background). This applied even with a prolonged counting time of 62 h and a decay period of more than 14 months, to enhance the ^{109}Cd ($t_{1/2} = 462.0$ d): ^{110m}Ag ($t_{1/2} = 249.8$ d) ratio. The result was an upper limit γ ^{109}Cd : ^{110m}Ag ratio of 0.0019 in the ^{110m}Ag stock solution. This value should be compared with the calculated theoretical ratio of 0.0016 (see below).

Source and quantitative estimation of ^{109}Cd and total cadmium in ^{110m}Ag stock

Once we had discovered that the ^{110m}Ag stock contained ^{109}Cd , we first worked out by theory [3] how this occurred. Neutron capture by ^{109}Ag results in ^{110m}Ag , which has a half-life of 249.7 d, and decays to stable ^{110}Cd . An unavoidable consequence of neutron activation of the two naturally occurring silver isotopes (^{107}Ag and ^{109}Ag) is the concomitant neutron capture by ^{107}Ag , resulting in ^{108}Ag , which decays with a half-life of 2.4 min to stable ^{108}Cd . This difference in half-life between ^{110m}Ag and ^{108}Ag means that ^{108}Cd accumulates together with ^{110m}Ag during neutron activation, thereby enabling a further neutron capture by ^{108}Cd yielding radioactive ^{109}Cd , which has a half-life of 462 d and decays to stable ^{109}Ag . The resulting radioactive product is thus ^{110m}Ag , and inevitably

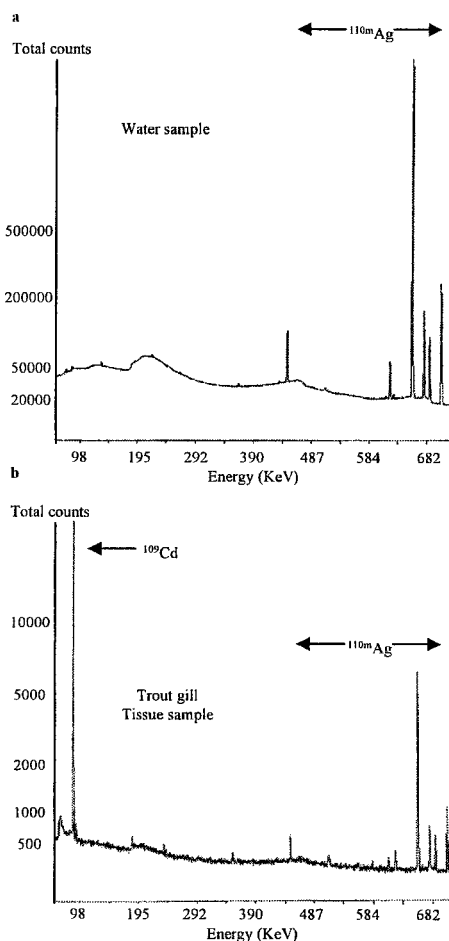


Fig. 1. (a) Spectrum of ^{110m}Ag stock solution applied to the exposure tanks and (b) spectrum of a randomly selected rainbow trout gill sample after 8 d of depuration. Note the distinct ^{109}Cd peak (88 keV) observed in the gill sample. No such peak was detectable in the stock solution.

contains radioactive ^{109}Cd as the result of a neutron transmutation of the original silver target.

Because of the very low concentration, measurement of the ^{109}Cd content in the ^{110m}Ag stock solution by direct spectrometry was impossible. Therefore, we calculated the amount of ^{109}Cd radioactivity and of total cadmium by using an up to six-step Bateman equation [4], as used by Selmann-Eggebert et al. [5]. The general Bateman equation is

$$A_n(t) = \lambda_n \cdot N_0^0 \cdot \left(\prod_{i=0}^{n-1} \Lambda_i^* \right) \left[\sum_{i=0}^n \left(e^{-\Lambda_i t} / \left(\prod_{\substack{j=0 \\ j \neq i}}^n (\Lambda_j - \Lambda_i) \right) \right) \right]$$

where A is activity, λ is a decay constant, N_0^0 is the initial number of target nuclides, t is irradiation time, n is number of transmutations, Λ^* is formation probability for a certain nuclide, Λ is disappearance probability for a certain nuclide, and i and j are summation and multiplication indices for the formation and disappearance probabilities.

We calculated the amount of both cadmium and silver isotopes by using the earlier listed fluence densities and irradiation time and nuclear data together with the effective fluence density from the measured amount of ^{110m}Ag produced. These calculations were corrected for the shielding effect from the target, assuming this effect to be the same for all the transitions involved.

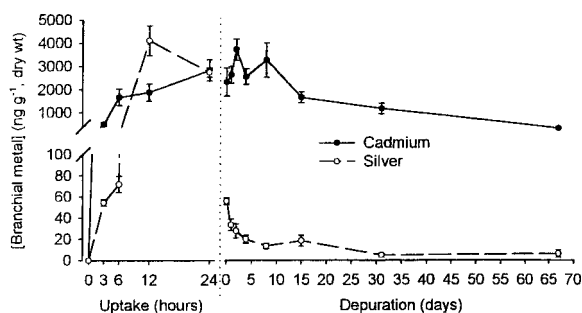
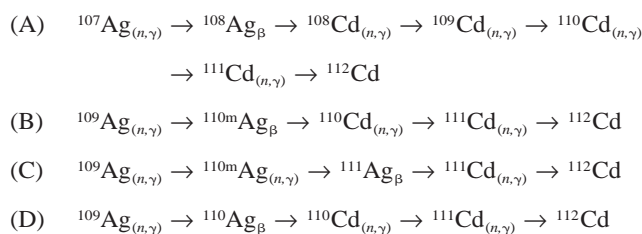


Fig. 2. Total cadmium and silver accumulation in rainbow trout gills during 24 h of waterborne exposure, and subsequent depuration for up to 67 d after transfer to clean water. Data were calculated from the respective ^{109}Cd and ^{110m}Ag radioactivity of the samples, together with their respective specific activities in the exposure water. Mean \pm standard error of mean ($n = 5-10$ at each time). Note the break on the y-axis.



From these production routes, the amount of ^{111}Ag activity was calculated to be 9.0% and the amount of ^{109}Cd activity as calculated to be 0.16% at time of calibration of the ^{110m}Ag stock solution. Adding these four lines (A–D) gives a cadmium composition in atom % of $^{108}\text{Cd} = 19.0$, $^{110}\text{Cd} = 80.7$, $^{111}\text{Cd} = 0.27$, and $^{112}\text{Cd} = 0.0015$, resulting in a total of 2.63 μmol . Similarly for silver, $^{107}\text{Ag} = 54.35$, $^{109}\text{Ag} = 45.35$, and $^{110m}\text{Ag} = 0.29$, resulting in a total of 34.1 μmol (Ag:Cd ratio of 13).

Originally, 36.7 μmol of silver was present. The total amount of measured ^{110m}Ag activity was 1,774 MBq, which gives 0.16% = 2.84 MBq of ^{109}Cd , and the following specific activities based on the above calculated values: $^{110m}\text{Ag} = 52.1$ kBq/nmol Ag and $^{109}\text{Cd} = 1.08$ kBq/nmol Cd.

From the above theoretical analyses, the absolute concentration of cadmium in the exposure water was calculated to be less than 100 ng/L, which is lower than cadmium concentrations previously reported to have no or very little effect on rainbow trout even during chronic exposure in soft water [6]. In contrast, the radioactivity of ^{109}Cd in trout gills could be as much as seven times higher than the corresponding ^{110m}Ag radioactivity, equivalent to an approximately 345 times higher absolute mass concentration measured in normalized weight units (ng/g dry wt).

Uptake and depuration of silver and cadmium in the gills

Rainbow trout gills accumulated more silver than eel gills during the initial 24-h ^{110m}Ag exposure, but, because of faster depuration of silver from trout gills compared to eel gills, the ^{110m}Ag activity levels (at 658 keV) in eel gill samples was higher than in trout gill samples throughout the 67-d depuration period, as illustrated by the selected samples in Table 1. A full comparison of the ^{110m}Ag patterns in trout versus eel gills is given by Wood et al. [1]. However, our focus here is on the ^{110m}Ag versus ^{109}Cd patterns in trout gills. Eel gills did not take up detectable amounts of ^{109}Cd .

Figure 2 shows a comparison of uptake and depuration patterns of ambient total silver and cadmium in trout gills,

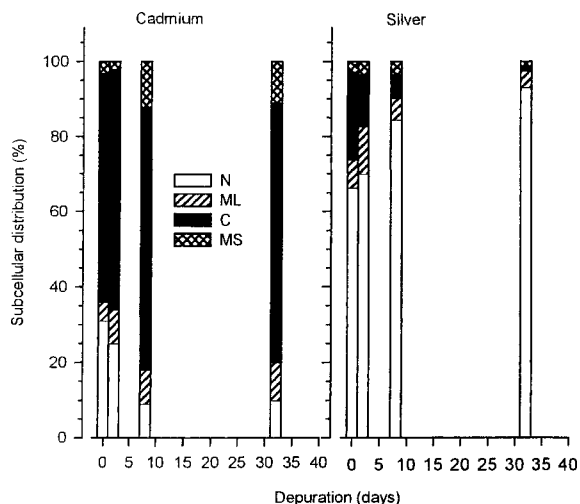


Fig. 3. Subcellular distribution (percent of total) of cadmium and silver as calculated from the respective ^{109}Cd and ^{110m}Ag radioactivity of the samples in rainbow trout gill samples after 0, 1, 2, 8, and 32 d of depuration. N = nuclear fraction (includes also cell debris, cell surface membranes, and mucus); ML = mitochondria and lysosomes; C = cytosolic fraction; MS = microsomes ($n = 5$).

with the results calculated based on the above specific activities. Note that no ^{109}Cd or ^{110m}Ag was present in samples obtained from nonexposed control fish. Although the uptake of silver peaks at 12 h, the concomitant uptake of cadmium continues to increase up to 24 h. However, the main difference seems to relate to the depuration process. After transfer to clean water, gill-associated silver is rapidly depleted, whereas cadmium persists in the gills in substantial amounts even after 67 d of depuration. The present results show an approximately 4,400 times greater concentration factor of branchial cadmium from the water, relative to silver from the water, in trout gill tissue after 8 d of depuration *in vivo*. This was due partly to an enhanced relative uptake during the 24-h incubation period (Fig. 2), resulting in comparable levels of branchial cadmium and silver despite the 13-fold difference in exposure concentration. However, more importantly, this was due to an approximately 100-fold slower depuration of cadmium compared to silver (Fig. 2).

The observed slow cadmium depuration is in excellent agreement with the work of Norey et al. [7], who showed very slow depuration from rainbow trout gills after transfer to cadmium-free water (time to half concentration > 60 d). This slow depuration explains why no ^{109}Cd could be detected in any of the internal tissues of the trout; the gills served as a barrier. The very rapid depuration of branchial silver (time to half concentration < 8 h) is in accord with the rapid recovery (a few hours) of branchial Na^+ transport function reported by Morgan et al. [8] and is associated with subsequent internal accumulation of silver [1].

The difference in depuration between silver and cadmium is furthermore illustrated in Figure 3, which compares the distribution patterns of the two metals among various trout gill cell components as calculated from the respective distributions of ^{110m}Ag and ^{109}Cd . Although ^{110m}Ag was concentrated in the nuclear fraction, which includes cell nuclei, cell debris, cell surface membranes, and mucus, ^{109}Cd was concentrated mainly in the cell cytosol. These patterns remained consistent over time, despite the great difference in depuration rates. The subcellular distribution of ^{109}Cd is in close agreement with

patterns previously reported in minnows [9], where dominating cytosolic ^{109}Cd was largely associated with metallothioneinlike proteins. In contrast, Wood et al. [1] suggested that ^{110m}Ag was cleared rapidly from the gills because it was not retained by the cytosolic fraction; only small amounts remained bound to unidentified nuclear components.

We now know that silver is a Na^+ antagonist [10], and that cadmium is a Ca^{2+} antagonist [11], in terms of gill transport function. Both Na^+ and Ca^{2+} enter the gill via different routes and possibly even different cells (Na^+ via pavement cells and Ca^{2+} via chloride cells); thus, it is not surprising that they exhibit marked differences in branchial handling. Although eel and trout take up Ca^{2+} via similar pathways [12], the virtual absence of exposed chloride cells in the gills of eels compared to trout [13] could well explain the absence of ^{109}Cd accumulation in eels in response to this short-term exposure.

CONCLUSION

Obviously, one cannot be too careful in interpreting the gross uptake of metal isotopes in experimental ecotoxicology. Only thanks to the differential bioconcentrative properties of the trout gills for ^{109}Cd and ^{110m}Ag , in combination with our comparison of γ and β detection techniques for a number of different tissues from two different species were we able to detect the ^{109}Cd in our ^{110m}Ag study. This ^{109}Cd in principle is unavoidable, as outlined above. Had we not detected this contamination problem, we would have completely misinterpreted the kinetics of silver loading and depuration in the trout gill. The lesson here is that β counting of ^{110m}Ag in biological samples always should be associated with control γ counting. Furthermore, it is essential that such γ counting is restricted to an energy window well above 88 keV so as to ensure no ^{109}Cd detection. We have adopted these precautions in our recent studies [1,10]. Nevertheless, it should be stressed that once ^{109}Cd has been determined to be absent, direct β counting is by far the most sensitive detection method, because of a much higher counting efficiency, even when the samples are just dried tissues. Scintillation counting that applies appropriate energy windows could avoid ^{109}Cd interference with ^{110m}Ag detection.

Acknowledgement—We are grateful for the excellent technical assistance of Alice Kjølhedde, Vibeke Jørgensen, and Tove Christensen during our stay at RISOE National Laboratory, Roskilde, Denmark. Financial support was provided by a grant from the Association of University Colleges of Canada Going Global Program, KODAK Canada, Eastman Kodak (USA), and the Natural Sciences and Engineering Research Council of Canada Industrially Oriented Research Program. We thank Joe Gorsuch for expediting this project and for helpful comments on the manuscript. C.M. Wood is supported by the Canada Research Chair Program. M. Grosell is supported by the Danish Natural Research Council (grant 21-01-0255).

REFERENCES

- Wood CM, Grosell M, Hogstrand C, Hansen HJM. 2002. Kinetics of radiolabelled silver uptake and depuration in the gills of rainbow trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*): The influence of silver speciation. *Aquat Toxicol* 56: 197–213.
- Bøtter-Jensen L, Nielsen SP. 1989. Beta multiscaler systems for low-level measurements of environmental samples. *Proceedings, XV Regional Congress of IRPA, Visby, Gotland, Sweden, September 10–14, 1989*, pp 459–464.
- Friedlander G. 1981. *Nuclear and Radiochemistry*, 3rd ed. John Wiley, New York, NY, USA.

4. Bateman H. 1910. Solution of a system of differential equations occurring in the theory of radioactive transformations. *Proc Camb Phil Soc* 15:423–427.
5. Selmann-Eggebert W, Flegelheimer J, Pfennig G. 1962. *Die Mathematische Behandlung der Zerfalls und Bildungsgesetze der Radioaktivität mit Grafisch Gelösten Beispielen*. KFK117. Kernforschungszentrum, Karlsruhe, Germany.
6. Hollis L, McGeer JC, McDonald DG, Wood CM. 2000. Effects of long term sublethal Cd exposure in rainbow trout during soft water exposure: Implication for biotic ligand modelling. *Aquat Toxicol* 51:93–105.
7. Norey CG, Brown MW, Cryer A, Kay J. 1990. A comparison of the accumulation, tissue distribution and secretion of cadmium in different species of freshwater fish. *Comp Biochem Physiol C* 96:181–184.
8. Morgan IJ, Henry RP, Wood CM. 1997. The mechanisms of acute silver nitrate toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*) is inhibition of gill Na⁺ and Cl⁻ transport. *Aquat Toxicol* 38:145–163.
9. Wicklund Glynn A, Olsson PE. 1991. Cadmium turnover in minnows (*Phoxinus phoxinus*) preexposed to waterborne cadmium. *Environ Toxicol Chem* 10:383–394.
10. Bury NR, Wood CM. 1999. The mechanism of branchial apical silver uptake by rainbow trout is via the proton coupled Na⁺-channel. *Am J Physiol* 277:R1385–R1391.
11. Verbost PM, Flik G, Lock RAC, Wendelaar Bonga SE. 1987. Cadmium inhibition of Ca²⁺ uptake in rainbow trout gills. *Am J Physiol* 253:R216–R221.
12. Flik G, van der Velden J, Dechering KJ, Verbost PM, Schoenmakers TJM, Kolar ZI, Wendelaar Bonga SE. 1993. Ca²⁺ and Mg²⁺ transport in gills and gut of tilapia, *Oreochromis mossambicus*: A review. *J Exp Zool* 265:356–365.
13. Perry SF, Goss GG, Fenwick JC. 1992. The interrelationship between gill chloride cell morphology and calcium uptake in freshwater teleosts. *Fish Physiol Biochem* 10:327–337.