Criteria of applicability for autoradiography of tritium

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Abstract. Autoradiography is an effective tool for the imaging of radionuclide distributions in various samples. In sophisticated applications with special preparation and development of sample-emulsion combinations and subsequent grain counts it can be highly quantitative, but it requires carefully controlled conditions and a variety of counter-checks, for example through scintillation spectroscopy. Less refined applications use X-ray films as detectors, and their seeming simplicity tends to invite artefacts and misinterpretations. Particular care needs to be taken, if one deals, or presumes to deal, with the low-energy β-emitter tritium. Because of the short electron ranges the film must be in intimate contact with the sample, which tends to produce chemographic artefacts; without added spectroscopic measurements it is impossible to discriminate the spurious signals from a blackening of the film due to tritium. Recent statements concerning autoradiographic tritium measurements in tree samples have created considerable public concern and have demonstrated the pitfalls of uncritical use. This paper presents order-of-magnitude criteria for the detection threshold in the autoradiography of tritium; they can serve as an exclusion principle for some of the more extravagant misinterpretations.

Introduction

The subsequent analysis needs to be prefaced by a brief justification that is outside the proper realm of science. A childhood leukaemia cluster near a nuclear reactor in the Federal Republic of Germany has motivated – and, indeed, requires – a search for possible causes. Two state governmental commissions have been charged with this task. Their work has, from the outset, been accompanied by breaches of procedural rules and by the failure to distinguish between

undocumented assertions and meaningful scientific evidence. A new level of misconception is reached in the interim report of one of the commissions [7]. This report suggests that certain purported autoradiographs of wood samples from the region of the leukaemia cluster might be indicative of a major tritium contamination, while it rejects the far more conclusive evidence of sophisticated low-level measurements that had shown normal tritium levels in analogous wood samples (Kim MA, Baumgärtner F Report to 'Niedersächsisches Sozialministerium'). The presumed autoradiographs had all the characteristics of artefacts, and, quite apart from this fact, they should have been rejected right away, because they were not accompanied by the minimum of documentation that is a precondition of technical or scientific discussion.

When unsupported evidence is blindly admitted, the mechanisms of scientific evaluation are bound to fail. But even then there remain certain criteria for the identification of erroneous results. Such criteria will here be given for the applicability – or rather inapplicability – of autoradiography to the detection of tritium in environmental samples.

General considerations

Autoradiography is an established technique for the analysis of radionuclide-labelled molecular or cellular samples. For low-energy β-ray emitters it is best performed by applying a fine layer of emulsion directly to a thin sample and by then storing the sample-film combination under carefully controlled conditions and at sufficiently low temperature for the exposure period. With this type of sophisticated *micro-autoradiography* one conducts precise grain counts after the development of the sample-film combination; for radionuclides with more energetic β-rays one can also identify and count individual particle tracks [12]. A useful survey of modern methods of autoradiography has been given by Baker [2], Wittendorp, Rechenmann, and co-workers have extended and demonstrated the potential of these methods in cell studies [11] and in their work on sensitised emulsions and improved processing techniques [8]. They have also compared various methods for the imaging of radioactive tracers, for example in plants, stressing the fact that autoradiography, even in its most sophisticated form, needs to be counter-checked by other, more specific methods, such as scintillation counting [12].

In simpler investigations (one may speak of *macro-autoradiography*) commercially available films are used. A quantitative evaluation of specific activities in the sample is then often not sought, and where it is required, for example in the evaluation of ^{3}H-labelled or ^{14}C-labelled electrophoresis gels, it is performed by densitometry rather than grain counts. For the autoradiography of tritium one can use special, commercially available films which are devoid of a protective surface layer and are brought into direct contact with the sample. Since this type of unprotected film requires great care to avoid chemographic or mechanical artefacts, one uses alternatively a film with inert surface foil. One must then fluor-impregnate the sample, so that the low-energy β-rays of tritium can produce fluorescence which is registered by the film. Without fluor-impregnation of the sample, these films are too insensitive to the short-ranged β-rays of tritium to be suitable.

The seeming simplicity of autoradiography invites, and has invited in the past, attempts to apply this technique in environmental sampling. Since it is easy to produce artefacts, one will usually obtain "images", and these will be accepted, if there is, as recently happened even on the official level [7], no assessment of the order of magnitude of activity that would be required for an actual autoradiograph. The subsequent considerations are to provide the relevant information on the order of magnitude of specific activities of tritium that can be detected with the commercial tritium-sensitive films. The result will show a striking discrepancy, by many orders of magnitude, between the assertions that have been made and the actual detection levels. Setting at rest the question of the purported tritium autographs, the result may also generate a more careful attention to scientific standards.

Definition of relevant quantities

Two conditions will be considered. The first refers to thin samples, i.e. to samples that are much thinner than 1 μm and that cause no appreciable self-absorption of β-rays. This is the usual condition for the specification of sensitivity of commercially available films, such as have been used in the recently attempted tritium autoradiography. The opacity versus activity relations are given for these films [1] with reference to thin samples, and these are characterised by the *surface specific activity*, i.e. their activity per unit area. The second condition refers to environmental measurements where one deals with thick samples which are characterised by their *mass specific activity*, i.e. the activity per unit mass. In the following a correspondence will be derived between the surface specific and the mass specific activity. This will then permit a translation of the available sensitivity specifications to the case of thick samples.

Let s_0 be the thickness and ρ_0 the density of the inert layer between sample and sensitive emulsion of the film. The relevant quantity is the product of layer thickness and density, and this quantity will, for simplicity, also be termed thickness and be designated by $x = s_0 \cdot \rho_0$. For a unit density material, the thickness 10^{-4} g/cm^2 corresponds to 1 μm. One can now compare the mean energy or the number of electrons to reach the film from thin or thick samples, and can consider in the comparison different separations, x. To deal with rigorously defined quantities [3], one can refer to the energy fluence rate, $\dot\psi$, or the electron fluence rate, $\dot\phi$, into the film, and express these quantities in terms of the activity of the sample and its separation, x, from the film. The quantities will be denoted by $\dot\psi(x)$ and $\dot\phi(x)$ in the case of thin samples. In the case of thick samples they will be denoted by $\dot\psi_x$ and $\dot\phi_x$. The surface specific activity of a thin sample will be denoted by a; the mass specific activity of a thick sample will be denoted by α. The relations will first be given as quantity equations, i.e. with no commitment to particular units or their multiples or submultiples.

The subsequent numerical results are obtained in terms of computer simulations of electron transport, with cross sections that are explained in the appendix. The energy spectrum of the β-rays of tritium is given in Fig. 1. To keep the discussion simple and to focus it on the point of current public interest, it will be confined to tritium, but it will be noted that the considerations can, with certain modifications, be extended to other radionuclides.

In the case of a thin sample with surface specific activity α, one has the following energy fluence rate, i.e. the energy transported into the film per unit
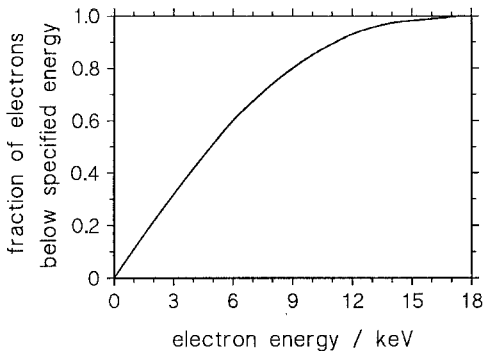
Fig. 1. Distribution of initial energies of the β-rays of tritium [9]
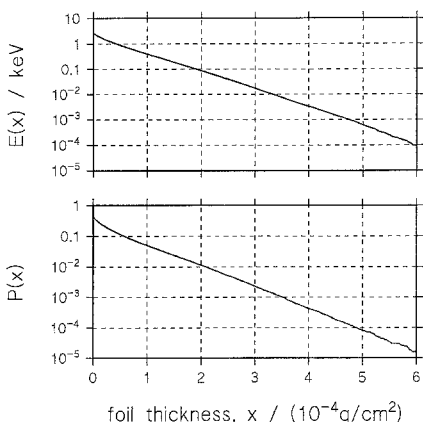
Fig. 2. The mean energy $E(x)$ and the fraction of electrons $P(x)$ per decay that reach the film, if it is separated from the point of decay by a mass layer of the specified thickness x. The results are obtained in computations with cross sections for water and are scaled to density (see appendix)

area and unit time:

$$\dot{\psi}(x) = E(x) \cdot a \tag{1}$$

$E(x)$ is the mean energy imparted to the film per decay. This quantity is plotted in the upper panel of Fig. 2.

The particle fluence rate, i.e. the number of β-rays entering the film per unit area and unit time, is

$$\dot{\phi}(x) = P(x) \cdot a \tag{2}$$

$P(x)$ is the fraction of β-rays reaching the film. This quantity is plotted in the lower panel of Fig. 2.

One notes that even a very thin protective foil reduces the energy fluence, and also the particle fluence, substantially. A protective layer of thickness x $= 2.4 \cdot 10^{-4}$ g/cm^2 (corresponding to 2.4 µm at unit density) is used in the commercially available fluor-^{3}H-autoradiography film [1]. The corresponding reduction factor is about 50; this factor of sensitivity is lost, when one uses this type of film (as was recently the case [7]) without fluoridation of the sample. However, it will be seen subsequently that these recent attempts were futile, quite apart from the reduction factor of 50.
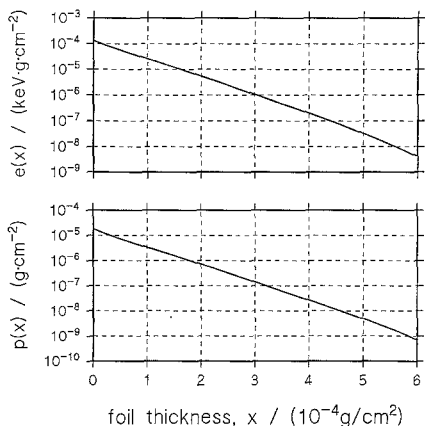
Fig. 3. Mean energy $e(x)$ and mean number $p(x)$ of electrons that reach the film, if a thick sample is separated from the film by a layer of thickness x. The quantities are given per cm^2 of film surface and per decay per gram of the sample
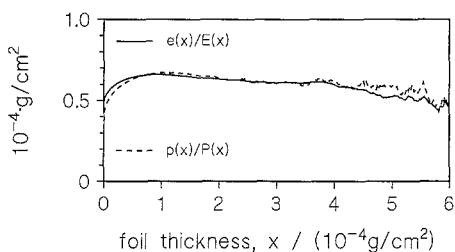
Fig. 4. The surface specific activity of a thin sample that delivers the same energy or the same number of electrons to the film as unit mass specific activity of the thick sample. The values are given for different layers x between sample and film

To obtain the analogous quantities for thick samples of mass specific activity α, one needs to integrate the expressions in (1) and (2) over the different depths. One has the following relations:

$$\dot{\psi}_x = \int_x^\infty E(x') \cdot \alpha \cdot \mathrm{d}x' = e(x) \cdot \alpha \tag{3}$$

and

$$\dot{\phi}_x = \int_x^\infty P(x') \cdot \alpha \cdot \mathrm{d}x' = p(x) \cdot \alpha \tag{4}$$

$e(x)$ has the dimension energy times mass divided by distance squared, $p(x)$ the dimension mass per distance squared. The two quantities are given in Fig. 3.

From the comparison of (1) and (3) one can derive the mass specific activity α of a thick sample that is equivalent (in terms of energy reaching the film) to a surface specific activity a of a thin sample:

$$\alpha = a / \frac{e(x)}{E(x)} \tag{5}$$

The ratio $e(x)/E(x)$ is the "virtual thickness" of the thick sample. The activity in this layer equals that of the equivalent thin sample. The quantity $e(x)/E(x)$ is plotted in Fig. 4. It has a value of about $6 \cdot 10^{-5}$ g/cm^2, largely independent of x. At unit density this corresponds to 0.6 µm.

The same equivalence can be established in terms of the number of β-rays to reach the film. One obtains

$$\alpha = a / \frac{p(x)}{P(x)} \tag{6}$$

$p(x)/P(x)$, too, can be considered as virtual thickness of the thick sample. The ratio is given as the dashed line in Fig. 4. It has roughly the same value of about 0.6 μm as $e(x)/E(x)$.

The value of 0.6 μm that has here been derived rigorously is, of course, fairly obvious in its order of magnitude. Even without detailed calculation one would estimate that the tritium β-rays from a thick sample come predominantly from a surface layer that is somewhat less than 1 μm thick, for a unit density material. Thus one could have obtained the approximate result without detailed computations.

Detection levels of specific activity

Sensitivity specifications for commercial films

The commercially available films to register tritium – and reference will here be made to Hyperfilm-³H and Hyperfilm-MP, marketed by Amersham [1] – are fairly insensitive compared with special sensitised emulsions that can be evaluated microscopically. These films are characterised by the producer in terms of their response to thin samples [1]. These characterisations can be translated into specifications for thick samples, i.e. into specifications for environmental sampling. To this purpose one applies the conversion formula that corresponds to (5) or (6). The mass specific activity, α, of a thick sample and the surface specific activity, a, of a corresponding thin sample are in the relation

$$\alpha = a / (6 \cdot 10^{-5} \text{ g/cm}^2) \tag{7}$$

The product information [1] is given in terms of the former unit μCi/cm² for the surface specific activity, a, per unit area. For conversion into the equivalent mass specific activity, α of a, thick sample, expressed in Bq/kg, one then obtains the relation

$$\alpha/(\text{Bq/kg}) = 1.7 \cdot 10^7 \cdot a/(\text{Bq/cm}^2) = 6.3 \cdot 10^{11} \cdot a/(\mu\text{Ci/cm}^2) \tag{8}$$

In terms of this conversion, one obtains the specification for Hyperfilm-³H (no cover foil) and Hyperfilm-MP (cover foil, $x = 2.4 \cdot 10^{-4}$ g/cm²) that is given in Fig. 5. The diagram shows the very high specific activities that are required with these films which are used for simple visual or densitometric evaluations.

According to the producers specification one can thus register, with the bare film (Hyperfilm-³H), specific activities down to about 10^{11} Bq/kg in thick samples, if Hyperfilm-MP is used without fluor-impregnated samples, the specific activity would have to be about 10^{12} Bq/kg.

It is hardly conceivable for environmental samples, for example, pieces of wood, to come even remotely close to specific activities of the above order of magnitude. Even for much longer exposure times (if they were practicable without producing artefacts) the detection levels would remain several million
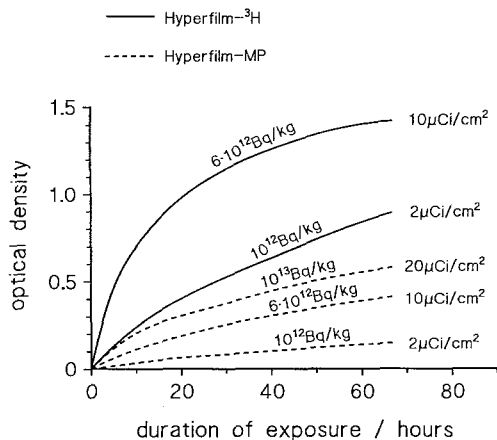
Fig. 5. Specification of response for Hyperfilm-3H and Hyperfilm-MP versus exposure time and surface specific activity of a thin sample or mass specific activity of a thick sample. The diagram is taken from the producer's specification [1] adding to the surface specific activity of a thin sample the equivalent mass specific activity in a thick sample

times higher than normal levels of organically bound tritium in wood or other biosamples, which are of the order of magnitude of 30 Bq/kg [4]. Tritium autoradiography is clearly not the method of choice for surveillance of environmental tritium levels. It would work with an intense local contamination, as might occur in a laboratory, but it is blind to environmental tritium levels, even if they are very substantially elevated.

One concludes that one would have needed truly horrendous environmental tritium levels – far beyond those that might be attainable in a reactor accident – to obtain the various black spots that have, in recent discussions, been tentatively ascribed to ambient tritium levels [7]. It is, therefore, apparent that these spots were not due to tritium but were artefacts of excessive storage times under inadequate conditions, such as room temperature.

Sensitivity under idealised conditions

The practical criteria for the applicability of tritium autoradiography must, of course, relate to the actual properties of the films. The above numerical example refers to the producer's specifications which have here not been experimentally verified. There may be better sensitivities in commercial films and, certainly, one can do considerably better in sophisticated studies, i.e. with suitable sensitised emulsion layers that are directly applied to the samples. In an earlier study [5] tritium was directly admixed to sensitive emulsions, and under suitable conditions it was possible to produce about one silver grain per decay. With the best techniques [8] one may even go somewhat further, but one may consider the effectiveness of one grain per electron in the emulsion as a limit that might be attainable under carefully controlled conditions, when a sample is brought into direct contact with the emulsion. One can then give the grain density as a function of the activity of the sample and the exposure time.

The following formulae will be given as value equations with conventional units of exposure time, T, in days; grain density, n, in grains per cm^2; surface specific activity, a, of the thin sample in Bq/cm^2; and specific activity, α, of the thick sample in Bq/kg.

For the thin sample one then obtains

$$n = 4.3 \cdot 10^4 \cdot a \cdot T \tag{9}$$

For the thick sample one has

$$n = 2.6 \cdot 10^{-3} \cdot \alpha \cdot T \tag{10}$$

For an illustration one might consider a surface specific activity of a thin sample of 30 Bq/cm² (corresponding to a mass specific activity of $5.1 \cdot 10^8$ Bq/kg). According to (9) one would then, with an exposure time of 10 days, obtain 0.13 grains per µm², which would be readily observed.

The formulae are given for application to specific circumstances that depend on grain size and sensitivity of the emulsions that might be used. Typical examples will, in agreement with these equations, show that one can do several hundred times better in proper micro-autoradiography studies, than in the work with the commercial films with simple visual or densitometric evaluation. But even under optimal conditions one would need extremely elevated specific activities to obtain tritium autoradiographs in biosamples, and in all cases one needs added evidence to ascertain that an observed count or film opacity is, indeed, due to tritium. While one can achieve direct identification of tracks with more energetic β-rays, one always needs to use added tools, such as scintillation measurements, in the attempt to determine tritium.

Conclusion

In the microbiological laboratory tritium autoradiography is performed with sophisticated techniques, and very small activities of tritium are then sufficient to produce intense labelling of microscopic samples. This may, without further reflection, lead to the expectation that small specific acitivities can be measured in macroscopic environmental samples. However, this is a misconception, since the film, even if it is in direct contact with the sample, sees effectively only the activity in a surface layer that is 0.6 µm thick in a material of unit density. Even with the advanced method of micro-autoradiography [2, 8, 11, 12], one requires high specific activities to register tritium. With the considerably less sensitive commercial films one would need specific activities far beyond those that could conceivably be encountered in environmental samples.

Established scintillation counting techniques permit the measurement of environmental tritium levels in biosystems with high precision [4, 6], and they are thus more sensitive than the autoradiographic method by at least the above factor. Autoradiography has a different purpose. It is not suitable for low-level measurements; its potential is the high spatial resolution down to microscopic dimensions. If autoradiography is, nevertheless, used for radioactivity measurements, it must always be complemented by careful spectroscopy, as it is possible with scintillation counting. This need is particularly critical with tritium, where one cannot achieve a discrimination against artefacts by the observation of particle tracks.

The considerations that have here been applied to autoradiography of tritium could similarly be related to the autoradiography of other β-ray emitters. However, the treatment is limited to tritium, because of the extraordinary confusion

that has led to the mistaken comparison [7] of careful and valid low-level scintillation measurements of tritium with purported tritium autoradiographs that should, on the basis of elementary order-of-magnitude considerations, have been readily recognised and discarded as fallacies.

Appendix

The computations were performed in terms of semi-empirical differential ionisation cross sections for electron scattering in water vapour that were derived by Rudd [10]. These cross sections are valid up to the electron eneregy 10 keV. Above 10 keV the relativistic asymptotic Bethe formula is used to extrapolate the total ionisation cross sections. The corresponding differential cross sections are normalised by the asymptotic values.

The elastic cross sections and excitation cross sections for electrons are taken from the input data set of a Monte Carlo program written by Zaider and Brenner [13]. The excitation cross sections are extrapolated with the non-relativistic asymptotic Bethe formula in the energy range between 1 and 10 keV. Above 10 keV the relativistic asymptotic Bethe formula is used for the extrapolation.

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