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October 10, 2008

Nuclear Regulatory Commission
Materials Inspection Branch
US Nuclear Regulatory Commission, Region III
2443 Warrenville Road, Suite 210
Lisle, IL 60532-4352

ATTN: Patrick L. Loudon

RE: NRC Inspection Report 030-05154/08-01(DNMS) and Notice of Violation –
Analytical Bio-Chemistry Laboratories, Inc.

Dear Mr. Loudon:

Please find enclosed the requested “BioAssay Procedure for Assessing
Exposure to Radioactive Material (OESH-813)”.

If you have any questions or need further information, please feel free to contact
me at 573-777-6070.

Sincerely,

A handwritten signature in blue ink that reads 'Sheila Hecht'.

Sheila C. Hecht
Director, Safety and Occupational Health

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Bioassay Procedure for Assessing Exposure to Radioactive Material (OESH-813)

ABC Laboratories uses radioactive material in labeled compounds that include carbon-14 (C-14) and hydrogen-3 (H-3). Exposure from conducting studies with these compounds is controlled well within regulatory limits due to environmental controls and proper laboratory techniques demonstrated by our laboratory personnel. To verify implementation of controls and monitor exposures all employees who work in the synthesis laboratory will submit bioassay (urinalyses) weekly. In addition, laboratory personnel working with levels of radioactivity of 10 millicuries and greater, will submit bioassays (urinalyses) on the work day following completion of work.

Definitions

Annual Limit on Intake (ALI): The derived limit for the amount of radioactive material taken into the body of an adult worker by inhalation or ingestion in a year. ALI is the smaller value of intake of a given radionuclide in a year by the reference man that would result in a committed effective dose equivalent of 5 rem (0.05 Sv) or a committed dose equivalent of 50 rem (0.5 Sv) to any individual organ or tissue. The ALIs are published in 10 CFR 20 Appendix B Table1.

Derived Air Concentration (DAC): The concentration of a given radionuclide in air which, if breathed by the reference man for a working year of 2,000 hours under conditions of light work (inhalation rate of 1.2 cubic meters of air per hour), results in an intake of one ALI. These values are also published in 10 CFR 20 Appendix B Table1.

Evaluation and Intake Monitoring Action Levels

In addition to controlling radiation dose within the regulatory limits, ABC Laboratories will limit radiation dose to employees to levels that are as low as readily achievable (ALARA). The ALARA program is implemented by establishing administrative controls for work at ABC Laboratories at radiation dose levels that are lower than the regulatory limits.

ABC Laboratories will recognize an indicated intake of 0.02 times the ALI as an **Evaluation Level** and 0.10 times the ALI as an **Action Level**. At the evaluation level, the contaminated associate will be required to submit daily or weekly bioassay samples so as to properly estimate total intake. If the action level is reached or exceeded, in addition to the more extensive biomonitoring and possible consultation with the corporate physician, the Radiation Safety Committee will meet to review the incident and decide if additional environmental controls are required in the area.

Values (From 10 CFR 20.1001 – 20.1204 and Appendix B, Table 1)

ALI for Carbon-14 and Hydrogen-3 (inhalation or ingestion)

Compound ^{14}C	$2 \times 10^3 \mu\text{Ci/year}$
$^{14}\text{CO}_2$	$2 \times 10^5 \mu\text{Ci/year}$
Compound and gas ^3H	$8 \times 10^4 \mu\text{Ci/year}$

DAC for Carbon-14 and Hydrogen-3

Compound ^{14}C	$1 \times 10^{-3} \mu\text{Ci/cc}$
$^{14}\text{CO}_2$	$9 \times 10^{-5} \mu\text{Ci/cc}$
Compound and gas ^3H	$2 \times 10^{-5} \mu\text{Ci/cc}$

Incidents and Activities requiring Biomonitoring at ABC Laboratories

- Spraying of test plots in greenhouses or open-air designated areas with labeled material.
- Procedures using 10 millicuries of carbon-14 compounds
- Individuals involved in an incident whereby they may have come into direct contact, via skin, inhalation, or consumption of activity levels equal to or greater than 0.02 times the ALI. An example of such an incident is where a chemist in the laboratory has checked out a stock solution with a total activity of 15 mCi. While transferring an aliquot of the material, the chemist accidentally knocks the storage container holding the material over, spilling a portion of the contents on him/herself. As the incident likely involved contact with more than 0.02 times the ALI of ^{14}C -compound (40 μCi), the individual must undergo biomonitoring.
- Individuals who are working in areas where air monitoring has demonstrated that they are exposed to concentrations of ^{14}C radioactivity sufficient to cause an inhalation exposure equal to or greater than 0.02 times the ALI (40 DAC-hrs).

Procedure for Submitting Urine Samples for Analysis

The routine weekly bioassay samples for employees working in the synthesis lab will be submitted on Fridays or on the last working day of the week, as applicable. Bioassays that are required due to the quantity of radioactive material that is handled or due to incidents will be submitted on the day following the activity or incident. Additional bioassay samples may be required by the Radiation Safety Officer.

If the urinalysis is being conducted because of a possible incident where an employee has been exposed to a significant amount of activity, the Radiation Safety Officer or, in their absence, the Radiation Safety Technician should be

contacted immediately. The incident should be reviewed for severity, based on the nature of the exposure (primary area of contact and the duration of the contact) and the level of activity to which the employee was exposed. If significant intake is viewed as unlikely, an initial sample plus an additional one at 24 hours will be sufficient. If the intake is estimated as being more serious, as may occur from the inhalation of dry material with a high specific activity, daily samples will be required for two weeks, or until no excreted radioactivity is detected in the daily bioassay samples.

When bioassay samples are required, the laboratory worker(s) will perform the following steps:

- Don a pair of clean new gloves.
- Obtain a sterile (unopened) urine sample container.
- Write name or ABC employee identification number, the date, the compound last used, and the time that the urine sample is taken on the urine sample container label.
- Fill the urine container approximately one half full and screw the lid back on tightly.
- Place the filled urine container in the appropriate collection box.

Counting the Urine Samples

- Radiation Safety will collect the urine containers daily, maintain chain-of-custody documentation, and perform the bioassay using a Liquid Scintillation Counter.
- One (1.0) milliliter of a urine sample will be pipeted into a 20-milliliter scintillation vial. Fifteen (15) milliliters of liquid scintillation cocktail (Beckman ReadyGel or equivalent) will be added, the vial capped, and the vial shaken to insure homogeneity. The identity of the sample must be recorded on the cap of the vial.
- The above step will be repeated for each individual urine sample.
- Two reagent controls containing 15 mL of scintillation cocktail solution (and one mL of deionized water) will also be prepared.
- The samples will be placed in a laboratory refrigerator for at least 90 minutes to decrease fluorescence in the samples.
- The samples will be counted in a Liquid Scintillation Counter per the instrument's SOP (typically for 5 minutes or to a 2 sigma/95% confidence). The activity (in disintegrations per minute) will be counted.

Analysis and Actionable Exposures

After the samples have counted the Radiation Safety Officer will review the bioassay results. If the indication is that no excreted radioactive material can be detected, no further samples need to be taken. If activity greater than 40 dpm above control samples was detected, at least three additional daily samples will be taken. The samples will be analyzed daily.

If significant activity is detected, urinalyses may continue daily until 14 calendar days after the incident, followed by weekly monitoring until the Radiation Safety Officer is satisfied that the committed dose equivalent has been determined.

Following the completion of the urinalyses, the contaminated associate will be requested to submit a written description of the incident. Information included in the incident report are: name, job title, manager, date and time of the incident, description of the incident, description of the contaminating material (including the activity of the material) and efforts made to prevent or limit the contamination. The incident report plus the results of the urinalyses will be provided to the Radiation Safety Officer to record the incident. In addition, the employee's manager will be requested to fill out an incident report form, documenting the incident and acknowledging actions which were taken.

If an employee's total effective dose for the year reaches 500 mrem or 10% of the ALI, an evaluation of the employee's work activities will be conducted by the Radiation Safety Committee. If the total exposure for the year reaches 2500 mrem or 50% of the ALI, the employee will be removed from working with radioactive materials and their work activities will again be reviewed by the Radiation Safety Committee for approval to continue working. If total exposure reaches 75%, the employee will be removed from working with radioactive material until the next calendar year, unless authorized by the Laboratory Director in writing. If the total dose reaches 4500 mrem, no further work with radiological materials is permitted until the following calendar year.

Calculation of Intake

The following table will be used to determine effective dose equivalent from all forms of H-3 and from compounds labeled with C-14 by multiplying the activity in dpm in one milliliter of urine times the appropriate factor for the radionuclide, gender, chemical form, and time since the incident.

If the indicated dose to an individual exceeds 100 mrem in a calendar quarter and there are at least five weeks of monitoring data available, or if the incident was more than 80 before the bioassay date, a Certified Health Physicist may calculate and document the dose of record for the individual in that calendar quarter.

Table 1: Bioassay Conversion Factors (mrem per dpm/ml)

days after intake	Male	Female	Male	Female	Male	Female	Male	Female
	HTO	HTO	carbonate	carbonate	cyanide	cyanide	benzene/anilene	
	BCF	BCF	BCF	BCF	BCF	BCF	BCF	BCF
1	7.6E-04	6.9E-04	1.5E+00	1.4E+00	1.1E-01	1.0E-01	8.4E-01	7.7E-01
2	8.1E-04	7.4E-04	1.6E+00	1.4E+00	1.1E-01	1.0E-01	8.5E-01	7.8E-01
3	8.6E-04	7.8E-04	1.6E+00	1.5E+00	1.2E-01	1.1E-01	8.7E-01	7.9E-01
4	9.2E-04	8.4E-04	1.7E+00	1.5E+00	1.2E-01	1.1E-01	8.8E-01	8.1E-01
5	9.7E-04	8.9E-04	1.7E+00	1.6E+00	1.2E-01	1.1E-01	9.0E-01	8.2E-01
6	1.0E-03	9.5E-04	1.8E+00	1.6E+00	1.2E-01	1.1E-01	9.1E-01	8.3E-01
7	1.1E-03	1.0E-03	1.8E+00	1.7E+00	1.2E-01	1.1E-01	9.3E-01	8.5E-01
8	1.2E-03	1.1E-03	1.9E+00	1.7E+00	1.3E-01	1.2E-01	9.5E-01	8.6E-01
9	1.3E-03	1.1E-03	2.0E+00	1.8E+00	1.3E-01	1.2E-01	9.6E-01	8.8E-01
10	1.3E-03	1.2E-03	2.0E+00	1.9E+00	1.3E-01	1.2E-01	9.8E-01	8.9E-01
11	1.4E-03	1.3E-03	2.1E+00	1.9E+00	1.3E-01	1.2E-01	1.0E+00	9.1E-01
12	1.5E-03	1.4E-03	2.2E+00	2.0E+00	1.4E-01	1.2E-01	1.0E+00	9.3E-01
13	1.6E-03	1.5E-03	2.3E+00	2.1E+00	1.4E-01	1.3E-01	1.0E+00	9.4E-01
14	1.7E-03	1.6E-03	2.4E+00	2.2E+00	1.4E-01	1.3E-01	1.1E+00	9.6E-01
15	1.8E-03	1.7E-03	2.5E+00	2.3E+00	1.4E-01	1.3E-01	1.1E+00	9.8E-01
16	1.9E-03	1.8E-03	2.6E+00	2.4E+00	1.5E-01	1.3E-01	1.1E+00	9.9E-01
17	2.1E-03	1.9E-03	2.8E+00	2.5E+00	1.5E-01	1.3E-01	1.1E+00	1.0E+00
18	2.2E-03	2.0E-03	2.9E+00	2.7E+00	1.5E-01	1.4E-01	1.1E+00	1.0E+00
19	2.3E-03	2.1E-03	3.1E+00	2.8E+00	1.5E-01	1.4E-01	1.1E+00	1.0E+00
20	2.5E-03	2.3E-03	3.3E+00	3.0E+00	1.6E-01	1.4E-01	1.2E+00	1.1E+00
25	3.4E-03	3.1E-03	4.5E+00	4.1E+00	1.7E-01	1.5E-01	1.3E+00	1.2E+00

26	3.6E-03	3.3E-03	4.8E+00	4.4E+00	1.7E-01	1.6E-01	1.3E+00	1.2E+00
27	3.8E-03	3.5E-03	5.2E+00	4.7E+00	1.8E-01	1.6E-01	1.3E+00	1.2E+00
28	4.1E-03	3.7E-03	5.6E+00	5.1E+00	1.8E-01	1.6E-01	1.3E+00	1.2E+00
29	4.4E-03	4.0E-03	6.0E+00	5.5E+00	1.8E-01	1.7E-01	1.4E+00	1.2E+00
30	4.6E-03	4.2E-03	6.5E+00	5.9E+00	1.8E-01	1.7E-01	1.4E+00	1.3E+00
35	5.6E-03	5.1E-03	8.2E+00	7.5E+00	1.9E-01	1.8E-01	1.5E+00	1.3E+00
40	7.6E-03	7.0E-03	1.3E+01	1.1E+01	2.1E-01	1.9E-01	1.6E+00	1.5E+00
45	1.0E-02	9.5E-03	2.0E+01	1.8E+01	2.3E-01	2.1E-01	1.7E+00	1.6E+00
50	1.4E-02	1.3E-02	3.2E+01	3.0E+01	2.5E-01	2.3E-01	1.9E+00	1.7E+00
55	1.9E-02	1.8E-02	5.3E+01	4.9E+01	2.8E-01	2.5E-01	2.1E+00	1.9E+00
60	2.7E-02	2.4E-02	8.8E+01	8.1E+01	3.0E-01	2.7E-01	2.3E+00	2.1E+00
65	3.6E-02	3.3E-02	1.5E+02	1.4E+02	3.3E-01	3.0E-01	2.5E+00	2.2E+00
70	5.0E-02	4.5E-02	2.5E+02	2.3E+02	3.6E-01	3.3E-01	2.7E+00	2.4E+00
75	6.8E-02	6.2E-02	4.2E+02	3.8E+02	3.9E-01	3.6E-01	2.9E+00	2.7E+00
80	9.3E-02	8.5E-02	7.1E+02	6.5E+02	4.2E-01	3.9E-01	3.2E+00	2.9E+00

Basis for Calculations of Effective Dose Equivalent

Physiology and Physical References

The body mass of the reference male, M_{body} , is **73 kg** and the body mass of the reference female is **60 kg**. The urine excretion rate of the reference male, V_U is **1600 mL/day** and the urine excretion rate of reference female is **1200 mL/day**. These values are obtained from ICRP Publication 89, pp 64 and 84.

The average decay energy of H-3 is **5.68E-3 MeV/dis** and the average decay energy of C-14 is **4.95E-2 MeV/dis**, according to ICRP Publication 38.

Effective Half Life and Urine Excretion Fractions

The urine excretion fraction for H-3 is set at 60% in the calculations. This factor is based on NUREG/CR 4884, page B-711, which applies a urine excretion factor of 60% for liquid HTO. The effective half life of H-3 is set at 11.1 days. Although NUREG/CR-4884 page B-711 applies an effective half life of 10 days, the ALI for

H-3 in 10 CFR 20 Appendix B Table 1 is consistent with 11.1 days. Hence the more conservative value was applied. The selected values of the urine excretion fractions and effective half lives for C-14 are detailed in Table 2 below. Note that barium carbonate is modeled by a two compartment model to maintain conservatism.

Dose Conversion Factors

The dose conversion factors for screening, DCF_{intake} given in units of mrem/uCi of intake, were taken from 10 CFR 20, Appendix B Table 1, by calculating the ratio of the annual dose limit divided by the ALI. For H-3, this is:

$$5000 \text{ mrem} / 8000 \text{ uCi} = \mathbf{6.375E-2 \text{ mrem/uCi}}$$

and for C-14:

$$5000 \text{ mrem} / 2000 \text{ uCi} = \mathbf{2.5 \text{ mrem/uCi}}$$

Having established the DCF_{intake} from 10 CFR 20, there must be a further calculation to determine the effective half-life, T_{eff} , that is consistent with this DCF. The T_{eff} can then be used to calculate intake retention fractions, IRF, for converting bioassay results to dose. First the physical constants can be consolidated in one term.

The physical conversion constant, C_{phy} , is:

$$C_{\text{phy}} = 10 \text{ mrem/erg/g} * 1.6E-6 \text{ erg/MeV} * 1440 \text{ min/day} * 2.22E6 \text{ dpm/uCi}$$

$$C_{\text{phy}} = 5.11E4 \text{ (mrem/uCi)-(dis/day) per (MeV/g)}, \text{ and}$$

$$DCF_{\text{intake}} = (T_{\text{eff}}/0.693) * C_{\text{phy}} * E \text{ (MeV/dis)} / M_{\text{body}} \text{ (g)}, \text{ where } T_{\text{eff}} \text{ is in days.}$$

Therefore $T_{\text{eff}} = (0.693/5.11E4) * DCF_{\text{intake}} * M_{\text{body}} / E$. Since 10 CFR 20 Appendix B provides one ALI for males and females, the T_{eff} will be calculated using reference man's physical quantities.

Hence T_{eff} for H-3 is $(0.693/5.11E4) * 6.38E-2 * (7.3E4/5.68E-3) = \mathbf{11.1 \text{ days}}$, and the T_{eff} for C-14 is $(0.693/5.11E4) * 2.5 * (7.3E4/4.95E-2) = \mathbf{50.0 \text{ days}}$.

The values of T_{eff} are larger than generally accepted values of ~10 days and 40 days, respectively. The calculated values are consistent with the implied DCF_{intake} in 10 CFR 20 and conservative.

For a **reference female**, adopting these values of T_{eff} results in a DCF_{intake} of **7.72E-2 mrem/uCi for H-3** and **3.04 mrem/uCi for C-14**. These values, which are greater than the reference male factors, are used in Table 1 to be conservative.

Bioassay Conversion Factor

The bioassay conversion factor (BCF_T) is an engineering conversion factor that is used for convenience to convert the concentration of radioactivity in a urine sample, in units of dpm/mL, at a time T , in days after an incident, into effective dose equivalent in mrem. The BCF_T is derived from the following equation, which is consistent with USNRC Regulatory Guide 8.9:

$$\text{Dose} = DCF_{\text{intake}} * A_T / IRF_T \text{ where } A_T \text{ is the activity in urine in day } T \text{ and}$$

$$A_T = C_{U,T} * V_{\text{urine}} / (2.22E6), \text{ uCi/day,}$$

where C_U is the urine concentration in dpm/mL. Therefore:

$$\text{Dose} = DCF_{\text{intake}} * C_U * V_U / (2.22E6) * IRF_T \text{ and, if we define}$$

$$BCF_T = DCF_{\text{intake}} * V_U / (2.22E6 * IRT_T), \text{ then}$$

$$\text{Dose} = BCF_T * C_{U,T}$$

The use of the BCF_T facilitates calculations based on the concentration in a urine sample. The IRT_T is calculated from the exponential decay and urine excretion fraction per the method of NUREG/CR 4884:

$$IRT_{T_2} = F_U * [\exp(-0.693T_1/T_{\text{eff}}) - \exp(-0.693T_2/T_{\text{eff}})] / (T_2 - T_1),$$

where F_U is the fraction of intake that is excreted in urine and T_1 and T_2 are the start and end of the period of urine collection relevant to the sample concentration.

Table 2: Urine Excretion Factors and Half Lives for C-14

Chemical Form	Citation or Basis
<p>Barium carbonate</p> <p>(1) The fraction of carbon excreted in the urine is 1% in the dose calculation procedure.</p> <p>(2) The turnover of carbonate in the blood is at least 10% per day, which is equivalent to a half-life of 6.6 days:</p> $0.1 = \exp(-.693/6.6)$ <p>(1, 3, 4) The rapid turnover compartment with 6.6 day half-life is limited to 90% of the intake to maintain conservatism for general dose calculations</p> <p>(5) The effective half-life for long term retention of the remaining 10% of intake is set at 50 days, which is consistent with the ALI in 10CFR20 Appendix B Table 1 and conservative compared to ICRP's 40 days.</p>	<p>(1) Comparative Excretion and Distribution of C-14-Labeled Carbonate and Formate in Large Albino Rats, FREDERICK SPERLING, ELIZABETH S. MAXWELL AND W. F. VON OETTINGEN, Am J Physiol. 174 (1): 33: C-14 carbonate-injected rats excreted, in the urine, somewhat more than 1 % of the dose. After 8 days, residues of C-14 did not exceed 0 to 4% (Table 1.)</p> <p>(2) Current Surgical Therapy, ed. John Cameron, Elsevier Mosby, 2001, p1159: Renal mechanisms function to regulate pH by reabsorbing filtered bicarbonate, preventing bicarbonate loss in the urine, and excreting H+ ions instead. Most bicarbonate (85%) is reabsorbed in the proximal tubule by excretion of H+ ions.</p> <p>(3) Structure of a physiologically based biokinetic model for use in 14C and organically bound tritium dosimetry, D. W. Whillians, Radiation Protection Dosimetry 105:189-192 (2003): Physiologically based biokinetic dosimetry models for ^{14}C must include rapid turnover compartments which, can dominate bioassay measurements at early times after intake. In this paper a ... model structure will be described for use in dose assessments for organic ^{14}C ... based on the literature of human carbon metabolism, and on direct measurements of human excretion.</p> <p>(4) Assessment of contributors to radiation dose following intakes of rapidly excreted [14C]-compounds, David M. Taylor* School of Chemistry, Cardiff University, Cardiff CF10 3TB, UK Radiation Protection Dosimetry 2007 127(1-4):440-443, The International Commission on Radiological Protection default biokinetic model for the assessment of radiation dose received following intakes of unspecified [^{14}C]-compounds (DCM) appears to overestimate the radiation doses delivered by many [^{14}C]-compounds.</p> <p>(5) Annals of the ICRP, Volume 20, Issue 2, 1989, Page 22: "All labeled organic compounds are assumed to be distributed rapidly and uniformly in all body organs and tissues of adults and retained with a rounded half-time of 40 days (ICRP, 1979)."</p>
<p>Benzene</p> <p>(1) The fraction of carbon excreted in the urine is 10% in the dose calculation procedure.</p>	<p>(1) Determination of dose coefficients and urinary excretion function for inhalation of carbon-14-labelled benzene, A. Krins, K. Karcher, D. Noke, P. Sahre and T. Schönmath, Radiation Protection Dosimetry 104:139-152 (2003)</p> <p>© 2003 Oxford University Press: "The fraction of activity removed via urine varies between 52 and 10% of the intake. ... A 14-day interval for the incorporation monitoring by urine activity counting seems to be reasonable.</p>

<p>(2) The effective half-life for long term retention is set at 50 days, which is consistent with the ALI in 10CFR20 Appendix B Table 1 and conservative compared to ICRP's 40 days.</p>	<p>(2) Annals of the ICRP, Volume 20, Issue 2, 1989, Page 22: "All labeled organic compounds are assumed to be distributed rapidly and uniformly in all body organs and tissues of adults and retained with a rounded half-time of 40 days (ICRP, 1979)."</p>
<p>Potassium cyanide</p> <p>(1) The fraction of carbon excreted in the urine is 75% in the dose calculation procedure.</p> <p>2) The effective half-life for long term retention is set at 50 days, which is consistent with the ALI in 10CFR20 Appendix B Table 1 and conservative compared to ICRP's 40 days.</p>	<p>(1) Excretion of 14C-labeled cyanide in rats exposed to chronic intake of potassium cyanide, Okoh, P.N., Appl. Pharmacol., Vol/Issue: 70:2, Sep 15 1983, OSTI ID: 6914257: "Urinary excretion was the main route of elimination of cyanide carbon in these rats, accounting for ... 89% of the total excreted radioactivity in 24 hr."</p> <p>(2) Annals of the ICRP, Volume 20, Issue 2, 1989, Page 22: "All labeled organic compounds are assumed to be distributed rapidly and uniformly in all body organs and tissues of adults and retained with a rounded half-time of 40 days (ICRP, 1979)."</p>
<p>Aniline</p> <p>(1) The fraction of carbon excreted in the urine is 10% in the dose calculation procedure.</p> <p>(2) The effective half-life for long term retention is set at 50 days, which is consistent with the ALI in 10CFR20 Appendix B Table 1 and conservative compared to ICRP's 40 days.</p>	<p>(1) Studies in Detoxification D. V. Parke, Department of Biochemistry, St. Mary's Hospital Medical School, London, W2, UK : The quantitative results for the metabolism of aniline in the rabbit are shown in Table 4. In 3-8 days after oral dosage with C-14 aniline an average of 70% of the radioactivity is eliminated from the body in urine.</p> <p>(2) Annals of the ICRP, Volume 20, Issue 2, 1989, Page 22: "All labeled organic compounds are assumed to be distributed rapidly and uniformly in all body organs and tissues of adults and retained with a rounded half-time of 40 days (ICRP, 1979)."</p>

Comparative Excretion and Distribution of C¹⁴-Labeled Carbonate and Formate in Large Albino Rats

FREDERICK SPERLING, ELIZABETH S. MAXWELL AND W. F. VON OETTINGEN

From the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service, Federal Security Agency, Bethesda, Maryland

THE FATE, excretion and retention of C¹⁴ incorporated in sodium carbonate has aroused much interest since a preliminary report on this question was published by Bloom, Curtis and McLean (1). It was expected that C¹⁴ incorporated in other simple molecules would have a different fate and for this reason a comparative study was undertaken of the behavior of sodium carbonate and sodium formate under strictly comparable conditions. In addition it was hoped that such study of the formate might throw some light on its metabolism, especially its rate of oxidation, which has given rise to much speculation in connection with the mechanism of the toxicity of methanol and other monosubstituted methane derivatives. For this reason the studies were made with single intraperitoneal injections and the animals observed for a period of 8 days. Armstrong, Schubert and Lindenbaum (2) had developed suitable methods which were followed in the present study.

Since the experiments to be reported below were undertaken, several papers have been published on the distribution of C¹⁴ after the administration of labeled carbonate, by various means, in mice (3, 4) and rats (5) and on its presence in glycogen, blood glucose and lactate (6), in amino acids (7) and in nucleic acid (8). The excretory rates by various routes have been reported in rats (2, 5, 6). Similarly certain phases of the fate and pulmonary excretion of C¹⁴ from labeled formate have been reported in rats (9-11). None of these latter studies considered the excretion and distribution of formate and none have direct bearing on the problem discussed below.

MATERIALS AND METHODS

Eleven male Osborne-Mendel rats, 12-18 months of age, weighing 400-537 gm, were used, thus eliminating

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variants due to estrus, growth and senility. The animals were fed a diet of 10% dextrose by weight in whole milk, which was introduced into the cage through a funnel with a stopcock, thus excluding atmospheric CO₂ and preventing the escape of respiratory CO₂. Each rat was placed on this diet exclusively for 10 days - 2 weeks prior to injection.

Each rat was injected interapritoneally with a single dose of a solution of either 0.03-0.05 mc of sodium carbonate or 0.07-0.10 mc of sodium formate. Six rats received 1 cc/100 gm body wt. of a solution containing 1.1×10^{-5} M/cc sodium carbonate. Four rats received similar doses in similar volumes of sodium formate. One rat received 40% of this dose of formate.

Immediately after injection each rat was placed in a glass cage of 12-liters capacity, similar to that used by Armstrong *et al.* (2), the cage sealed, and the rat kept there for 8 days. The air, which was drawn through the cage, was first filtered through soda lime to remove CO₂ and then through a solution of barium hydroxide, which served to indicate the complete absorption of atmospheric CO₂ by the soda lime. Air-flow rate was measured by means of a 'rotameter,' and was kept at about 1 l/min. All metal and rubber surfaces in the cage were coated with 'Glyptal' to prevent reaction and leakage of CO₂. Respiratory CO₂ in the air passing through the cage was absorbed in 500 cc of a 20% solution of sodium hydroxide in a glass tower. The air was then passed through a wash bottle containing barium hydroxide solution.

Urine and feces were separately collected as described by Armstrong *et al.* (2). Those fecal pellets which stuck to the sides of the cage were collected at the end of the experiment and separately analyzed. The hair in the cage, which the animals had shed because of self-grooming, was also removed and analyzed.

The towers were changed at the end of 1, 2, 3, 6 and 24 hours and then daily, for the determination of CO₂. Urine was collected at 7 hours, at 24 hours and then daily. Feces were collected daily when possible. Occasionally the amount of feces passed during a 24-hour period was inadequate for analysis so that two-day samples had to be used.

The plan of analysis was similar to that of Armstrong *et al.* (2). Aliquots of all materials, with the exception of exhaled CO₂, underwent wet combustion by the method of Van Slyke and Folch (12). The products of combustion were passed through granulated zinc and then into a solution of normal, CO₂-free sodium hydroxide. Formates, when oxidized by this method, yield both CO and CO₂. To obtain complete recovery

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Determination of dose coefficients and urinary excretion function for inhalation of carbon-14-labelled benzene

A. Krins, K. Karcher, D. Nozke, P. Sahre and T. Schönmath

Based on existing pharmacokinetic models for benzene, the distribution and retention of activity after inhalation of ^{14}C -labelled benzene in humans were studied. Six different benzene concentrations from 0.1 to 10,000 ppm (corresponding to activity concentrations between 9.6×10^6 and 9.6×10^{11} Bq m^{-3}) and five exposure times from 0.1 to 1000 min were considered. The cumulated activities in the different organs and tissues and the urinary excretion rates were observed to depend non-linearly on the activity intake. The fraction of activity removed via urine varies between 52 and 10% of the intake. Nevertheless, for times that are long compared to the exposure duration the urinary excretion rate is determined by the activity clearance from adipose tissue and thus decreases at a constant rate. This decrease is common for all exposure conditions examined and thus allowed determining a mean urinary excretion rate and corresponding dose coefficients for committed equivalent doses as well as for the effective dose. The uncertainty of the dose coefficients is estimated to be about 50% for the exposure range covered. A 14-day interval for the incorporation monitoring by urine activity counting seems to be reasonable.

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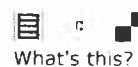
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Subject	560305 -- Chemicals Metabolism & Toxicology-- Vertebrates-- (-1987) ;551001 -- Physiological Systems-- Tracer Techniques. ;CYANIDES-- EXCRETION;CYANIDES-- INGESTION; BREATH;CARBON 14 COMPOUNDS;CHRONIC INTAKE;FECES;LABELLED COMPOUNDS;MEASURING METHODS;POTASSIUM COMPOUNDS;RATS;TIME DEPENDENCE;TRACER TECHNIQUES;URINE
Related Subject	ALKALI METAL COMPOUNDS;ANIMALS;BIOLOGICAL MATERIALS;BIOLOGICAL WASTES;BODY FLUIDS;CLEARANCE;INTAKE;ISOTOPE APPLICATIONS;LABELLED COMPOUNDS;MAMMALS;MATERIALS;RODENTS;VERTEBRATES;WASTES
Description/Abstract	The excretion of an acute dose of ¹⁴ C-labeled cyanide in urine, feces, and expired air was studied in rats exposed to daily intake of unlabeled KCN in the diet for 6 weeks. Urinary excretion was the main route of elimination of cyanide carbon in these rats, accounting for 83% of the total excreted radioactivity in 12 hr and 89% of the total excreted radioactivity in 24 hr. The major excretion metabolite of cyanide in urine was thiocyanate, and this metabolite accounted for 71 and 79% of the total urinary activity in 12 hr and 24 hr, respectively. The mean total activity excreted in expired air after 12 hr was only 4%, and this value did not change after 24 hr. Of the total activity in expired air in 24 hr, 90% was present as carbon dioxide and 9% as cyanide. When these results were compared with those observed for control rats, it was clear that the mode of elimination of cyanide carbon in both urine and breath was not altered by the chronic intake of cyanide.
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Table 5. Occurrence of radioactivity in the tissues of rabbits receiving [¹⁴C]aniline

Expt. no.	6			7		
	5			8		
Dose of ¹⁴ C (μc)	67			109		
Time of death (days after dosing)	5			8		
	Wt. of wet tissue (g.)	Specific activity of tissue (μc/g.)	Percentage of dose present in tissue	Wt. of wet tissue (g.)	Specific activity of tissue (μc/g.)	Percentage of dose present in tissue
Lungs	15	0.001	0.02	14	0.001	0.01
Liver	70	0.001	0.10	53	0.003	0.15
Kidney	11	0.002	0.03	12	0.004	0.05
Heart	7	< 0.001	< 0.01	7	< 0.001	< 0.01
Spleen	2	0.005	0.02	1.5	0.004	0.01
Blood	350	0.002	1.1	350	0.003	1.0
Muscle, voluntary	700	0.001	1.1	750	0.001	0.8
Muscle, involuntary	200	0.001	0.3	200	0.001	0.2
Fat	470	0.004	2.8	550	0.005	2.5
Brain	35	< 0.001	< 0.05	40	0.002	0.8
Ovary	1	0.001	< 0.01	1	< 0.001	< 0.01
Bile	—	—	—	1	< 0.001	< 0.01
Stomach contents	105	0.002	0.3	70	0.005	0.4
Intestinal contents (small intestine)	130	0.002	0.4	210	0.004	0.8
Intestinal contents (caecum)	60	0.002	0.2			
Faeces	75	0.010	1.1	60	0.027	1.5
Total in tissues and faeces	—	—	7.4	—	—	8.2
Total in expired air	—	—	< 0.4	—	—	< 0.4
Total in urine	—	—	77	—	—	73
Total accounted for	—	—	85	—	—	81

Table 6. Elimination of metabolites in the urines of dogs receiving [¹⁴C]aniline orally

Expt. no.	1	2
Dose of aniline (mg./kg.) ...	175	200
Dose of ¹⁴ C (μc/animal) ...	12.5	15
Duration of expt. (hr.) ...	16*	41*
Aniline (total)	2.8	12.8†
Aniline (free)	0.4	8.9†
Acetanilide	< 0.1	< 0.1
<i>o</i> -Aminophenol	10.7	25.2
<i>m</i> -Aminophenol	< 0.1	< 0.1
<i>p</i> -Aminophenol	7.1	11.3
Sum of metabolites	20.6	49.3
Total radioactivity in urine	18.6	48.5

* Both animals showed symptoms of methaemoglobinemia and were killed when distressed.

† Some of this was from vomit.

aminophenol accounts for 51 % of the dose. Other metabolites are *o*-aminophenol (9 %), phenylsulphamic acid (5.5 %), aniline-*N*-glucuronide (3.5 %), *m*-aminophenol (0.1 %) and acetanilide (0.2 %). 4-Aminoresorcinol, which was tentatively suggested by Smith & Williams (1949) as a metabolite of aniline, was not found, and none of the other dihydroxyanilines was detected. No anilide other than acetanilide was present and no evidence of methylation or deamination was obtained. There was no evidence of the formation of a mercapturic acid since the isotope-dilution procedure for *p*-

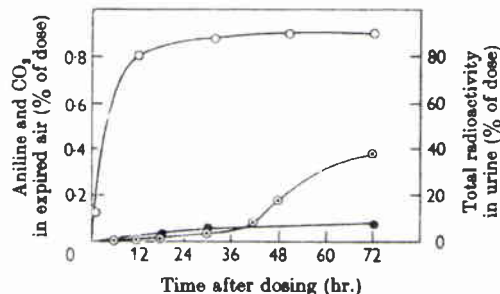


Fig. 1. Excretion of aniline (●) and CO₂ (○) in the expired air, and total radioactivity (○) in the urine, of rabbit no. 3 dosed orally with [¹⁴C]aniline (200 mg./kg.).

aminothiophenol was negative, and steam-distillation of alkali-hydrolysed urines did not yield any thiophenols. No evidence of nitrosobenzene, azoxybenzene, azobenzene or benzidine was found in the urines, but two unidentified metabolites, equivalent to about 0.1 and 0.2 % of the dose, were found by paper chromatography in the urines of both rabbits and dogs. The same two compounds have also been found in chromatograms of the urines of rabbits dosed with nitrosobenzene and with azoxybenzene (D. Robinson, personal communication) and this therefore gives some support to the claim of Kiese (1959a, b) that aniline is oxidized to nitroso-

Reference values for body mass

Age	Mass (kg)	
	Male	Female
Newborn	3.5	3.5
1 year	10	10
5 years	19	19
10 years	32	32
15 years	56	53
Adult	73	60

4.2.2. Surface area of the body

(79) Several authors have developed formulae to estimate the surface area of the body (Dubois and Dubois, 1916; Boyd, 1935; Gehan and George, 1970; Haycock et al., 1978; Lentner, 1984). These formulae generally are of the form:

$$SA = \alpha_0 H^{\alpha_1} M^{\alpha_2}$$

where SA is surface area (m^2), H is height (cm), and M is mass (kg).

(80) The reference values for body surface tabulated below are based on the above formula, using the age- and gender-specific reference heights and masses tabulated earlier. The following parameter values were applied: $\alpha_0 = 0.0235$, $\alpha_1 = 0.42246$, and $\alpha_2 = 0.51456$. These values were derived by Gehan and George (1970) from measurements on 401 subjects with surface areas ranging from 0.11 to 2 m^2 . Parameter values derived from studies of Dubois and Dubois (1916), Boyd (1935), or Haycock et al. (1978) give reasonably similar results.

Reference values for body surface area

Age	Area (m^2)	
	Male	Female
Newborn	0.24	0.24
1 year	0.48	0.48
5 years	0.78	0.78
10 years	1.12	1.12
15 years	1.62	1.55
Adult	1.90	1.66

(81) Estimates of Boyd (1935) of portions of the total surface area associated with various subdivisions of the body are given in Table 4.4.

prevent decomposition of aniline conjugates. One-half of the urine (pH 8.0) was treated with an excess of a saturated aq. soln. of basic lead acetate, and the glucuronides were separated as described by Kamil, Smith & Williams (1951). This glucuronide fraction was dissolved in ethanol and chromatographed on paper in solvent C (see Table 3). The other half of the urine was treated with 20 ml. of an aq. 10% (w/v) soln. of Ba(OH)₂, the excess of barium was removed with solid CO₂ and the solution was filtered and concentrated to 10 ml. *in vacuo*. The concentrated solution was then treated with 50 mg. of (NH₄)₂SO₄, the BaSO₄ was filtered off and the filtrate (the phenylsulphamate fraction) was chromatographed on paper in solvent C. Both fractions showed spots corresponding to glucuronic acid (*R_F* 0.02), aniline-*N*-glucuronide (*R_F* 0.08, equivalent to 5% of the dose) and phenylsulphamic acid (*R_F* 0.26, 10% of dose).

RESULTS AND DISCUSSION

The quantitative results for the metabolism of aniline in the rabbit are shown in Table 4. In Expts. 3, 6 and 7 some 80–100% of the dose of radioactivity was accounted for. In 3–8 days after oral dosage with [¹⁴C]aniline an average of 70% of the radioactivity is eliminated from the body in the urine: 1% is in the faeces, less than 0.5% is in the expired air and about 5% or more remains in the body, from which it is slowly eliminated via the urine and respiratory carbon dioxide. The urinary excretion of metabolites is fairly rapid; in Expt. 3

over 80% was eliminated in the first 12 hr. after dosing (see Fig. 1); it is usually complete in the first 2 days, but the urines are still slightly radioactive after even 8 days. The radioactivity remaining in the carcass (see Table 5) was evenly distributed throughout the tissues except for a slightly higher concentration in the spleen and fat, and in one case in the gut contents.

Expired air. The radioactivity eliminated in the expired air was very small. Aniline could not be detected within the limits of the method (less than 0.02% of the dose/day) and no other organic metabolites were present. Radioactive carbon dioxide was present in the expired air on the second and subsequent days after dosing, but this amounted to less than 0.4% of the dose in 3 or 4 days (see Fig. 1), although at the termination of the experiments the excretion of ¹⁴CO₂ was still maximal at about 0.2% of the dose/day. Since aniline (b.p. 184°) itself is not excreted in the expired air, it is unlikely to occur in the free state in the blood, for iodobenzene (b.p. 189°) and 1:3:5-trichlorobenzene (b.p. 209°) after oral administration are exhaled in the breath of rabbits to the extent of 3% and 10% of the doses respectively (Azouz, Parke & Williams, 1952; Parke & Williams, 1960).

Metabolites in urine. The metabolites of aniline excreted by the rabbit are shown in Table 4; *p*-

Table 4. Elimination of metabolites by rabbits receiving [¹⁴C]aniline

Doses were given orally. Metabolites were determined in bulked urines collected for 3 days. Tissues were examined after 3, 5 and 8 days in Expts. 3, 6 and 7 respectively. See Table 7 for Expts. 8–10. The s.e.m. is given in parenthesis.

Expt. no.	1	2	3	4	5	6	7	Percentage of dose		Average of 10 expts.
										< 0.2	< 0.2	
Dose of aniline (mg./kg.)	160	200	200	250	250	250	500			
Dose of ¹⁴ C (μC/animal)	9	34	35	41	15	67	109			
Duration of expt. (days)	3	3	3	6	4	5	8			
Respiratory CO ₂			—	—	0.4	—	—	< 0.2	< 0.2			
Aniline (total)			1.2	10.8	19.2*	11.7	6.5	14.5*	4.3*	8.2	(1.8)	
Aniline (free)			0.8	2.5	6.4	1.6	—	—	1.0	1.8	(0.7)	
Phenylsulphamic acid			—	7.6	8.9	8.2	—	8.0	2.4	5.4	(1.0)	
Aniline- <i>N</i> -glucuronide			—	4.6	10.8	3.3	—	4.2	1.9	3.5	(1.3)	
Acetanilide			0.07	0.2	0.2	—	0.3	0.1	0.3	0.2	(0.03)	
<i>o</i> -Aminophenol (total)			5.5	10.1	11.0	9.6	8.4	12.8	9.8	9.1	(0.7)	
<i>m</i> -Aminophenol (total)			< 0.1	< 0.1	0.16	—	< 0.1	0.06	0.09	0.1	(0.03)	
<i>p</i> -Aminophenol (total)			43	58	66	56	46	47	56	51	(2.6)	
<i>p</i> -Aminophenylglucuronide			—	—	9.0	—	—	—	22.2	—		
<i>p</i> -Acetamidophenylglucuronide			—	—	17.2	—	—	—	24.6	—		
Total metabolites in urine			50	81	97	77	61	72	71	68	(4.7)	
Radioactivity in urine			60	80	90	73	69	77	73	70	(4.1)	
Radioactivity in faeces			—	—	0.7	—	—	1.1	1.5	—		
Radioactivity in tissues			—	—	3.0	—	—	6.3	6.7	—		
Total accounted for			—	—	94	—	—	85	81	—		

* Less than 0.2% in the expired air.

Studies in Detoxication

84. THE METABOLISM OF [¹⁴C]ANILINE IN THE RABBIT AND OTHER ANIMALS*

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(Received 28 March 1960)

The fate of aniline in the rabbit was investigated by Smith & Williams (1949), who showed that glucuronides and ethereal sulphates of *o*- and *p*-aminophenol, and possibly 4-aminoresorcinol, were metabolites accounting for 40–45% of the aniline fed; but the main metabolite, more than 50% of the dose, was considered to be a labile glucuronide of aniline itself. This labile *N*-glucuronide was also thought to be the principal metabolite of aniline by Ishidate, Hogiya, Hashimoto & Takitani (1958); and another conjugate of aniline, phenylsulphamic acid, was detected in the urine of rabbits dosed with aniline (Boyland, Manson & Orr, 1957). The metabolism of aniline by liver slices from rat, rabbit, dog and cat has yielded *o*- and *p*-aminophenol and 4-aminoresorcinol (Sato, Suzuki, Fukuyama & Yoshikawa, 1955); rat-liver and cat-liver microsomes in the presence of reduced triphosphopyridine nucleotide and oxygen gave *o*- and *p*-aminophenol (Booth & Boyland, 1957; H. S. Posner, unpublished results), and rabbit-liver microsomes under the same conditions gave only *p*-aminophenol (Mitoma, Posner, Reitz & Udenfriend, 1956). The hydroxylation of aniline to *p*-aminophenol has been effected by ascorbic acid in the presence of ferrous iron, ethylenediaminetetra-acetic acid and oxygen (Brodie, Axelrod, Shore & Udenfriend, 1954), and it appears likely that ascorbic acid is involved in the hydroxylation of aniline *in vivo* (Axelrod, Udenfriend & Brodie, 1954; Volkova, 1958). The present work with [¹⁴C]aniline has shown that the principal fate of aniline in mammals is hydroxylation to *o*- and *p*-aminophenol, with the *N*-glucuronide of aniline as only a minor metabolite.

The use of [¹⁴C]aniline has made possible the accurate estimation in urine of the isomeric aminophenols, aniline *N*-glucuronide, phenylsulphamic acid and acetanilide, and of the amounts exhaled in the breath and retained in the faeces and tissues. In this way some 80–100% of a single dose of aniline has been accounted for. In addition, the principal metabolites have been estimated in several animal species and the proportion of *o*- to *p*-aminophenol excreted by different animals has been shown to vary widely.

* Part 83: Baldwin, Robinson & Williams (1960).

MATERIALS AND METHODS

Melting points are corrected.

Preparation of [¹⁴C]aniline. [¹⁴C]Aniline randomly labelled with ¹⁴C in one carbon atom was prepared from [¹⁴C]nitrobenzene by catalytic transfer-hydrogenation with cyclohexane and palladium black according to Braude, Linstead & Wooldridge (1954) (yield 91%, b.p. 184°).

Aniline N-glucuronide. Salts of aniline-*N*-glucosiduronic acid have been previously described (Thierfelder, 1889; Bergmann & Wolff, 1923; Ishidate *et al.* 1958), but unequivocal characterization of these unstable compounds was first established by Heyns & Baltes (1958), who described a sodium salt, C₁₁H₁₄O₆NNa, $\frac{1}{2}$ C₆H₇N, m.p. 201–203° (decomp.), and a potassium salt, C₁₁H₁₄O₆NK, C₆H₇N, H₂O, m.p. 175° (decomp.). An hydrated ammonium salt, m.p. 130° (decomp.), $[\alpha]_D^{25} -55^\circ \rightarrow -15^\circ$ (c, 2.3 in water), was described but not adequately characterized by Ishidate *et al.* (1958). The ammonium salt used in experiments described in this paper was prepared as follows: to a solution of 5 g. of glucuronic acid in 15 ml. of water was added 1.9 ml. of aq. NH₃ soln. (sp.gr. 0.88) followed by 5 ml. of aniline in 10 ml. of ethanol. After mixing, the solution was left at room temperature until a precipitate began to form. The mixture was then kept at 0° for 8 hr. and the precipitate was filtered off and washed with a little cold ethanol. Recrystallization from aq. 50% (v/v) ethanol gave ammonium aniline-*N*-glucosiduronate, containing 1 mol.prop. of aniline of crystallization, as colourless needles, m.p. 122° (decomp.); $[\alpha]_D^{25} -77^\circ \rightarrow -17^\circ \pm 1^\circ$ (c, 2.0 in water) (Found: C, 54.7; H, 6.65; N, 10.4; aniline, 47.3. Calc. for C₁₁H₁₄O₆N₂.C₆H₇N, H₂O: C, 54.4; H, 6.85; N, 10.6; aniline, 46.9%). The total aniline content was estimated by refluxing 0.05 g. of the salt with 10 ml. of 2*N*-HCl for 1 hr., steam-distilling the solution after adjusting to pH 10 with 5*N*-NaOH, and determining the difference in light absorption of the steam-distillate at 280 m μ in 0.1*N*-HCl and 0.1*N*-NaOH (aniline in 0.1*N*-NaOH showed light-absorption at 280 m μ , ϵ 1300; in 0.1*N*-HCl at 280 m μ , ϵ 0). The ammonium salt is readily soluble in cold water, less soluble in ethanol and insoluble in ether and ethyl acetate. It slowly reduces Benedict's reagent but reduces more readily after preliminary treatment with *N*-HCl. It gives the naphtharesorcinol reaction for glucuronic acid and shows the colour reactions characteristic of free aniline. The structure of the ammonium salt is similar to that of the potassium salt and not the sodium salt, as would be expected, since the radius of the ammonium ion, 1.48 Å, is closer to that of potassium (1.33 Å) than that of sodium (0.95 Å).

Other compounds. 3-Aminocatechol-HCl and 7-hydroxy-2-phenylbenzoxazole, m.p. 192° (Henrich, 1921); 4-aminocatechol-HBr (Jacobs, Heidelberger & Rolf, 1919) and

Bicarbonate excretion

<http://www.lib.mcg.edu/edu/eshuphysio/program/section7/7ch12/7ch12p21.htm>

Bicarbonate is freely filtered across the glomerular membrane. bicarbonate reabsorption in the proximal tubule depends on the activity in the luminal membrane of a secondary active Na^+ , H^+ antiporter, a primary active H^+ -ATPase. Also important is the presence of CA in the proximal ICF and in the proximal luminal membrane. Intracellular CA facilitates the hydration of intracellular CO_2 to H_2CO_3 which then dissociates rapidly into H^+ and HCO_3^- ions. Na^+ moving down its concentration gradient from tubular fluid into the cell provides the energy for the secondary active secretion of H^+ from the cell into the lumen. ATP provides the energy for the primary active secretion of H^+ from the cell into the lumen.

In the lumen H^+ combines with filtered HCO_3^- to form H_2CO_3 which then is dehydrated to form H_2O and CO_2 . This reaction is facilitated by the presence of CA located in the brush border of the luminal membrane. All cell membranes are highly permeable to CO_2 and the CO_2 formed in the lumen rapidly enters the cell. There it enters the cellular CO_2 pool and its rapid hydration to H_2CO_3 is facilitated by intracellular CA. The intracellular dissociation of H_2CO_3 provides additional H^+ for the secretory process. The intracellular HCO_3^- formed by this sequence of reactions moves into the peritubular fluid where it is electrically balanced by a reabsorbed cation, primarily Na^+ . The major pathway is via a basolateral membrane $\text{Na}^+ - 3\text{HCO}_3^-$ cotransporter and additional Na^+ is provided by the basolateral $\text{Na}^+ .\text{K}^+ - \text{ATPase}$.

In this system for each H^+ ion secreted an HCO_3^- is reabsorbed and the secreted H^+ ion appears in the urine as water and the urine pH is not changed. The amount of H_2O added to the tubular fluid in this process is insignificant relative to the filtered load. About 80 to 90 % of the filtered HCO_3^- is reabsorbed in the proximal tubule by this mechanism. Because of the rapidity of the reactions catalyzed by CA and the high permeability to CO_2 of the luminal membrane, HCO_3^- is reabsorbed preferentially over Cl^- early in the proximal tubule.

Carbonate Excretion Rate

Renal mechanisms function to regulate pH by reabsorbing filtered bicarbonate, preventing bicarbonate loss in the urine, and excreting H⁺ ions instead. Most bicarbonate (85%) is reabsorbed in the proximal tubule by excretion of H⁺ ions.

Source: Current Surgical Therapy, ed. John Cameron, Elsevier Mosby, 2001, p1159

Arthur E. Desrosiers III M.D.

Carbonate-injected rats excrete about 95% of the injected dose in 8 days. Formate-injected rats excrete about 90%.

Carbonate-injected rats excreted, in the urine, somewhat more than 1% of the dose in 8 days; most of this during the first 7 hours. Formate-injected rats excreted almost 2% in the first 7 hours, and more than 5% during the 8 days.

Fecal excretion for both groups of animals was similar in rate, but greater by the formate-injected group. In both groups, the feces fat contained far less C^{14} than did the fat-free portion.

The biological half-life of the retained C^{14} , 24 hours after injection, was calculated to be about 14 days for the carbonate group and about 7 days for the formate group.

The retained C^{14} was distributed throughout all tissues in both groups of rats, but the formate group retained a greater percentage at the end of 8 days.

In both groups, testes fat had a higher concentration of C^{14} than the fat from most other tissues. The fats from the lungs, spleen, heart, kidneys and testes in the formate group, had the highest concentration of C^{14} , and that from the spinal cord and depot fat had the lowest.

The pattern of concentration of C^{14} in the proteins, in both groups of rats, was similar. Stomach, spleen, kidney, testes and liver had the highest concentration, while spinal cord had the lowest.

In both groups, defatted bone had a low concentration of C^{14} , but the formate group had a higher concentration than the carbonate group.

We would like to express our appreciation to Robert McLaughlin and especially to Walter S. Cool for technical assistance, and to Marvin Schneiderman of the Section on Biometrics of the National Institutes of Health for his advice on the statistical evaluation of our data.

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injected rats had excreted 94.8% and the formate-injected rats, 75.1%. For the remainder of the 8 days the $C^{14}O_2$ excretion by the carbonate-injected rats totaled about 1%, while that of the formate-injected group totaled about 9%. It should be noted that although the carbonate-injected rats excreted a larger total quantity of $C^{14}O_2$ than did the formate-injected rats, the excretion rate patterns in both groups were similar.

Urinary excretion of C^{14} in the two groups of rats followed a pattern similar to that for pulmonary $C^{14}O_2$; rapid excretion during the first 24 hours after injection was followed by a slower rate for the remainder of the experiment. The carbonate-injected rats excreted in the urine an average of 0.7% in 7 hours and a total of 1.2% in 8 days. The formate-injected rats excreted 1.6% during the first 7 hours, and at the end of 8 days had excreted 5%.

Most of the C^{14} in the feces of the carbonate-injected rats was found in the fat-free residue, from which 0.05-0.9% was recovered in 8 days. On the other hand little C^{14} was found in the feces fat. In 2 of the 6 rats the C^{14} in the feces fat was insufficient for calculation, while in the other 4 the average recovered after 8 days was 0.03%. Similarly the formate-injected rats also excreted less C^{14} in the feces fat than in the fat-free residue. An average total of 0.13% was recovered from the fat and between 1.1 and 1.8% from the residue. Although the C^{14} content in both feces fat and feces residue fluctuated daily in both groups, it showed a slow, steady decline.

The total C^{14} excreted by the lungs, kidney and bowel during the 8 days of the experiment (table 1), by the carbonate-injected group, was about 96% and about 90% by the formate-injected rats. Of these totals, the former excreted 95% during the first 24 hours after injection, and the latter, 78%. In both groups, the greatest proportion was excreted during the first 7 hours after injection. The biological half-life of the remainder was calculated to be 14 days in the carbonate group and 7 days in the formate group.

The radioactive carbon retained by both groups of rats was distributed in all tissues of each animal. In the carbonate-injected rats each tissue or tissue constituent exhibited a low order of radioactivity. In the formate-

injected rats, these exhibited relatively high counting rates. An end-window Geiger tube passed over the surface of the carbonate-injected rats, just prior to sacrifice, gave no indication of radioactivity. When the tube was passed over the formate-injected rats, it registered several hundred counts per minute over background.

The amount of C^{14} in the fat extracted from the various tissues of the carbonate-injected rats was, with the exception of the testes fat, too low to be counted. Aliquots of the

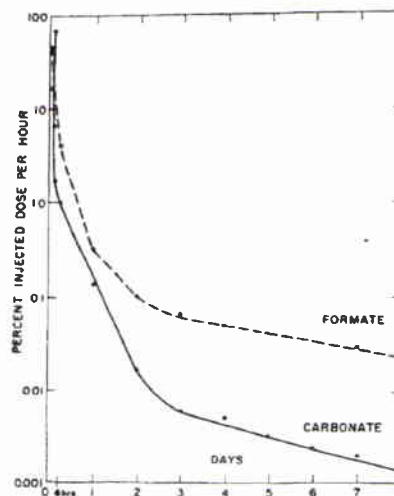


FIG. 1. Rate of $C^{14}O_2$ excretion (percentage of injected dose).

testes fats gave between 13 and 20 counts/min. over background. It was calculated that the fats from each pair of testes contained approximately 0.001%. In contrast, all the fats for the tissues of the formate-injected rats exhibited appreciable radioactivity. The aliquot counts ranged from 150 to as much as 1500/min. and the fats from each pair of testes contained between 0.002 and 0.17%. The testes fats in this group showed a greater concentration of C^{14} than all other fats except that from the kidney. In addition, the fats of the heart, spleen, and lungs showed relatively high concentrations of C^{14} , while the spinal cord and depot fat had the lowest concentrations. The other tissue fats were intermediate between these two extremes (table 2). The total fats, in each rat of this group, contained about 2%.

the combustion gases were passed over hot copper oxide before entering the absorption vessel, in order to convert CO to CO₂. The contents of the absorption vessels were then added to a hot aqueous solution of M/4 barium chloride-ammonium chloride and the precipitated barium carbonate was filtered off in sintered crucibles which were then dried and weighed.

For the determination of respiratory CO₂, aliquots were taken from the tower contents and added directly to the barium-ammonium chloride solution. Fat was extracted from the feces by the method of Hawk and Bergheim (13). This and the fat-free residue were separately oxidized. Urine and the hair from the cage were dried and weighed before oxidation. Aliquots of the injected materials were converted to barium car-

bonate injected dose and will hereafter be noted as percent. Tissue constituents were further calculated in terms of percentage of injected C¹⁴/mg dry wt. of each fraction. Many of the tissue constituents in the carbonate-injected group produced very low counts. Those which counted at a rate of 10 or less/min. over background were arbitrarily considered to have no activity worthy of calculation. Since this was the case, and since significance tests would be required for over a thousand separate determinations, a method (16) of ranking was employed to compare concentrations of C¹⁴ among the tissue constituents and between the two groups of animals.

Each tissue constituent from each animal was ranked according to the percent of injected dose found in that

TABLE I. C¹⁴ RECOVERED AT THE END OF 8 DAYS IN PERCENTAGE OF INJECTED DOSE

Animal No.	Carbonates						Formates				
	1	2	3	4	5	6	7*	8	9	10	11‡
Total CO ₂ exhaled	94.1	93.9	98.9	96.5	95.6	95.5	74.1	82.1	78.4	89.1	84.5
Total urine	2.3	1.1	1.0	2.7	0.6	0.7	5.7	3.9	3.0	7.0	6.0
Total feces residue	0.1†	0.9	0.9	0.1	0.1	0.3	0.7	0.7	1.1	1.6	1.8
Total feces fat	0.0†	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.3	0.1	0.3
Total excreted	96.5	96.0	100.8	99.3	96.3	96.5	80.6	86.8	82.8	97.8	93.5
Total all fats							1.4	2.3	2.3	2.0	2.7
Total all proteins	0.2	0.3	0.2		0.2	0.3	6.7	3.9	7.9	5.8	8.2
Blood							‡	‡	1.4	0.5	0.9
Glycogen							‡	‡	0.1	0.1	0.1
Residues							3.7	1.6	2.6	3.9	3.2
Total recovered	96.7	96.3	101.0		96.5	96.8	92.4	94.6	97.1	110.1	108.6

* 5 days only. † Cage feces lost. ‡ Lost. § Received 40% of dose.

bonate by the same technique, in order to determine their activities, and, for direct comparison with the samples from the rats.

At the end of 8 days, the rat was removed from the cage and injected intraperitoneally with 4 mg of pentothal sodium/100 gm body wt. When anesthetized, 7-10 cc of heart blood were withdrawn. The abdomen of the living animal was then opened and a portion of the liver removed, blotted, weighed and placed in hot potassium hydroxide solution for glycogen extraction by the method of Stetten and Boxer (14). Similarly, samples of the abdominal and thigh muscles were removed immediately and treated in the same way. The other organs were then removed, blotted, weighed and the fats and proteins extracted (2, 15). These tissue constituents and the residues remaining from the various extractions, were then oxidized and converted to barium carbonate.

The barium carbonate was ground in alcohol and the slurry poured into planchets to make infinitely thick plates, which were dried and counted in a gas-flow proportional counter which registered about 35 background counts/min. Each sample received two consecutive counts. If 10,000 counts/sample could not be recorded in a reasonable time, then two consecutive 1-hour counts were made. The average of the two counts was used in the calculations.

All calculations were made in terms of percentage of

tissue constituent. The sum of the numerical positions in rank for each constituent from each rat in the group was calculated and the constituents regrouped according to this sum. The rankings between the two groups were then compared.

RESULTS

The sum of the C¹⁴ recovered from all avenues of excretion and from all tissues ranged from 96-101% among the carbonate-injected rats, and from about 92-110% among the formate-injected rats. All data from the formate-injected rat which received 40% of the dose matched those of the other animals in the group (table 1).

The rate of pulmonary excretion of C¹⁴O₂ in both groups of rats is illustrated in figure 1. The mean CO₂ excretion during the first hour by the carbonate-injected rats was about 72%; by the formate-injected rats, 42%. At 24 hours the hourly rates had fallen to 0.14 and 0.34% respectively and at the end of 8 days these had dropped to about 0.002 and 0.025%. At the end of 24 hours, the carbonate-

Annals of the ICRP

ICRP PUBLICATION 89

Basic Anatomical and Physiological Data for Use in Radiological Protection: Reference Values

Editor
J. VALENTIN

PUBLISHED FOR
The International Commission on Radiological Protection

By




PERGAMON

$f_1 = 1.0$ VAPOR OR LIQUID ($^3\text{H}_2\text{O}$) HALFLIFE = $4.49\text{E}+03$ DAYS HYDROGEN 3

TIME AFTER SINGLE INTAKE DAYS	FRACTION OF INITIAL INTAKE IN:		
	TOTAL BODY	24-HOUR URINE	ACCUMULATED URINE
1.00E-01	9.93E-01		4.19E-03
2.00E-01	9.86E-01		8.34E-03
3.00E-01	9.79E-01		1.25E-02
4.00E-01	9.72E-01		1.66E-02
5.00E-01	9.66E-01		2.06E-02
6.00E-01	9.59E-01		2.47E-02
7.00E-01	9.52E-01		2.87E-02
8.00E-01	9.46E-01		3.27E-02
9.00E-01	9.39E-01		3.66E-02
1.00E+00	9.36E-01	3.85E-02	3.85E-02
2.00E+00	8.73E-01	3.76E-02	7.61E-02
3.00E+00	8.14E-01	3.51E-02	1.11E-01
4.00E+00	7.60E-01	3.27E-02	1.44E-01
5.00E+00	7.09E-01	3.05E-02	1.74E-01
6.00E+00	6.61E-01	2.85E-02	2.03E-01
7.00E+00	6.17E-01	2.66E-02	2.29E-01
8.00E+00	5.75E-01	2.48E-02	2.54E-01
9.00E+00	5.37E-01	2.31E-02	2.77E-01
1.00E+01	5.01E-01	2.16E-02	2.99E-01
2.00E+01	2.50E-01	1.08E-02	4.48E-01
3.00E+01	1.25E-01	5.37E-03	5.22E-01
4.00E+01	6.23E-02	2.68E-03	5.59E-01
5.00E+01	3.11E-02	1.34E-03	5.77E-01
6.00E+01	1.55E-02	6.69E-04	5.85E-01
7.00E+01	7.76E-03	3.34E-04	5.89E-01
8.00E+01	3.87E-03	1.67E-04	5.90E-01
9.00E+01	1.93E-03	8.33E-05	5.91E-01
1.00E+02	9.66E-04	4.16E-05	5.90E-01
2.00E+02	9.30E-07	4.01E-08	5.89E-01

NUREG CR 4884



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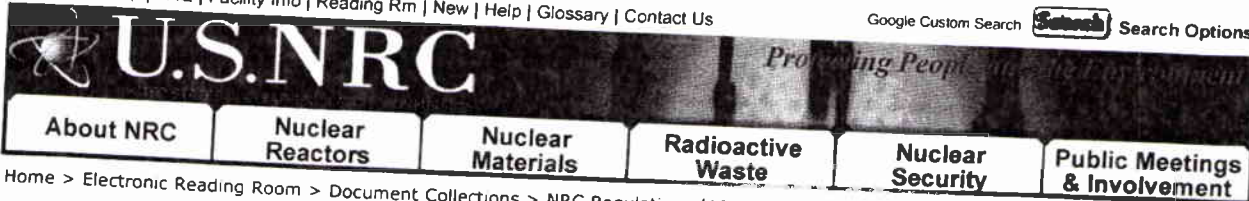
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Carbon-14

Atomic No.	Radionuclide	Class	Table 1 Occupational Values			Table 2 Effluent Concentrations		Table 3 Releases to Sewers
			Col. 1	Col. 2	Col. 3	Col. 1	Col. 2	Monthly Average Concentration (µCi/ml)
			Oral Ingestion ALI (µCi)	Inhalation		Air (µCi/ml)	Water (µCi/ml)	
6	Carbon-14	Monoxide		-	2E+6			7E-4
		Dioxide	-	2E+5	9E-5	3E-7	-	-
		Compounds	2E+3	2E+3	1E-6	3E-9	3E-5	3E-4

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Hydrogen-3

Atomic No.	Radionuclide	Class	Table 1 Occupational Values			Table 2 Effluent Concentrations		Table 3 Releases to Sewers	
			Col. 1	Col. 2	Col. 3	Col. 1	Col. 2	Monthly Average Concentration (µCi/ml)	
			Oral Ingestion ALI (µCi)	Inhalation		Air (µCi/ml)	Water (µCi/ml)		
1	Hydrogen-3	Water, DAC includes skin absorption		8E+4	8E+4			DAC (µCi/ml)	2E-5
Gas (HT or T ₂) Submersion ¹ : Use above values as HT and T ₂ oxidize in air and in the body to HTO									

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1975) also provides the values for carbon balance of 100 g/d, 200 g/d and 300 g/d for the 1 year old, 10 year old, and adult, respectively. Age-dependence on CO₂ production and carbon losses into urine and faeces suggest that the daily carbon balance does not differ by more than 10% from that of the 1 year old. Thus, values for carbon balance are extrapolated and/or interpolated for the infant, 5 year old, and 15 year old and assumed here to be 90 g/d, 160 g/d, and 270 g/d, respectively.

Dose coefficients

(59) From the above information, the half-times given in Table 2.-1 were derived. Dose coefficients derived from the biokinetic data in Table 2.-1 are summarized in Table 2.-2.

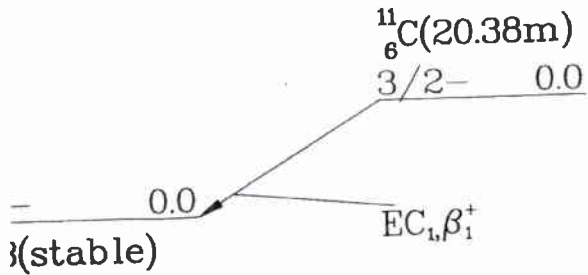
Table 2.-1. Biokinetic data for ¹⁴C

Age	Distribution (%) Total body	Biological half-time (d)
3 months	100	8
1 year	100	15
5 years	100	19
10 years	100	26
15 years	100	32
Adult	100*	40*

*Values from *ICRP Publication 30* (ICRP, 1979).

ICRP, Volume 20, Issue 2, 1989

CARBON



6-CARBON-11

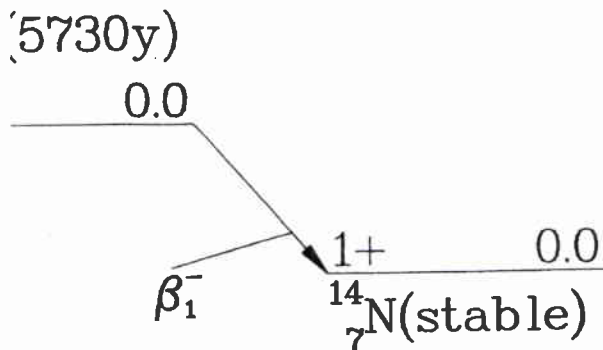
HALFLIFE = 20.38 MINUTES
DECAY MODE(S): EC, β^+

29-MAR-78

RADIATION	y(i) (Bq-s) ⁻¹	E(i) (MeV)	y(i) × E(i)
β^+ 1	9.98E-01	3.855E-01*	3.85E-01
γ^\pm	2.00E 00	5.110E-01	1.02E 00
K α_1 X-ray	1.62E-06	1.833E-04	2.97E-10
K α_2 X-ray	8.10E-07	1.833E-04	1.48E-10

LISTED X, γ AND γ^\pm RADIATIONS 1.02E 00
LISTED β , ce AND Auger RADIATIONS 3.85E-01
LISTED RADIATIONS 1.40E 00

* AVERAGE ENERGY (MeV)
BORON-11 DAUGHTER IS STABLE.



6-CARBON-14

HALFLIFE = 5730 YEARS
DECAY MODE(S): β^-

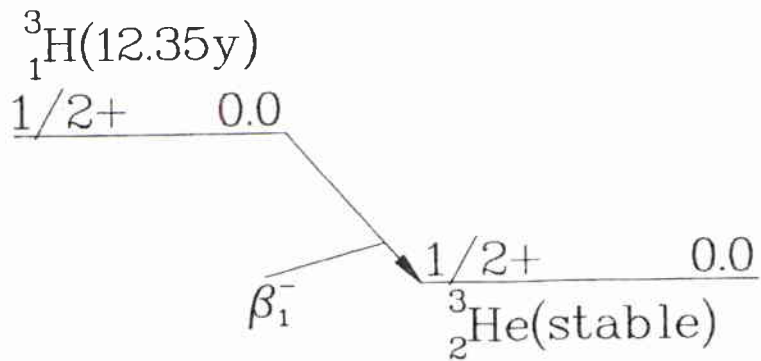
13-OCT-77

RADIATION	y(i) (Bq-s) ⁻¹	E(i) (MeV)	y(i) × E(i)
β^- 1	1.00E 00	4.945E-02*	4.95E-02

LISTED β , ce AND Auger RADIATIONS 4.95E-02
LISTED RADIATIONS 4.95E-02

* AVERAGE ENERGY (MeV)
NITROGEN-14 DAUGHTER IS STABLE.

HYDROGEN



1 - HYDROGEN - 3

HALFLIFE = 12.35 YEARS
 DECAY MODE(S): β

RADIATION	$\frac{y(i)}{(\text{Bq} \cdot \text{s})^{-1}}$	$\frac{E(i)}{(\text{MeV})}$	$\frac{y(i) \times E(i)}{5.68\text{E}-03}$
β 1	1.00E 00	5.683E-03*	5.68E-03

LISTED β , α AND Auger RADIATIONS	5.68E-03
LISTED RADIATIONS	5.68E-03

* AVERAGE ENERGY (MeV)
 HELIUM-3 DAUGHTER IS STABLE.

RADIATION PROTECTION

ICRP PUBLICATION 38

Radionuclide Transformations

Energy and Intensity of Emissions

Report of a Task Group of Committee 2
of the International Commission on Radiological Protection
on data used in
ICRP Publication 30

PUBLISHED FOR

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For a normal diet, water loss in faeces is typically about 100 (50–150) ml/day (ICRP, 1975; Guyton, 1982). This can increase dramatically as a consequence of diarrhoea or vomiting.

(131) The following estimates for the average adult male were based on the above information and the constraint that daily intake plus metabolic production of water must balance losses in urine, faeces, insensible losses, and sweat: intake in food and fluids, 2600 (2000–3000) ml; oxidation of food, 300 (200–400) ml; urinary excretion, 1600 (1200–2000) ml; insensible losses, 690 (300–1000) ml, with equal losses through the lungs and skin; faeces, 110 (55–165) ml; and sweat, 500 (300–700) ml. The central estimates are adopted as reference values for water balance in adult males. Reference values for adult females are taken as 75% of the corresponding values for males except for faeces, which is assumed to be 85% of that for males for consistency with reference values for the mass of faeces excreted per day by adult males and females.

Reference values for water balance in adults

	Male	Female
Water intake in food and fluids (ml/day)	2600	1960
Oxidation of food (ml/day)	300	225
Losses (ml/day)		
Urine	1600	1200
Insensible loss ^a	690	515
Sweat	500	375
Faeces	110	95

^a Assumed to be divided equally between the lungs and skin.

4.5. Comparisons of reference values with Asian data

(132) A considerable amount of information has been published during the past decade on characteristics of several Asian populations. Included are reports on Japanese populations (Tanaka and Kawamura, 1979, 1996; Tanaka, 1992), Chinese populations (Wang et al., 1999), and Indian populations (Jain, 1995). The most extensive effort has been the 5-year effort conducted under the auspices of the IAEA. In this effort, characteristics of populations in Bangladesh, China, India, Japan, Republic of Korea, Pakistan, Philippines, and Vietnam were examined and compared (IAEA, 1998). This IAEA report presented comparative information on height, weight, other anthropomorphic measurements, organ masses, daily dietary intake, pulmonary function, and water balance. Also included in the IAEA report were the results of a model prepared by Tanaka giving suggested reference values for Asian male and female subjects at these six ages: newborn; 1, 5, 10, and 15 years; and adult.

(133) The authors of this report noted major questions that arose relating to the adequate and appropriate characterisation of reference values for Asian populations. These uncertainties included: (1) significant variations between and even within national populations and (2) secular trends within a given population as a result of

Reference values for body mass

Age	Mass (kg)	
	Male	Female
Newborn	3.5	3.5
1 year	10	10
5 years	19	19
10 years	32	32
15 years	56	53
Adult	73	60

4.2.2. Surface area of the body

(79) Several authors have developed formulae to estimate the surface area of the body (Dubois and Dubois, 1916; Boyd, 1935; Gehan and George, 1970; Haycock et al., 1978; Lentner, 1984). These formulae generally are of the form:

$$SA = \alpha_0 H^{\alpha_1} M^{\alpha_2}$$

where SA is surface area (m^2), H is height (cm), and M is mass (kg).

(80) The reference values for body surface tabulated below are based on the above formula, using the age- and gender-specific reference heights and masses tabulated earlier. The following parameter values were applied: $\alpha_0 = 0.0235$, $\alpha_1 = 0.42246$, and $\alpha_2 = 0.51456$. These values were derived by Gehan and George (1970) from measurements on 401 subjects with surface areas ranging from 0.11 to 2 m^2 . Parameter values derived from studies of Dubois and Dubois (1916), Boyd (1935), or Haycock et al. (1978) give reasonably similar results.

Reference values for body surface area

Age	Area (m^2)	
	Male	Female
Newborn	0.24	0.24
1 year	0.48	0.48
5 years	0.78	0.78
10 years	1.12	1.12
15 years	1.62	1.55
Adult	1.90	1.66

(81) Estimates of Boyd (1935) of portions of the total surface area associated with various subdivisions of the body are given in Table 4.4.

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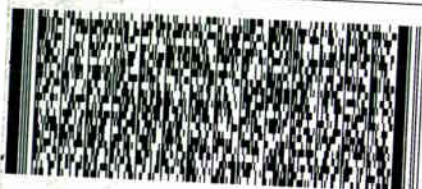


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