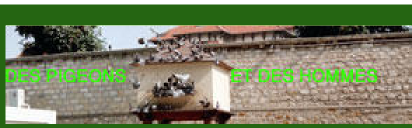

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entrée

Généralités :
accueil
Présentation de l'espèce
Les maladies du pigeon
Histoire du pigeonnier
Histoire du pigeon voyageur
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Le pigeonnier de ville moderne :
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quelques photos de pigeonniers :
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PROCÉDÉS À BASE DE GAZ CARBONIQUE

caisson à CO 2 **agrandir la photo**

Beaucoup d'entreprises de dépigeonnage tuent les pigeons capturés au moyen du gaz carbonique CO2. Elles prétendent que ce procédé ne fait pas souffrir les oiseaux. Une d'elle me disait : *on endort les pauvres oiseaux, ils ne sentent rien*, c'est tout juste si je n'allais pas sortir mon mouchoir....

Ces entreprises, en fait font ce qu'elles veulent, sans contrôle en apparence des services vétérinaires, par ce qu'il n'existe **aucune réglementation** française portant sur cette activité.

Voulant en savoir plus je cherchais sur internet des informations sur le sujet quand je tombai sur ce texte :

USA-Le caractère humain de la mise à mort des Oiseaux par le Dioxyde de Carbone contesté

Communiqué de presse - **United Poultry Concerns**
United Poultry Concerns PO Box 150 Machipongo, VA 23405
Phone: 757-678-7875 Fax: 757-678-5070

Pour parution immédiate
European Vegetarian and Animal News Agency (EVANA)
30 mars 2005

Le caractère humain de la mise à mort des Oiseaux par le Dioxyde de Carbone contesté par la science.

Il est peu probable que le nouveau système d'Alberta pour détruire des poules pondeuses en fin de ponte soit bénin et rapide, malgré ce qui est affirmé.

Machipongo, Va. - Le 21 mars, les Producteurs d'Oeufs d'Alberta ont annoncé leur soutien à un système de destruction d'un grand nombre de poules en fin de ponte qui les déverse d'un coup par centaines dans un réservoir profond qui peut contenir 650 oiseaux et en les tuant simultanément par le dioxyde de carbone (CO2).

Les producteurs d'oeufs prétendent qu'en plus d'être bon marché et efficace, le CO2 est une manière très humaine de traiter les oiseaux. Cependant, cette affirmation entre en conflit avec l'évidence scientifique qui montre que le CO2 provoque une extrême souffrance.

Dans un séminaire présenté au Département d'Agriculture le 16 décembre 2004, le Dr. Mohan Raj, chercheur principal associé à la Division des Animaux de Ferme du Département des Sciences Vétérinaires Cliniques de l'Université de Bristol en Angleterre, décrit les effets du CO2 sur le corps. Le CO2 induit l'essoufflement, une détresse respiratoire du sujet connue comme dyspnée. Selon le Dr Raj, la dyspnée chez les oiseaux et les mammifères active des régions du cerveau associées avec la douleur et induit une réponse émotionnelle de panique.

C'est à cause du fait que le CO2 accroît le rythme et la profondeur de la respiration pour expirer le CO2 des poumons, respirer accroît en réalité la prise de CO2, ainsi le désir de respirer pour évacuer le CO2 toxique provoque une suffocation lente et douloureuse.

En contraste, un système étourdir/tuer fondé sur l'utilisation d'un gaz inerte argon ou azote, connu comme Etourdissement en Atmosphère Contrôlée, élimine ou réduit grandement la souffrance occasionnée par le CO2 pur.

Tandis que le CO2 induit une suffocation douloureuse, des gaz tels que l'argon ou l'azote induisent un manquent d'oxygène, ou anoxie, résultant en une mort sans douleur selon le Dr. Raj.

La différence cruciale entre anoxie (manque d'oxygène) et dyspnée (essoufflement) est qu'à la différence de l'anoxie, pour laquelle les oiseaux et les mammifères manquent de récepteurs, l'essoufflement implique des récepteurs qui enregistrent la séparation physique du tractus respiratoire de l'atmosphère extérieure. Dans les expériences en Amérique du Nord et le Royaume-Uni, les poussins et les dindes exposées à de hauts niveaux (40 pour-cent ou plus) de CO_2, halètent, secouent leurs têtes et étirent leurs cous pour respirer.

Dans un courriel transmis à des protecteurs des animaux le 26 mars, le Dr Raj a réitéré que l'exposition au dioxyde est stressante et douloureuse pour les animaux et devrait par conséquent être évitée.

Alternativement, il a dit que les poules en fin de ponte pourraient être tuées avec moins de souffrance dans des systèmes alimentés avec un mélange de 80 % de volume d'argon et 20 % de dioxyde de carbone, un mélange universellement disponible comme mélange de gaz pour soudure.

Les partisans du bien-être animal concèdent que la mise à mort des poules à la ferme est préférable que leur transport vers l'abattoir sans nourriture ni eau, la majorité souffrant de fractures osseuses ou autres blessures encourues en étant serrées à l'étroit dans des cages et manipulées de manière rude.

Cependant, une exigence clé du bien-être pour un tel dispositif à la ferme est que les poules soient tuées dans leurs cages plutôt que d'être sorties des cages, jetées dans un chargeur frontal et déversées dans un énorme réservoir en étant pleinement conscientes pour y être étouffées par le dioxyde de carbone.

Le système pourrait être économique, mais il ne peut pas être appelé humain dit Karen Davis, Président de United Poultry Concerns, une organisation qui fait la promotion du traitement compatissant des oiseaux de basse-cour. Si les producteurs veulent réellement réduire la souffrance des oiseaux, ils les tueront dans leurs cages en utilisant de l'argon ou de l'azote. Ce n'est pas seulement ce que la conscience dicte, mais aussi ce que la science dicte.

United Poultry Concerns a produit un rapport sur l'extermination de 19 millions d'oiseaux en Colombie-Britannique pour contrôler l'épidémie de grippe aviaire en 2004. La Crise de la Gripppe Aviaire au Canada : Ethique du Contrôle des Maladies des Animaux de Ferme est disponible sur www.upc-online.org/poultry_diseases

Contact: Karen Davis, President - 757-678-7875

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United Poultry Concerns est une organisation sans but lucratif qui fait la promotion du traitement respectueux et compatissant des oiseaux de basse-cour.

Voulant vérifiant cette déclaration j'explorais le web en langue anglaise et effectivement la mise à mort par CO_2 d'après une grande majorité d'experts ferait souffrir les oiseaux.

Plus bas vous trouverez une **traduction du rapport 2000** de l'American Veterinary Medical Association (dont le sujet concerne toutes les méthodes d'euthanasie existantes). C'est une traduction de la partie traitant de toutes les méthodes à base de gaz où vous vous apercevrez que certains gaz peuvent ne pas faire souffrir les animaux. Ce rapport date un peu car ses conclusions sur le CO_2 sont contredites par les travaux récents actuels, ses conclusions : *Le gaz carbonique est recommandé pour certaines espèces (voir les tableaux 1 et 2 du rapport)* - sur ces tableaux les oiseaux sont mentionnés.

Pour prendre connaissance des recherches récentes vous devrez lire avec attention ma traduction du **rapport complet** de l'utilisation de l'anhydride carbonique (CO_2) pour l'euthanasie des animaux de laboratoire du Bureau of Animal Welfare, DPI Victoria (Australie Melbourne) qui passe en revue la littérature scientifique courante sur l'utilisation de l'anhydride carbonique (mélanges purs et divers) pour l'euthanasie des animaux de laboratoire. Dans ce texte on apprend que :

- l'utilisation du CO_2 pour tuer les volailles est interdite dans les abattoirs Britanniques depuis 1995

- le CCAC (Canadian Council for Animal Care) reconnaît que ni l'induction lente et ni l'induction rapide par anhydride carbonique est sans stress (HSUS 2002)

- il existe un consensus ultra majoritaire dans les travaux publiés (y compris par conférences) sur les animaux pour montrer une évidence de l'agressivité bien que le gaz carbonique soit encore préconisé par beaucoup d'institutions dans le monde
C'est la vue à laquelle est arrivée aussi l'European Food Standards Authority's Scientific Panel on Animal Health and Welfare ainsi que l'UK's Farm Animal Welfare Council.

- Le National Consultative Committee for Animal (Australien) a passé en revue les méthodes d'euthanasie de volaille en 2005. Ce rapport reconnaît qu'il y a une contradiction croissante entre les plus récents travaux scientifiques et quelques recommandations internationales (NCCAW 2005)

Le rapport termine sur ces conclusions :
L'évidence de la souffrance et des réactions animales à l'agressivité associées à l'utilisation de l'anhydride carbonique comme agent anesthésique ou comme agent exclusif pour l'euthanasie des animaux de laboratoire est considérée comme acquise par un certain nombre de revues scientifiques récentes et indépendantes.
Il est recommandé que l'anhydride carbonique soit couplé à un pré-anesthésique inhalant non toxique, comme l'isoflurane, le halothane ou le methoxyflurane si utilisé pour l'euthanasie.

En Europe le comité scientifique sur la santé et le bien-être animal de l'autorité européenne de sécurité des aliments a rendu un **avis en juin 2004** sur toutes les techniques d'étourdissement et d'abattage pratiquées sur les principales espèces d'animaux utilisés dans le commerce en considération de la directive 93/119/CEE.
Il conclut :
Les recherches suggèrent que les atmosphères contrôlées contenant des concentrations de plus de 30 % de CO_2 sont aversives et peuvent causer de la douleur et une détresse respiratoire avant la perte de connaissance.

Mais ce rapport date un peu. Car l'autorité européenne de sécurité des aliments a rendu un **autre avis** récent du 14/11/2005 qui porte sur les aspects biologiques et de bien-être des animaux utilisés dans la recherche scientifique dans la perspective d'une révision de la directive 86/609/CEE.

Et là les choses changent le gaz carbonique devenant inacceptable pour tous les vertébrés :

Le CO_2 est aversif à tous les vertébrés, utilisés dans la recherche, qui ont été examinés. Quelques espèces ont même de l'aversion pour de basses concentrations (10-20 % par volume en air), indépendamment de toutes additions. On ne peut pas le recommander comme méthode unique de mise à mort humanitaire pour toutes les espèces. Le CO_2 peut être employé comme méthode d'euthanasie secondaire sur les animaux sans connaissance.

L'anhydride carbonique n'en devrait pas être employé comme agent exclusif dans les procédés d'euthanasie à moins que l'animal n'ait été d'abord rendu sans connaissance

Il serait inadéquat de placer un animal entièrement conscient dans un environnement gazeux connu comme nocif et dont il ne pourrait pas s'échapper

De nouvelles méthodes de mise à mort humanitaire des animaux qui utilisent des mélanges de gaz autres que ceux contenant du CO_2 doivent être développées de façon urgente.

Dans le rapport, le tableau n° 4 page 37 indique :

Les méthodes suivantes ne doivent pas être employées pour mettre à mort les oiseaux

.... décompression (caisson à vide) .... anhydride carbonique (CO_2)

Table 4 - Characteristics of methods for euthanasia of birds

*« The following methods are not to be used for killing birds: neck crushing, **decompression**, exsanguination, **carbon dioxide**, nitrous oxide, diethyl ether, chloroform, cyclopropane, hydrogen cyanide gas, trichlorethylene, methoxyflurane, chloral hydrate, strychnine, nicotine, magnesium sulphate, ketamine and neuromuscular blocking agents »*

Finalement le gaz carbonique, d'après les recherches scientifiques récentes, fait bien souffrir les oiseaux
Et les entreprises de dépigeonnage seraient avisées de ne pas le mentionner comme argument publicitaire. Comme aucune autorité ne les contrôle il est plus que probable que certaines utilisent n'importe comment les bouteilles de CO2 achétées dans le commerce; la lecture du rapport 2000 DE L'AVMA mettant l'accent sur une formation adéquate du personnel pour diminuer au maximum les souffrances.

Pour finir vous trouverez **ci-dessous** une revue de la législation française et européenne (floues) ainsi que les recommandations de l'Office international des épizooties O.I.E portant sur les méthodes d'euthanasie et d'étourdissement au moyen du gaz.

Le rapport 2000 de l'American Veterinary Medical Association

le rapport complet en pdf ici

Les gaz inhalés doivent avoir une concentration adaptée dans les alvéoles pour être efficace. Donc l'euthanasie par ces gaz prend un certain temps. La convenance d'un gaz particulier dépend de l'existence d'une détresse animale entre le moment ou l'animal commence à inhaler le gaz et le moment où il perd connaissance. Certains gaz induisent des convulsions mais celles-ci arrivent généralement après la perte de conscience. Certains gaz provoquent ses convulsions avant l'inconscience et sont de ce fait inacceptables pour l'euthanasie. Certaines considérations sont communes à tout gaz :

- dans la plupart des cas la perte de conscience est plus rapide et donc l'euthanasie plus humaine si l'animal est rapidement exposé à une concentration élevée du mélange gazeux

- le matériel qui fournit et maintient cette concentration élevée doit être dans un bon état d'entretien et conforme aux règlements de l'état. Un équipement qui fuit ou défectueux peut engendrer une mort lente et douloureuse et peut être dangereux pour les autres animaux et le personnel en raison des risques : d'explosion (par exemple l'éther), de narcose (halothane), d'hypoxémie (azote et oxyde de carbone), addiction ou dépendance (protoxyde d'azote) ou problème de santé résultant d'une exposition chronique (protoxyde d'azote et monoxyde de carbone)

- Une bonne concentration alvéolaire est difficile à obtenir avec un animal qui a une ventilation diminuée; cette phase de concentration durant plus longtemps et cause plus souvent de l'agitation pendant l'induction. D'autres méthodes non basées sur les mélanges inhalant devraient être envisagés pour de tels animaux

- les animaux nouveaux nés semblent être résistants à l'hypoxie et comme finalement tous les mélanges gazeux provoquent une hypoxie les animaux nouveaux nés mettent plus de temps pour mourir par ce procédé que les adultes......... la commission recommande de ne pas utiliser d'agent inhalant pour les animaux de moins de 16 semaines sauf pour provoquer une perte de conscience qui sera suivie d'une autre méthode de mise à mort

- les écoulements rapides de gaz peuvent produire un bruit qui effraye les animaux. Si un haut débit s'avère nécessaire l'équipement devrait être conçu pour réduire au minimum le bruit.

- Les animaux placés ensemble dans les chambres devraient être des mêmes espèces, et, si nécessaires, devraient être retenus afin qu'ils ne se blessent. Les chambres ne devraient pas être surchargées et maintenues propres pour réduire au maximum les odeurs qui pourraient affecter les animaux prochainement euthanasiés

- pour les reptiles, les amphibiens et les oiseaux et mammifères pouvant évoluer sous l'eau, sans oxygène et dont le métabolisme a une capacité anaérobique l'induction de l'anesthésie et le temps de la perte de conscience en utilisant des gaz peut être considérablement prolongé. D'autres techniques peuvent être plus appropriées pour ces espèces.

Monoxyde de Carbone CO

L'oxyde de carbone (Co) est un gaz sans couleurs et inodore. Il est ininflammable et non explosif à moins que les concentrations excèdent 10 %. Il se fixe sur l'hémoglobine pour former la carboxyhémoglobine ce qui bloque la prise d'oxygène O_2 par les érythrocytes amenant une hypoxémie mortelle.

Dans le passé beaucoup d'euthanasies de masse ont été faites en utilisant 3 moyens pour produire du CO :
(1) interaction chimique entre le formiate de sodium et l'acide sulfurique
(2) vapeurs d'échappement d'un moteur à explosion au ralenti
(3) Bouteilles de gaz liquide commercialisées. Les 2 premières techniques génèrent des problèmes tels que une production d'autres gaz, des concentrations insatisfaisantes de monoxyde de carbone, un refroidissement insatisfaisant du gaz et des problèmes d'entretien des appareils

Par conséquent, la seule source acceptable est le Co comprimé dans des bouteilles.
Dans une expérience effectuée par Ramsey et Eilmann, 8 % de CO ont fait s'effondrer des cobayes en 40 secondes à 2 minutes et la mort s'est produite dans un délai de 6 minutes. Le monoxyde de carbone a été utilisé pour euthanasier des visons et des chinchillas. Ces animaux se sont effondrés en 1 minute, la respiration a cessé en 2 minutes et le coeur s'est arrêté de battre en 5 à 7 minutes. Dans une étude évaluant la physiologie et le comportement caractéristiques des chiens exposés à 6 % de Co/air, Chalifoux et Dallaire n'ont pu déterminer précisément le moment de la perte de connaissance. Les électroencéphalogrammes ont indiqué 20 à 25 secondes de fonction corticale anormale avant la perte de connaissance C'était dans cette période que les chiens sont devenus agités et ont vocalisé.

On ne sait pas si des animaux ressentent de la douleur; cependant des humains dans cette phase n'ont ressenti aucune douleur. Des expériences suivantes ont révélé qu'une tranquilisation avec de l'acepromazine diminue de façon significative les réponses comportementales et physiologiques des chiens euthanasiés avec du CO.

Dans une étude comparative, du monoxyde de carbone issu des gaz d'échappement d'un moteur à essence et 70 % de CO_2 plus 30 % de O_2 ont été employés pour l'euthanasie des chats. L'euthanasie a été divisée en 3 phases. La phase I était le temps du contact initial, du début des signes cliniques (par exemple, bâillement, chancellement, ou tremblement). La phase II s'est étendue de la fin de la phase I jusqu'à ce que l'animal se couche et ne puisse plus se relever (recumbency) et la phase III de la fin de la phase II jusqu'à la mort. L'étude a montré que les signes d'agitation avant la perte de connaissance étaient plus grands avec le CO2 plus O2.. Les convulsions se sont produites pendant des phases II et III avec les deux méthodes. Cependant, quand la chambre d'euthanasie a été pré-remplie avec du CO (par ex les gaz d'échappement) les convulsions ne se sont pas produites dans la phase III. Le temps de la complète immobilisation était plus grand avec le mélange CO2 plus O2 (approximativement 90 secondes) qu'avec le CO seul (approximativement 56 secondes).

Chez les porcs nouveaux nés l'excitation avant la perte de connaissance était plus probable si les porcs étaient exposés à une élévation rapide de concentration de CO. Cette agitation a été réduite à des débits inférieurs ou quand le CO a été combiné avec de l'azote.

Pour les humains les symptômes les plus communs d'une intoxication au CO sont le mal de tête, les vertiges et la faiblesse. Au fur à mesure que la concentration de la carboxyhémoglobine augmente ces signes peuvent être suivis d'une diminution de l'acuité visuelle, acouphènes, de nausées, d'une dépression progressive, d'une confusion et d'un effondrement. Par ce que le CO stimule les centres moteurs du cerveau la perte de connaissance peut-être accompagnée par des convulsions et des spasmes musculaires.

L'oxyde de carbone est un poison cumulatif. Les signes d'intoxication au CO ne sont pas évidents jusqu'à la concentration de CO à 0.05 %/air et les signes aigus peuvent ne pas se développer jusqu'à la concentration de CO d'environ 0.2 %/air. Chez l'homme une exposition à 0.32 % CO et 0.45 % de CO pendant une heure induira une perte de connaissance et la mort, respectivement. L'oxyde de carbone est extrêmement dangereux pour le personnel parce qu'il est très toxique et difficile à détecter. Une exposition chronique à de basses concentrations d'oxyde de carbone peut être un risque sanitaire, particulièrement en ce qui concerne les maladies cardiovasculaires et les effets tératogéniques. Un système efficace de ventilation est essentiel pour prévenir les expositions accidentelles des humains

Avantages :

(1) l'oxyde de carbone induit une perte de connaissance sans douleur et avec un minimum de malaise
(2) L'hypoxemie induite par le CO est insidieuse de sorte que l'animal semble en être ignorant.
(3) La mort se produit rapidement si des concentrations de 4 à 6 % sont employées.

Inconvénients :

(1) des précautions doivent être prises pour empêcher l'exposition du personnel
(2) Les éléments. électriques exposé au CO (par ex lumières, ventilateurs) doivent être anti-déflagrants

Recommandations :
L'oxyde de carbone utilisé pour euthanasier individuellement ou collectivement les animaux est acceptable pour les chiens, les chats et les autres petits mammifères si le CO utilisé provient de bouteilles vendues dans le commerce et si les précautions suivantes sont prises :
(1) le personnel utilisant le CO doit être formé complètement sur son utilisation et il doit comprendre ses risques et limitations.
(2) la chambre doit être d'une grande qualité de construction et devrait prendre en compte la séparation des individus animaux
(3) la source de CO et la chambre doivent être situées dans un environnement bien-aéré, de préférence hors des ouvertures
(4) la chambre doit être bien éclairée et avoir des ouvertures qui permettent au personnel l'observation directe des animaux
(5) le débit du CO devraient être adéquat pour réaliser rapidement un concentration uniforme de CO d'au moins 6 % après que les animaux soient placés dans la chambre, bien que quelques espèces (par ex porcs nouveau-nés) soient probablement moins agités avec une élévation progressive de la concentration de CO
(6) si la chambre est à l'intérieur des dispositifs surveillant le niveau de Co doivent être placés dans la pièce afin d'avertir le personnel d'une concentration dangereuse. Il est essentiel que l'utilisation du CO soit en conformité avec les lois et règlements de l'état où est utilisé le dispositif

CO2

L'air ambiant contient 0.04 % de dioxyde de carbone (Co2) qui est plus lourd que l'air et presque inodore. L'inhalation de Co2 à une concentration de 7.5 % augmente le seuil de douleur et des concentrations plus élevées de Co2 ont un effet anesthésique rapide.

Leake et Waters ont parlé de l'utilisation expérimentale du CO2 comme anesthésique sur des chiens. Au concentration de 30 % à 40 % de CO2 par rapport à l'oxygène (O2) l'anesthésie a été induite 1 à 2 minute habituellement sans lutte, haut-le-coeur, ou vomissements. Pour des chats une inhalation de 60 % de CO2 entraîne une perte de conscience en 45 secondes et un arrêt respiratoire en 5 minutes. Les signes de l'efficacité de l'anesthésie étant ceux associés en chirurgie à une anesthésie profonde telle que la disparition du réflexe opticopalpébral (de clignement) et de retrait.

Le temps de la perte de conscience est réduit avec des concentrations plus élevées de CO2 de 80 à 100 % qui engendrent une anesthésie en 12 à 33 secondes chez les rats et avec une concentration de 70 % de CO2 par rapport à l'oxygène (O2) induit une anesthésie en 40 à 50 secondes. Le temps pour perdre connaissance sera plus long si la concentration est augmentée lentement plutôt que de soumettre l'animal à une pleine concentration immédiatement.

Plusieurs chercheurs ont suggéré qu'une concentration importante de CO2 peut faire souffrir les animaux par ce que le gaz carbonique se dissout en humidité dans les muqueuses nasales. Et le produit résultant, de l'acide carbonique (H2 CO3), peut activer les nocicepteurs dans la muqueuse nasale. Des humains ont été exposés à des concentrations de 50 % de CO2 et ont rapporté que respirer ce mélange est désagréable et que des concentrations plus élevées sont nocives. Une brève étude sur les porcs a étudié le côté agressif du CO2 et a trouvé qu'une concentration de 90 % était agressive pour les porcs tandis que 'une de 30 % ne l'était pas. Pour les rats une exposition croissante au CO2 (33 % après 1 minute) effectuée dans leur cage où ils vivent ne produit aucun stress évident. On estime le stress en observant le comportement de l'animal et en mesurant les concentrations d'ACTH, de glucose et de cortisone dans le sang

Le gaz carbonique a été employé pour euthanasier des groupes de petits animaux de laboratoires comprenant des souris, rats, cobayes, poulets et des lapins et pour rendre des porcs inconscients avant de les abattre de façon décente. La combinaison de 40 % de CO2 et d'environ 3 % de CO (monoxyde de carbone) a été utilisée expérimentalement pour l'euthanasie de chiens.

Le gaz carbonique a été utilisé en particulier dans des caissons conçus spécialement pour euthanasier différents chats et d'autres petits animaux de laboratoire. Des études portant sur des poussins d'un jour ont indiqué que le CO2 est un produit d'euthanasie efficace. L'inhalation de CO2 cause peu de détresse aux oiseaux, et supprime l'activité nerveuse et provoque la mort en 5 minutes.

Puisque la respiration commence pendant le développement de l'embryon, l'environnement des poulets non éclos peut être normal avec une concentration aussi haute que 14 %. Ainsi la concentration de CO2 pour euthanasier les poulets nouvellement éclos et les nouveaux-nés d'autres espèces devrait être spécialement haute. Une concentration de 60 à 70 % avec un temps d'exposition de 5 minutes semble être optimale.

Dans les recherches sur le vison des concentrations élevées de Co2 tuaient rapidement mais une concentration de 70 % de CO2 induisait une perte de conscience sans les tuer.

Des animaux creusant des terriers, comme les lapins Oryctolagus, ont aussi un temps de survie prolongé quand ils sont exposés au CO2. Des animaux creusant des terriers (par ex : lapins) et des animaux pouvant évoluer temporairement sous l'eau (dit de plongée) ont des mécanismes physiologiques leur permettant de faire face à l'hypoxémie. Par conséquent, il est nécessaire d'avoir une concentration de Co2 suffisante pour tuer l'animal par hypoxémie après l'induction anesthésique par C02.

Avantages :

- l'effet depresseur, analgésique et anesthésique du CO_2 est bien établi.

- l'anydride carbonique est facile à trouver et peut s'acheter en bouteille de gaz comprimé.

- Le gaz carbonique est peu cher, ininflammable, non explosif et pose un minimums de risques au personnel quand un équipement correctement conçu est utilisé.

- le gaz carbonique ne s'accumule pas dans les tissus (tissue residues) dans le cadre d'une production de viande

- l'euthanasie par l'anhydride carbonique ne modifie pas les marqueurs murins

-l'euthanasie d'anhydride carbonique ne tord pas les marqueurs cholinergiques murins ni la concentration de corticosterone

Inconvénients :

Puisque le CO_2 est plus lourd que l'air, un remplissage incomplet d'une chambre à gaz peut permettre aux animaux de monter ou soulever leur tête au dessus des hautes concentrations ce qui leur permet d'éviter l'exposition au gaz.

- certaines espèces comme les poissons et les animaux creusant des terriers (par ex : lapins) et les mammifères de plongée peuvent avoir une résistance extraordinaire au CO_2.

- La respiration lente des reptiles et des amphibiens pose problème pour l'usage du CO_2.

- L'euthanasie par exposition au CO_2 peut prendre plus de temps qu'une euthanasie par d'autres méthodes.

- L'induction de la perte de conscience aux concentrations inférieures à 80 % peut produire des lésions pulmonaires et des voie aériennes supérieures.

- des concentrations élevées de CO_2 peuvent énormément faire souffrir certains animaux.

Recommandations :

Le gaz carbonique est recommandé pour certaines espèces (voir les tableaux 1 et 2 du rapport).

Le CO_2 comprimé en bouteille est la seule source recommandée car l'apport du gaz dans la chambre peut être contrôlé avec précision. Le gaz carbonique produit par d'autres méthodes comme la glace carbonique, la neige carbonique des extincteurs ou par des produits chimiques (par ex anti-acides) est inacceptable. Les espèces devraient être séparées et les chambres non surchargées. Pour un animal dans la chambre le débit optimal devrait être de 20 % du volume par minute. La perte de conscience peut-être induite plus rapidement en exposant les animaux à une concentration de 70 % ou plus en préférant une chambre pour des espèces en qui cela n'a pas entraîner de souffrance. L'écoulement devrait être maintenu pendant au moins un minute après la mort clinique (apparente). Il est important de vérifier qu'un animal est mort avant de l'enlever de la chambre. Si un animal n'est pas mort, la narcose par CO_2 doit être suivie par une autre méthode d'euthanasie. Rajouter de l'oxygène O_2 au CO_2 peut ou ne peut pas prévenir les signes de souffrance. Le rajout d'O_2, cependant, prolongera le temps de la mort et rendra plus difficile la détermination de la perte de connaissance. Il apparaît ici qu'il n'y a aucun avantage à combiner l'oxygène O_2 avec le gaz carbonique pour euthanasier.

argon azote

L'azote (N_2) et l'argon (Ar) sont des gaz sans couleur, sans odeur car ils sont inertes, ininflammables et non explosifs L'azote représente 78 % de l'air et l'argon moins de 1 %. L'euthanasie est induite en plaçant l'animal dans un conteneur fermé qui a été pré-rempli avec de l'azote (N_2) ou de l'argon (Ar) ou dans lequel le gaz alors est rapidement apporté. L'azote/argon remplace l'O_2 et de ce fait engendre la mort par hypoxémie.

Des expériences effectuées par Herin et son équipe ont montré que : des chiens sont devenus inconscients en 76 secondes quand une concentration de N_2 de 98.5 % a été achevée en 45 à 60 secondes. L'électroencéphalogramme (EEG) est devenu isoélectrique (plat) en 80 secondes après et la tension artérielle artérielle était indétectable à 204 secondes. Bien que tous les chiens aient souffert d'hyperventillation avant la perte de conscience, les expérimentateurs ont conclu que cette méthode provoque la mort sans douleur. Après la perte de conscience, chez quelques chiens, se sont développées des vocalisations, des halètements, des convulsions et des tremblements musculaires. À la fin de 5 minutes d'exposition tous les chiens étaient morts. Pour les lapins et les visons les résultats étaient semblables. Avec du N_2 diffusé à une vitesse de 39 % du volume de la chambre par minute les rats se sont effondrés approximativement en 3 minutes et la respiration a cessé en 5 à 6 minutes. Quel que soit le débit les signes de panique et de souffrance étaient évidents pour les rats avant qu'ils s'effondrent et meurent. On peut s'interroger dans de telles circonstances sur la réalité d'une mort sans douleur.

L'administration du tranquillisant acepromazine en conjonction avec l'azote N_2 a été étudié par Quine et son équipe pour euthanasier les chiens. Grâce à l'enregistrement d'électrocardiogrammes et d''électroencéphalogrammes ils ont observé que ces chiens ont eu un temps de survie plus long que sans administration d'acepromazine avant exposition au N_2. Pour un chien l'activité ECG a continué pendant 51 minutes. Quine a aussi abordé le problème de la souffrance associée à l'azote en retirant des chiens et des chats de la chambre après la perte de connaissance, en leur permettant ainsi de récupérer. Quand ces animaux ont été mis de nouveau dans la chambre ils n'ont pas semblé effrayés ou anxieux.

Des recherches sur l'aversion des porcs et de la volaille à l'argon Ar ont montré que ces animaux tolèrent de respirer 90 % d'argon Ar avec 2 % d'oxygène O. Swine a utilisé une chambre contenant ce mélange en y plaçant de la nourriture comme récompense et il les a seulement sortis quand ils sont devenus ataxiques. Ils ont réintégré immédiatement la chambre et ont continué à manger. La volaille aussi a été décrite dans une chambre contenant ce mélange avec de la nourriture placée comme récompense et qui a continué à manger jusqu'à ce qu'elle s'effondre.

Quand de l'argon Ar était utilisé pour euthanasier des poulets par une exposition en chambre préremplie avec un mélange argon Ar plus oxygène O_2 à une concentration de 2 % cela a provoqué un changement et un effondrement de l'EEG en 9 à 12 secondes. Des oiseaux retirés de la chambre après 15 à 17 secondes n'ont pas répondu au pincement de la crête du coq. L'exposition qui a continué a provoqué des convulsions en 20 à 24 secondes. Les potentiels évoqués ont disparu en 24 à 34 secondes après et l'EGG plat en 57 à 66 secondes. Les convulsions ont commencé après la perte de connaissance (effondrement et absence de réponse au pincement de la crête du coq), ce qui semblerait montrer que ce procédé est une méthode décente pour euthanasier les poulets.

En dépit de la disponibilité d'un certain nombre de recherches il reste beaucoup à apprendre sur l'utilisation de l'azote N_2 et de l'argon Ar.

Avantages :

L'azote et l'argon sont faciles à trouver en bouteille et les risques pour le personnel minimes.

Inconvénients :

La perte de connaissance est précédée par une hypoxémie et une stimulation ventilatoire ce qui peut faire souffrir l'animal. Le rétablissement d'une faible concentration d'oxygène O2 (6 % ou plus) dans la chambre avant la mort permettra un rétablissement immédiat.

Recommandations :

L'azote et l'argon peuvent être pénibles pour quelques espèces (par ex les rats)

Par conséquent, cette technique est conditionnellement acceptable si des concentrations d'oxygène O2 inférieures à 2 % sont réalisées rapidement et si on a donné un fort sédatif aux animaux ou si on les a anesthésiés au préalable. Avec une forte sédation ou anesthésie il faudrait reconnaître quand la mort est retardée. Bien que l'azote N2 et l'argon Ar soient efficaces d'autres méthodes sont préférables.

gaz anesthésiants

Les gaz anesthésiants

Les gaz anesthésiants (par exemple l'éther, l'halothane, le methoxyflurane, l'isoflurane, le sevoflurane, le desflurane et l'enflurane) ont été employés pour euthanasier beaucoup d'espèces.

L'halothane induit l'anesthésie rapidement et est le gaz anesthésique le plus efficace pour l'euthanasie. L'enflurane est moins soluble dans le sang que l'halothane mais en raison de sa plus petite pression de vapeur saturée et de sa plus petite efficacité, les taux d'induction peuvent être identiques à ceux du halothane. Les animaux en profonde anesthésie peuvent avoir des problèmes très graves de santé (attaque). C'est un agent efficace pour l'euthanasie mais ses effets secondaires peuvent être dangereux pour le personnel. L'isoflurane est moins soluble que l'halothane et il devrait induire une anesthésie plus rapide. Cependant il a une odeur légèrement piquante. Les animaux souvent retiennent leur respiration retardant ainsi le début de la perte de connaissance. L'isoflurane comparé à l'halothane exige plus de produits pour tuer un animal. Bien que l'isoflurane soit acceptable pour euthanasier l'halothane est préférable. Le sevoflurane est moins soluble que l'halothane et n'a pas d'odeur. Il est moins efficace que l'isoflurane ou le halothane et a une pression de vapeur saturée inférieure. Des concentrations anesthésiques peuvent être réalisées et maintenues rapidement Le desflurane est actuellement le gaz le moins soluble dans le sang mais son odeur piquante peut ralentir l'induction. Ce produit est si volatil qu'il pourrait remplacer l'oxygène (O2) et induire une hypoxémie pendant l'induction si de l'oxygène supplémentaire n'était pas fourni. Le methoxyflurane est fortement soluble et l'induction anesthésique lente. Son utilisation peut s'accompagner de troubles. C'est sous conditions un agent acceptable pour l'euthanasie des rongeurs. L'éther est très soluble dans le sang et induit lentement l'anesthésie. Il irrite les yeux et le nez et pose de sérieux risques d'incendie et d'explosion et il a été utilisé pour créer un modèle de stress.

Pour les gaz anesthésiques l'animal peut être placé dans un réceptacle fermé contenant du coton ou de la gaze imbibée d'une quantité appropriée du produit ou le gaz peut être introduit par un inhalateur. Cette dernière méthode peut engendrer un plus long temps d'induction. Les vapeurs sont inhalées jusqu'à ce que la respiration cesse et que la mort s'en suive. Puisque l'état liquide de la plupart des gaz anesthésiques est irritant les animaux devraient toujours être exposés aux vapeurs. En outre il faut fournir suffisamment d'air ou d'oxygène pendant la période d'induction pour éviter pendant cette période l'hypoxémie.

Dans le cas de petits rongeurs placés dans un grand récipient, il y aura suffisant d'oxygène dans la chambre pour empêcher l'hypoxémie. De plus grandes espèces placées dans de petits récipients peut avoir un besoin d'air supplémentaire ou d'oxygène.

Le protoxyde d'azote (N2O) peut être employé avec un autre inhalant pour accélère le début de l'anesthésie mais utilisé seul il n'induit pas d'anesthésie chez les animaux même concentré à 100 %. Utilisé seul le protoxyde d'azote induit une hypoxémie avant l'arrêt respiratoire pouvant faire souffrir les animaux avant la perte de conscience.

L'exposition professionnelle aux gaz anesthésiques constitue un risque sanitaire. Des avortements spontanés et des anomalies congénitales ont été associés avec l'exposition de femmes aux agents anesthésiques pendant le début de la grossesse.

Pour ce qui concerne l'exposition humaine aux gaz anesthésiques les concentrations de l'halothane, de l'enflurane et de l'isoflurane devraient être de moins de 2 ppm et de moins de 25 ppm pour le protoxyde d'azote. Il n'existe aucune étude prouvant que ces concentrations sont sans danger; ces concentrations ont été fixées parce que ce sont celles constatées en condition réelle à l'hôpital. Des procédures efficaces doivent être mises en place pour protéger le personnel des vapeurs des gaz anesthésiques.

Avantages :

Les gaz anesthésiques sont particulièrement adaptés pour euthanasier les petits animaux (inf à 7 kg) ou pour les animaux difficilement injectables

L'halothane, l'enflurane, l'isoflurane, l'sevoflurane, le desflurane, le methoxyflurane, et le N2O sont ininflammables et sont non explosifs en conditions courantes

Inconvénients :

(1) les animaux peuvent lutter et devenir angoissés pendant l'induction de l'anesthésie parce que les vapeurs anesthésiques peuvent être irritantes et induire une excitation.

(2) l'éther est inflammable et explosif. Des explosions se sont produites quand des animaux euthanasiés avec de l'éther ont été placés dans un réfrigérateur ou un congélateur ordinaire (pas à l'épreuve des explosions) et quand des animaux mis en sac ont été placés dans un incinérateur. (3) L'induction avec le methoxyflurane est bien trop lente pour certaines espèces.

(4) le protoxyde d'azote augmentera la combustion.

(5) le personnel et les animaux peuvent compromettre leur santé en étant exposés à ces gaz.

(6) Une utilisation abusive comme drogue de ces gaz est possible spécialement le N2O

Recommandations :

dans l'ordre de préférence , halothane, enflurane, isoflurane, sevoflurane, methoxyflurane, et desflurane, avec ou sans protoxyde d'azote, sont acceptable pour l'euthanasie des petits animaux (inf à 7 kilogrammes). L'éther devrait seulement être employé dans des cas particuliers conformément aux aux règlements fédéraux de l'état. Acceptables sous conditions. Le protoxyde d'azote ne devrait pas être utilisé seul , en attendant encore d'autres études scientifiques sur sa convenance à l'euthanasie animale.

Bien qu'ils soient acceptables ces agents anesthésiques ne sont généralement pas employés chez les grands animaux en raison de leur coût et de leur difficulté d'administration.

le rapport complet en pdf ici

L'utilisation de l'anhydride carbonique pour l'euthanasie des animaux de laboratoire

Ce document passe en revue la littérature scientifique courante sur l'utilisation de l'anhydride carbonique (mélanges purs et divers) pour l'euthanasie des animaux de laboratoire. Il décrit les différents paramètres que les comités animaux victoriens d'éthique devraient considérer en faisant des recommandations.

Sommaire :

La littérature sur l'utilisation de l'anhydride carbonique pour l'euthanasie des animaux de laboratoire peut être considérée séparément pour les rongeurs et la volaille. Elle traite de l'évaluation de l'aversion à l'anhydride carbonique à différentes concentrations et avec différentes méthodes d'utilisation, de l'importance de la formation des utilisateurs et des différences parmi les directives internationales existantes. La littérature récente recommande l'utilisation de l'halothane pour les rats et de l'enflurane pour les souris en tant que méthodes plus humanitaires pour euthanasier par inhalation. Pour des poulets un mélange d'argon de 60 % et d'anhydride carbonique de 30 % par rapport à l'air est considéré comme la méthode la plus humanitaire.

Le code d'instructions victorien pour le logement et les soins des souris de laboratoire, des rats, des cobayes et des lapins déclare que cette euthanasie par inhalation qui utilise l'anhydride carbonique pour les rats et les souris est une méthode acceptable. L'utilisation de l'halothane, de l'isoflurane et du methoxyflurane est déclarée acceptable avec des réserves. Ces réserves sont basées sur les considérations de salubrité et de sûreté professionnelle qui peuvent être surmontées par une utilisation d'un l'équipement approprié d'extraction.. Basé sur la littérature scientifique, la promotion de ces agents d'inhalation en tant que méthode d'euthanasie préférée est recommandée.

Un tableau de recommandations des diverses méthodes d'euthanasie pour les rongeurs adultes en fonction des besoins expérimentaux basé sur la littérature existante est fourni. La littérature scientifique indique également que cette euthanasie gazeuse effectuée dans la cage de résidence parmi les congénères a un avantage mesurable en matière de bien-être animal.

Introduction

L'anhydride carbonique est utilisé généralement pour euthanasier les rats, les souris et les poulets, adultes, en laboratoire, espèces étudiées dans cette revue. Son utilisation est un sujet de polémiques au sein de la communauté scientifique. Les études décrivent comment le gaz carbonique est agressif, avec un rapport linéaire entre concentration et souffrance et/ou de sensation de douleur aux concentrations s'échelonnant de 7-100 % (HSUS 2002). Ce sujet a soulevé de l'inquiétude quant au caractère humanitaire d'une utilisation de l'anhydride carbonique pour l'euthanasie des animaux, ce qui a été le sujet de nombreuses études et publications récentes.

Littérature scientifique sur l'utilisation et les effets de l'anhydride carbonique

Passer en revue le mécanisme physiologique réel d'un excès d"anhydride carbonique aide à comprendre les signes cliniques associés qui peuvent changer entre les espèces et les individus à un certain degré. Le mécanisme primaire de l'anesthésie et de la mort est l'action directe de l'anhydride carbonique sur les systèmes essentiels (Pritchett et autres, 2005).

Pour simplifier, la molécule de gaz se répand essentiellement dans le sang, le corps et le cerveau par les poumons. Confronté aux niveaux excessifs du gaz, la capacité inhérente du sang d'être tampon pour l'anhydride carbonique est dépassée, ce qui a comme conséquence l'acidose (l'abaissement du pH du sang et des fluides associés). Une concentration de faible à modéré d'anhydride carbonique cause (s'étendant de 5-35 %, Conlee et autres, 2005) une acidose respiratoire douce menant à une augmentation compensatoire de l'intensité et du rythme de la respiration pour expulser la quantité d'anhydride carbonique excessive (hyperventilation) avec changements du rythme cardiaque et de la tension artérielle. De plus hautes concentrations mènent alors à une acidose respiratoire plus profonde, réprimant les centres respiratoires du cerveau menant à un modèle respiratoire lent et haletant. Sans la capacité tampon du sang, le pH du fluide cérébrospinal (CSF) baisse brusquement ce qui est directement lié à la profondeur de l'anesthésie et à l'insensibilité à la douleur qui suit, à la stupeur et finalement à l'inconscience et à la mort. En outre, un autre mécanisme est la dépression d'acidose induite du muscle cardiaque provoquant des arythmies de coeur et l'arrêt.

En ce qui concerne le bien-être animal, cependant, on s'intéresse à la durée et à l'effet avant l'insensibilité et la perte de connaissance (par ex. anesthésie). Un certain nombre d'articles se réfèrent non seulement à la sensation et aux signes de la détresse et de l'asphyxie respiratoire (y compris quelques indications histologiques d'un état équivalent à la noyade consciente) mais aussi à l'acidification des muqueuses (par exemple yeux, bouche, système respiratoire). Une capacité sensorielle particulière de la muqueuse nasale associée à différents niveaux de malaise et de douleur a été rapportée chez l'homme et chez les animaux (Conlee et autres 2005).

Les études sur l'utilisation de l'anhydride carbonique pour les rats et les souris diffèrent de celle pour les poulets ce qui est dû aux différences comportementales entre les rongeurs et les poulets et aussi aux méthodes de mise à mort des volailles pour la production de nourriture.

Les limitations méthodologiques créent une tendance à comparer divers agents d'inhalation plutôt que de comparer d'autres méthodes d'euthanasie ou aspects du processus de l'euthanasie.. Les études anciennes impliquant des rongeurs changent selon les méthodes employées à évaluer le bien-être tandis que les études les plus récentes et les plus vigoureuses incluent une exploration du comportement afin d'améliorer l'analyse des facteurs d'agressivité.

En comparaison, les études publiées sur l'euthanasie du poulet sont plus nombreuses en raison de son utilisation comme viande. La nature de la recherche est la plupart du temps applicable à l'industrie alimentaire mais est encore utile en considérant la gestion du laboratoire. Cette littérature s'est déplacée au-delà des indicateurs comportementaux qui indiquent que l'anhydride carbonique est agressif. On se concentre actuellement sur l'efficacité des gaz moins agressifs et on utilise un mélange d'électroencéphalogrammes (ECG) et de potentiels évoqués (SEP) comme indicateurs des niveaux de la conscience et de l'établissement de la mort.

Rongeurs

Les recherches sur les rongeurs ont étudié au niveau comportemental, physiologique et histologique les changements associés à l'utilisation d'anhydride carbonique. Ils ont examiné les effets des différentes concentrations, ajoutant de l'oxygène et en utilisant des chambres préremplies par rapport à une induction progressive. Les observations qui ont été rapportés avec l'utilisation d'anhydride carbonique sont celles qui peuvent indiquer une souffrance ou douleur, elles incluent : locomotion accrue, excitation et agitation sérieuse, élevage accru, défécation et urinage, irritation des muqueuses (lavage fréquent y compris), hyperventilation, halètement et « têtes tournées vers le haut et vers l'arrière ». Ces signes augmentent généralement en fréquence et en intensité avec l'augmentation de la concentration de l'anhydride carbonique de 25-100 %, jusqu'à ce que l'anesthésie soit induite. Les travaux sur les effets histologiques sont largement passés en revue par Conlee et autres en 2005.

Les travaux récents sur les rats et les souris concluent que l'anhydride carbonique est le moins préféré des agents d'inhalation comparé aux halothane, isoflurane, enflurane, desflurane, sevoflurane et argon. L'aversion d'exposition de ces espèces à l'anhydride carbonique aux concentrations assez hautes pour causer une perte de connaissance. Les variations dans l'utilisation comprenant l'humidification, le mélange avec de l'argon ou de l'oxygène, l'utilisation de chambres pré remplies par rapport à l'augmentation progressive de la concentration. Tous ont échoué à faire disparaître les effets agressifs

(Leach et autres 2002a ; Leach et autres 2002b). Le degré d'aversion des agents d'inhalation précédemment mentionnés a été établi en évaluant la bonne volonté des rongeurs à entrer et à demeurer dans les chambres qui contiennent ces agents. Les seuls agents que les auteurs ont conclus comme non agressifs sont le halothane et le sevoflurane pour les rats. Les auteurs ont conclu (Leach et Morton 2004) : tous ces agents d'inhalation ont eu quelques degrés d'agressivité dans le cas des souris.

Par conséquent, basé sur les résultats de ces travaux l'agent anesthésique recommandé pour les rats est l'halothane et pour les souris l'enflurane car aux concentrations appropriées ils provoquent une induction rapide et efficace avec un minimum de détresse. La méthode d'euthanasie recommandée employant un agent simple serait l'argon. Cependant en induisant une perte de connaissance avec un anesthésique volatil halogène (par ex halothane et enflurane) et plus tard en tuant rapidement avec de l'anhydride carbonique après que les animaux soient sans connaissance, cela peut être considéré comme plus humanitaire que l'utilisation de l'argon seul. Bien que l' animal soit exposé à l'anhydride carbonique sans connaissance, l'anhydride carbonique étant un agent efficace pour tuer, cela n'en fait pas un agent recommandable en matière de bien-être animal.

Bien qu'en dehors du sujet de ce rapport, l'impact sur le bien-être animal par la façon d'utiliser les agents d'inhalation mérite attention. Il y a une évidence pour suggérer que l'utilisation de la cage où réside habituellement l'animal et l'inclusion d'un familier de l'animal peut offrir un avantage significatif de bien-être pendant le processus de l'euthanasie (Maguire et Arthur 2003). C'est conforme à tous les travaux sur le stress animal. L'utilisation de la télémétrie et des nouvelles technologies peut permettre d'augmenter notre vision, spécifiquement utilisant ces sortes de paramètres pendant le processus d'euthanasie (Williams 2004).

Poulets

L'anatomie et la physiologie spécialisées du système respiratoire aviaire qui est fortement adapté à un échange gazeux efficace, rend la volaille extrêmement sensible à l'inhalation de gaz et il est essentiel qu'on démontre le côté humanitaire des méthodes d'inhalation. L'anhydride carbonique pour les oiseaux adultes et vieux est actuellement une technique toujours utilisée généralement pour l'euthanasie d'un grand nombre de volailles, bien que cela soit non permis dans les abattoirs Britanniques depuis 1995 (NCCAW 2005). Il existe une importante étude internationale, récente, sur les techniques d'étourdissement par le gaz (CAS - controlled atmospheric stunning) dans la pratique commerciale de mise à mort des poulets qui fournit une alternative au processus plus courant de l'étourdissement électrique ou de l'utilisation de l'anhydride carbonique seul. Il y a un certain nombre de différents mélanges de gaz qui étourdissent les oiseaux par différents mécanismes comportant diverses combinaisons d'anhydride carbonique, de gaz inertes (argon ou azote) et d'oxygène ou d'air, et les mélanges doivent être soigneusement contrôler. Le choix entre ces mélanges est indiscutablement un sujet de controverses parmi toutes celles portant sur étourdissement par le gaz (Wathes 2004).

L'utilisation de la technologie EEG et SEP (potentiel évoqué) a mi en évidence que l'inhalation de gaz carbonique est plus agressive pour les poulets que l'argon et l'argon avec du gaz carbonique mélangé (Raj et autres 1998). L'utilisation de l'argon à 90 % en air a comme conséquence une plus grande période pour avoir un EEG isoélectrique et une cessation d'SEPs liés une perte de connaissance et à la mort qu'avec un mélange de 60 % d'argon et de 30 % de gaz carbonique par rapport à l'air.

Les niveaux d''oxygène résiduel quand les poulets sont euthanasiés avec de l'argon à 90 % en air sont critiques si les niveaux d'oxygène sont plus grands que 2 % car cela peut augmenter le temps de la mort et diminuez sensiblement les taux de mortalité (Raj et Whittington 1995). Cet effet critique de l'oxygène résiduel ne se produit pas avec un mélange d' argon à 60 % et d'anhydride carbonique à 30 % en euthanasie de poulets en grands nombres (Raj et autres 1992).

Il y a cependant toujours, quelques avis différents portant sur l'interprétation de certains indicateurs de bien-être concernant l'euthanasie des poulets par inhalation. La plupart des experts et les récentes études considèrent les comportements spécifiques tels qu'haleter, secouer la tête, agiter les ailes, la défécation comme indicateurs de l'aversion, mais certains les considèrent toujours comme des réponses (involontaires) autonomes. D'autres résultats des recherches sont en suspens. On espère aussi sur une nouvelle technique statistique, validée dans l'anesthésie humaine qui pourra fournir d'autres informations sur les tracés complexes d'EEG et dans quelles mesures ils indiquent le niveau de la conscience (Wathes 2005).

Directives, codes et groupes d'enquêteurs scientifiques

Le recueil d'instructions australien des soins et des usages des animaux pour la recherche scientifique conseille que les décisions concernant la protection des animaux doivent être basées sur 'hypothèse que quand la douleur et la détresse ne peuvent pas être facilement évaluées chez les animaux, on doit supposer que les animaux éprouvent la douleur d'une façon semblable aux humains à moins qu'il soit évident que cette hypothèse soit fausse. Ce principe est en conformité avec la politique des services de santé publique des États-Unis sur les soins et l'utilisation humanitaire des animaux dans les laboratoires. À la lumière de ces politiques et des travaux scientifiques passés en revue, diverses directives internationales sembleraient être en conflit avec une utilisation de l'anhydride carbonique comme unique agent d'euthanasie :

Les directives 2001 sur l'euthanasie des animaux dans la recherche scientifique dans l'ANZCCART (Australian and New Zealand Council for the Care of Animals in Research and Teaching) recommande l'utilisation de l'anhydride carbonique au moyen d'un équipement spécialisé pour les rongeurs, préférant l'induction progressive mais reconnaissant et acceptant les divergences portant sur les différentes études et sur les différentes méthodologies d'utilisation de l'anhydride carbonique. Basé sur ces directives, l'AVA (Australian Veterinary Association) a actuellement également « approuvé » une telle utilisation de l'anhydride carbonique. L' AVMA (American Veterinary Medical Association) dans son rapport 2000 sur l'euthanasie recommande le CO_2 à 70 % dans une chambre préremplie pour l'euthanasie des rongeurs, comme le rapport de la Commission européenne de 1996. Le code britannique (Home Office Code of Practice) en 1997, qui est actuellement en cours de modification, indique que ce gaz carbonique est seulement approprié pour les rongeurs, les lapins et les oiseaux jusqu'à 1.5 kg et recommande l'exposition à une concentration progressive. L'UFAW (UK University Federation of Animal Welfare) et le CCAC (Canadian Council for Animal Care) reconnaissent que ni l'induction lente et ni l'induction rapide par anhydride carbonique sont sans stress (HSUS 2002). Un auteur fait ce commentaire au nom de 2 comités scientifiques indépendants (Morton, 2005) :

il existe un consensus ultra majoritaire dans les travaux publiés (y compris par conférences) sur les animaux pour montrer une évidence de l'agressivité.

C'est la vue à laquelle est arrivée aussi l'European Food Standards Authority’s Scientific Panel on Animal Health and Welfare ainsi que l'UK’s Farm Animal Welfare Council

L'équipe de la protection des animaux de McDonald également a récemment examiné la littérature scientifique portant sur les méthodes existantes d'étourdissement et d'abattage de volaille et tend vers l'utilisation des atmosphères contrôlées en utilisant des gaz inertes (McDonald 2005). Le National Consultative Committee for Animal (Australien) a passé en revue les méthodes d'euthanasie de volaille en 2005. Ce rapport reconnaît qu'il y a une contradiction croissante entre les plus récents travaux scientifiques et quelques recommandations internationales (NCCAW 2005)

Conclusions :

L'évidence de la souffrance et des réactions animales à l'agressivité associées à l'utilisation de l'anhydride carbonique comme agent anesthésique ou comme agent exclusif pour l'euthanasie des animaux de laboratoire est considérée comme acquise par un certain nombre de revues scientifiques récentes et indépendantes. Leach et son équipe (2004) en arrivent spécifiquement à cette conclusion en exposant des rats et des souris à l'anhydride carbonique sous toute forme pour l'anesthésie, qui est susceptible de causer une douleur et une détresse considérable et est donc inacceptable quand des solutions de rechange efficaces et plus humanitaires sont facilement disponibles.

Il est recommandé que l'anhydride carbonique soit couplé à un pré-anesthésique inhalant non toxique, comme l'isoflurane, le halothane ou le methoxyflurane si utilisé pour l'euthanasie. Pour l'utilisation d'autres combinaisons gazeuses pour l'euthanasie des rongeurs ou des poulets cela est également préféré au lieu d'une utilisation unique de l'anhydride carbonique. Cela serait très bien que l'argon/oxygène/air et les divers mélanges d'argon/gaz carboniques soient disponibles dans le commerce par le groupe BOC.

La formation appropriée du personnel dans toutes les techniques impliquant l'euthanasie est également fondamentale.

D'une manière importante le bien-être des rongeurs et des poulets subissant une euthanasie par inhalation a pu être encore augmenté par la rédaction de recommandations institutionnelles portant sur les pratiques préférées.

En résumé, il est recommandé que la communauté scientifique reconsidère soigneusement l'utilisation courante de l'anhydride carbonique comme agent exclusif pour l'anesthésie ou l'euthanasie et adopte ces pratiques plus humanitaires dans la mesure du possible.

Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals

The EFSA Journal (2004), 45, 1-29, Welfare aspects of the main systems of stunning and killing the main commercial species of animals

The EFSA Scientific Panel on Animal Health and Welfare was asked by the Commission services to report on the welfare aspects of the main systems of stunning and killing in the main commercial species of animals with consideration of Directive 93/119/EC. Species referred to in the present opinion are: cattle, sheep, pigs, poultry, horses and farmed fish. Welfare aspects of the systems for stunning other species, such as rabbits, deer, ratites or goats, have not been included in the present opinion.

Autorité européenne de sécurité des aliments

Avis du comité scientifique sur la santé et le bien-être animal sur une demande de la commission concernant les aspects de bien-être animal portant sur toutes les techniques d'étourdissement et d'abattage pratiquées sur les principales espèces d'animaux utilisés dans le commerce

Le comité scientifique sur la santé et le bien-être animal de l'autorité européenne de sécurité des aliments a été sollicité par les services de la commission pour faire un rapport sur les aspects de bien-être animal portant sur toutes les techniques d'étourdissement et d'abattage pratiquées sur les principales espèces d'animaux utilisés dans le commerce en considération de la directive 93/119/CEE. Les espèces visées par ce rapport : bétail, moutons, porcs, volaille, chevaux et poissons d'élevage. Les aspects de bien-être animal portant sur l'étourdissement ne sont pas inclus dans ce présent rapport pour les autres espèces : telles que les lapins, les cerfs communs, les rats ou les chèvres.

L'avis complet ici

5 - METHODS FOR STUNNING AND STUN / KILLING POULTRY SPECIES (CHICKENS AND TURKEYS)

5.3 Méthodes d'étourdissent ou de mise à mort par gaz

5.3.1 - Conclusions

Les recherches suggèrent que les atmosphères contrôlées contenant des concentrations de plus de 30 % de $CO2$ sont aversives et peuvent causer de la douleur et une détresse respiratoire avant la perte de connaissance. L'hypoxie induite avec de l'argon et/ou de l'azote avec moins de 2 % d'oxygène n'est pas aversive pour la volaille.

Une atmosphère contrôlée contenant moins de 30 % de $CO2$ avec de l'argon et/ou de l'azote avec moins de 2 % d'oxygène semble convenir pour étourdir/tuer la volaille. Une méthode alternative additionnelle, d'anesthésie des oiseaux avec une atmosphère de 30 à 40 % de $CO2$ (avec $O2$ et $N2$) pendant une minute suivie pendant deux minutes par une concentration de 80 % ou plus de $CO2$ pour tuer est en cours d'évaluation.

Quelque soit le mélange utilisé, les temps d'exposition ne sont pas connus pour étourdir efficacement la volaille sans la tuer et/ou sans insuffisamment l'étourdir. La durée de la perte de connaissance induite avec les mélanges connus de gaz est très courte et donc il sera difficile d'éviter la reprise de connaissance avant ou pendant la saignée. Les oiseaux qui montrent des signes de connaissance après l'étourdissement devront être ré-étourdis efficacement en préférant le pistolet à tige perforante plutôt que la dislocation du cou.

5.3.2 - Recommendations

En raison de l'absence de certitude scientifique évidente concernant la profondeur et la durée de la perte de connaissance avec les mélanges de gaz, les conditions minimums pour étourdir la volaille ne pourront pas être définies.

Quand on utilise des mélanges de gaz pour étourdir/tuer, la volaille vivante devrait être introduite dans le mélange de gaz par des caisses de transport ou par des tapis roulants en moins de 10 secondes en partant de l'air atmosphérique. Dans aucun cas du gaz glacial doit entrer dans la chambre. Il est recommandé que les concentrations de gaz soient surveillées sans interruption, au niveau des oiseaux, à l'intérieur.

Les mélanges recommandés de gaz sont : (a) un minimum de 2 minutes d'exposition pour l'argon, l'azote ou d'autres gaz inertes, ou pour tout mélange de ces gaz, en air atmosphérique avec un maximum de 2 % d'oxygène par volume. (b) un minimum d'exposition de 2 minutes à tout mélange d'argon, d'azote, ou d'autres gaz inertes avec de l'air atmosphérique et du $CO2$ à condition que la concentration en $CO2$ n'excède pas 30 % par volume et que la concentration d'oxygène n'excède pas 2 % par volume. Tous les oiseaux devraient être tués par les mélanges de gaz et en aucune circonstance ils ne devraient montrer des signes de rétablissement de connaissance une fois passés dans la chambre.

Etourdir et mettre à mort dans les cages de transport (par hypoxie et immobilisation des animaux inconscients) engendreraient moins de souffrance puisque cela permettrait non seulement d'éliminer la phase d'immobilisation des oiseaux vivants mais aussi mettre à mort effectivement tous les oiseaux. A cet égard, l'utilisation de l'hypoxie (avec moins de 2 % d'oxygène par volume), induite par de l'argon, de l'azote, d'autres gaz inertes ou des mélanges de ceux-ci, peut être la meilleure solution, du point de vue du bien-être animal.

5.3.3 - Priorités élevées des recherches

La technique d'étourdissement de la volaille avec les mélanges de gaz demande plus de recherches scientifiques afin de déterminer le mélange qui fait le moins souffrir les oiseaux, mais aussi afin de connaître la durée de la perte de connaissance, le laps de temps maximum qu'on doit recommander entre

l'étourdissement et la coupure du cou ou la coupe des carotides, et le moment du début de la mort cérébrale.
Les mélanges gazeux pour étourdir devraient être améliorés et validés ainsi ils pourraient être rapidement, couramment employés en abattoir, diminuant ainsi les souffrances dont celles engendrées par les manipulations d'immobilisation..
Les techniques de saignées sans immobilisation des oiseaux étourdis au gaz doivent être évaluées et développées.

Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes"

EFSA-Q-2004-105
Adopted by the AHAW Panel on 14 November 2005

The EFSA Journal (2005) 292, 1-46 - Opinion on the "Aspects of the biology and welfare of animals used for experimental and other scientific purposes"

EFSA was invited by the EU Commission to produce a scientific opinion concerning the "Revision of the Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes".

L'autorité européenne de sécurité des aliments a été sollicitée de donner un avis concernant la révision de la directive 86/609/CEE sur les animaux utilisés dans la recherche scientifique

Avis du comité scientifique sur la santé et le bien-ête animal sur une demande de la commission concernant les aspects biologiques et de bien-être portant sur les animaux utilisés dans la recherche scientifique

L'avis complet ici

4 - QUESTION ON HUMANE METHODS OF EUTHANASIA

4.5.5 - Méthodes gazeuses

4.5.5.1.Exposition aux mélanges d'anhydride carbonique

Conclusions :

Le CO2 est aversif à tous les vertébrés, utilisés dans la recherche, qui ont été examinés. Quelques espèces ont même de l'aversion pour de basses concentrations (10-20 % par volume en air), indépendamment de toutes additions. On ne peut pas le recommander comme méthode unique de mise à mort humanitaire pour toutes les espèces. Le CO2 peut être employé comme méthode d'euthanasie secondaire sur les animaux sans connaissance.

Des foetus de souris ne sont pas tués in utero dans un délai de 20 minutes quoique la mère ait est été tuée avec du CO2, mais il est possible de tuer les formes néonatales avec du CO2.

Recommandations :

L'anhydride carbonique n'en devrait pas être employé comme agent exclusif dans les procédés d'euthanasie à moins que l'animal n'ait été d'abord rendu sans connaissance, c'est-à-dire qu'il devrait être mis à mort aussitôt que possible. Il est important que des méthodes aussi efficaces et non aversives déjà partiellement exploitées soient développées plus à fond. Et il est temps que les personnes qui pratiquent les anciennes méthodes adoptent ces nouveaux mélanges de gaz plus humanitaires.

Il serait inadéquat de placer un animal entièrement conscient dans un environnement gazeux connu comme nocif et dont il ne pourrait pas s'échapper

Recherches futures :

La recherche sur l'euthanasie des animaux devrait suivre les directives de l'association internationale pour l'étude de la douleur (International Association for the Study of Pain). De nouvelles méthodes de mise à mort humanitaire des animaux qui utilisent des mélanges de gaz autres que ceux contenant du CO2 doivent être développées de façon urgente. Le temps pour induire une perte de connaissance a été habituellement déterminé sur la base des comportements (par exemple : ataxie) mais cette période d'induction doit être plus clairement définie grâce à des critères neurophysiologiques.

Une méthode objective pour mesurer la dyspnée est nécessaire pour démontrer et qualifier la dyspnée chez les animaux de laboratoire (particulièrement les rongeurs), qui permette une quantification de la durée et de la sévérité de la détresse des animaux exposés à tout mélange de gaz.

4.5.5.2 L'argon et l'azote comme gaz inertes induisant l'hypoxie

Conclusions :

il est proposé que l'utilisation de l'anoxie pour mettre à mort soit humanitaire et bien plus pratique pour les porcs et la volaille, et probablement les rongeurs. Une expérience pratique est indispensable. En raison de l'affinité élevée de l'hémoglobine pour l'oxygène chez les animaux au stade foetal ou néonatal, la mise à mort peut prendre plus de temps que chez les animaux adultes de la même espèce. Il n'existe actuellement aucune étude prenant en compte les aspects de bien-être animal. Plus de recherches scientifiques sont donc nécessaires pour l'azote.

Recommandations :

Des recherches sur les mélanges de gaz hypoxiques devraient être faites de façon urgente, il faudrait étudier les méthodes pratiques sur les petits animaux, comme les rongeurs.

Recherches futures :

Les recherches scientifiques, portant sur une utilisation humanitaire des mélanges de gaz hypoxiques et anoxiques, s'avèrent indispensables

4.5.5.3. Protoxyde d'azote

Conclusions :

Pour des raisons de salubrité humaine et de sûreté, le protoxyde d'azote n'est pas approprié pour l'euthanasie.

Recommandations :

(voir les tableaux 1 - 8)

Recherches futures :

(probablement les espèces transportées)

4.5.5.4. Monoxyde de Carbone

Conclusions :

Pour des raisons de salubrité humaine et de sûreté, le monoxyde de carbone est dangereux pour les mises à mort humanitaires

Recommandations :

Dans des conditions contrôlées l'oxyde de carbone peut être employé pour les chiens, les chats et les visons, toutefois il n'est pas recommandé pour des raisons de salubrité et de sûreté humaine, et également de bien-être animal.

4.5.5.5 Surdosage de gaz anesthésiques

Conclusions :

Le surdosage d'un gaz anesthésique connu, à la concentration appropriée, peut causer une détresse mineure pour quelques espèces, mais tous les gaz peuvent être aversifs aux concentrations élevées. Cependant ils ont l'avantage de ne pas exiger de contraintes dans leur administration.
Des foetus de souris ne sont pas tués dans l'utérus dans un délai de 20 minutes quoique la mère ait été tuée par un surdosage, mais les formes néonatales (1-7) sont tuées.

Recommandations :

Le surdosage d'un gaz anesthésique devrait être considéré comme une manière humanitaire de tuer les animaux en donnant quelques avertissements pour que la concentration et le côté aversif soient pris en compte.

Recherches futures :

L'aversion pourrait être évaluée chez quelques espèces pour quelques gaz (par exemple le furet)

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voir aussi le tableau n° 4 page 37 :
Les méthodes suivantes ne doivent pas être employées pour mettre à mort les oiseaux
.... décompression (caisson à vide) .... anhydride carbonique (CO_2)

Table 4 - Characteristics of methods for euthanasia of birds

*« The following methods are not to be used for killing birds: neck crushing, **decompression**, exsanguination, **carbon dioxide**, nitrous oxide, diethyl ether, chloroform, cyclopropane, hydrogen cyanide gas, trichlorethylene, methoxyflurane, chloral hydrate, strychnine, nicotine, magnesium sulphate, ketamine and neuromuscular blocking agents »*

LEGISLATION FRANCAISE ET EUROPEENNE

On va passer en revue les textes qui mentionnent les procédés à base de gaz.

EN FRANCE

I - Animaux élevés ou détenus pour la production de viandes, de peaux, de fourrures ou d'autres produits et procédures de mise à mort des animaux en cas de lutte contre les maladies contagieuses

En France c'est l'**arrêté du 12 décembre 1997** relatif aux procédés d'immobilisation, d'étourdissement et de mise à mort des animaux et aux conditions de protection animale dans les abattoirs qui définie les méthodes autorisées à base de gaz.

On y apprend qu'on peut utiliser pour étourdir les porcs du CO2 d'une concentration minimale de 70 %, le texte n'exige pas une durée précise d'exposition ("ils doivent être exposés à celui-ci pendant une durée assez longue pour qu'ils restent inconscients jusqu'à leur mise à mort")

Pour mettre à mort les animaux (aucune espèce spécifiée) on peut utiliser une "atmosphère gazeuse appropriée" sans précision

Quant aux animaux à fourrure on peut utiliser pour la mise à mort des mustélidés et des chinchillas (aucun pourcentage de concentration ni durée d'exposition n'est exigé si ce n'est ces exigences générales : le gaz doit d'abord provoquer une profonde anesthésie générale et, enfin, entraîner la mort à coup sûr et Les animaux doivent rester dans le puits jusqu'à ce qu'ils soient morts) :

- le monoxyde de carbone
- dioxyde de carbone (CO2) concentration : *Les animaux ne soient introduits dans le puits que lorsque l'atmosphère présente la plus forte concentration possible en dioxyde de carbone fournie par une source de dioxyde de carbone à 100 %*
- chloroforme (uniquement les chinchillas)

Pour la mise à mort des poussins et des embryons refusés dans les couvoirs on peut utiliser le dioxyde de carbone avec une concentration maximum (100 % ?) :
Les animaux doivent être placés dans une atmosphère présentant la plus forte concentration de dioxyde de carbone possible fournie par une source de dioxyde de carbone à 100 % ;

Les procédés utilisés dans les abattoirs doivent être agréés par une commission consultative de vérification de conformité des matériels (article R 214-76 du code rural)

II - Animaux de compagnie

La convention européenne pour la protection des animaux de compagnie indique dans son article 11 que les méthodes d'asphyxie sont interdites sauf si le gaz utilisé induit une anesthésie générale profonde avant de provoquer la mort et qu'il ne provoque aucune souffrance (*Tout sacrifice doit se faire avec le minimum de souffrances physiques et morales*).

DIRECTIVES EUROPEENNES

La France a adapté (copié) sa législation sur les conventions ou les directives européennes.

Par exemple la Directive du Conseil 93/119/EC du 22 décembre 1993 sur la protection des animaux au moment de leur abattage ou de leur mise à mort qui fixe des normes minimales où il n'existe aucune différence entre la réglementation française et la directive européenne.

RECOMMANDATIONS INTERNATIONALES

l'Office international des épizooties O.I.E. publie des lignes directrices pour l'abattage des animaux sensées prendre en compte le bien-être animal

I. lignes directrices pour l'abattage d'animaux à des fins de consommation humaine
annexe 3.7.5.

voir plus bas

étourdissement au gaz :

- Étourdissement des porcs au CO2 :
Il préconise une concentration de 90 % (pas inférieure à 80 %) et un temps d'exposition de 3 minutes

- **Mélanges de gaz inertes pour les porcs :**
Il conseille d'éviter les fortes concentrations de CO2 qui sont agressives et peuvent entraîner une détresse chez les animaux. C'est pourquoi l'utilisation de mélanges gazeux non agressifs est en cours d'étude. Ces études portent sur les mélanges suivants :

- un maximum de 2 % V/V d'oxygène dans de l'argon, de l'azote ou d'autres gaz inertes, ou
- jusqu'à un maximum de 30 % V/V de dioxyde de carbone et un maximum de 2 % V/V d'oxygène dans les mélanges avec du dioxyde de carbone et de l'argon, de l'azote ou d'autres gaz inertes.

- Étourdissement des volailles au gaz :

il préconise les mélanges suivants :

- un minimum de 2 minutes d'exposition à 40 % de dioxyde de carbone, 30 % d'oxygène et 30 % d'azote, puis minimum d'une minute d'exposition à 80 % de dioxyde de carbone dans l’air, ou

- un minimum de 2 minutes d’exposition à tout mélange d’argon, d’azote ou d’autres gaz inertes avec de l’air atmosphérique et du dioxyde de carbone, sous réserve que la concentration de dioxyde de carbone ne dépasse pas 30 % V/V et que la concentration d’oxygène résiduel ne dépasse pas 2 % V/V, ou

- un minimum de 2 minutes d’exposition à l’argon, l’azote ou d’autres gaz inertes ou tout mélange de ces gaz avec de l’air atmosphérique, avec un maximum de 2 % d’oxygène résiduel V/V, ou

- un minimum de 2 minutes d’exposition à au moins 55 % de dioxyde de carbone dans l’air.

Il ne donne pas de recommandation pour les mises à mort au gaz, l'étourdissement devant rapidement être suivi d'une saignée de l'animal.
Il note que l'utilisation de ces gaz posent des problèmes de souffrance animale pour :
les mélanges CO_2/air/O_2 et les mélanges CO_2/gaz inertes qui ont comme inconvénients une agressivité des fortes concentrations de CO_2 et qu'ils provoquent une détresse respiratoire, quant aux gaz inertes ils sont sources d'une reprise de conscience prématurée.

II. lignes directrices pour l'abattage d'animaux à des fins prophylactiques
annexe 3.7.6.

Mise à mort par un mélange CO2 / air

L’inhalation de dioxyde de carbone (CO_2) induit une acidose respiratoire et métabolique et réduit par conséquent le pH du liquide céphalo-rachidien (LCR) et des neurones, entraînant une perte de conscience et la mort après une exposition prolongée.

Pour les mises à mort par container ou caisson les animaux préconisés sont : les volailles ainsi que pour les ovins, les caprins et les porcs nouveau-nés, Pour le gazage en poulailler les volailles.
Comme inconvénients sont cités une agressivité des fortes concentrations de CO_2, une perte de conscience non immédiate et un risque de suffocation si les animaux sont trop nombreux (pour le caisson uniquement)

Mise à mort par mélanges d’azote ou de gaz inerte avec du CO2

Le CO_2 peut être mélangé en proportions diverses avec de l’azote ou un gaz inerte comme l’argon. L’inhalation de tels mélanges entraîne une hypoxie par hypercapnie et la mort lorsque la concentration d’oxygène est inférieure à 2 % (V/V). Avec cette méthode, il faut introduire les animaux dans un conteneur ou un appareil contenant ces gaz.

Ces mélanges n’entraînent pas de perte de conscience immédiate, de sorte que l’agressivité de certains mélanges gazeux contenant des concentrations élevées de CO_2 et la détresse respiratoire qui s’ensuit pendant la phase d’induction posent de graves problèmes de protection animale.

Chez les porcs et les volailles, les faibles concentrations de CO_2 ne s’avèrent pas très agressives. Aussi peut-on employer des mélanges d’azote ou d’argon contenant moins de 30 % V/V de CO_2 et moins de 2 % (V/V) de O_2 pour la mise à mort des volailles et des ovins, caprins ou porcs nouveau-nés.

animaux : volailles, ovins, caprins et porcs nouveau-nés.

avantages et inconvénients :
Les faibles concentrations de CO_2 sont peu agressives et, associées à l’azote ou à un gaz inerte, elles induisent une perte de conscience rapide.
La perte de conscience n’est pas immédiate et les temps d’exposition requis pour la mise à mort sont considérables.

Mise à mort par mélanges Azote et/ou gaz inertes

Cette méthode consiste à introduire les animaux dans un conteneur ou un appareil contenant de l’azote ou un gaz inerte tel que l’argon. L’atmosphère contrôlée produite conduit à la perte de conscience et à la mort par hypoxie.

Les recherches ont montré que l’hypoxie n’est pas une phase agressive pour les porcs et les volailles et qu’elle n’induit pas de détresse respiratoire avant la perte de conscience.

animaux : volailles, ovins, caprins et porcs nouveau-nés.

avantages et inconvénients :
Les animaux sont incapables de détecter l’azote ou les gaz inertes, et l’induction d’une hypoxie par cette méthode ne constitue pas une phase agressive.

La perte de conscience n’est pas immédiate et les temps d’exposition requis pour la mise à mort sont considérables.

ANNEXE 3.7.5. - LIGNES DIRECTRICES POUR L'ABATTAGE D'ANIMAUX À DES FINS DE CONSOMMATION HUMAINE

Article 3.7.5.7.

Méthodes d'étourdissement

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4 - Étourdissement au gaz

a. Étourdissement des porcs par exposition au dioxyde de carbone (CO2)

La concentration de CO_2 à utiliser pour l'étourdissement doit en principe être de 90 % V/V mais en aucun cas inférieure à 80 %. Après leur pénétration dans la chambre d'étourdissement, les animaux doivent être convoyés jusqu'au point où la concentration gazeuse est maximale et y être maintenus jusqu'à ce qu'ils soient morts ou plongés dans un état d'inconscience persistant jusqu'à la mort par saignée. Dans les conditions idéales, les porcs doivent être exposés à cette concentration de CO_2 pendant 3 minutes.

Quoi qu'il en soit, la concentration gazeuse doit être de nature à réduire autant que possible tout stress avant la perte de conscience.

La chambre d'exposition au CO_2 et le matériel de convoyage doivent être conçus, fabriqués et entretenus de manière à éviter toute blessure ou tout stress inutile aux animaux. La densité des animaux dans la chambre doit être telle que les animaux ne risquent pas de s'entasser les uns sur les autres.

Le convoyeur et la chambre doivent être correctement éclairés pour que les animaux puissent voir autour d'eux et si possible se voir les uns les autres.

Il faut prévoir la possibilité d'inspecter la chambre à CO_2 en cours d'utilisation et d'accéder aux animaux en cas d'urgence.

La chambre sera pourvue d'un dispositif de mesure et d'affichage continu de la concentration de CO_2 au point d'étourdissement et du temps d'exposition. Un signal d'alerte clairement visible et audible devra signaler toute chute de la concentration de CO_2 en dessous de la limite requise.

b. Mélanges de gaz inertes pour l'étourdissement des porcs (à l'étude)

L'inhalation de fortes concentrations de dioxyde de carbone est agressive et peut entraîner une détresse chez les animaux. C'est pourquoi l'utilisation de mélanges gazeux non agressifs est en cours d'étude.

Ces mélanges gazeux comprennent :

- un maximum de 2 % V/V d'oxygène dans de l'argon, de l'azote ou d'autres gaz inertes, ou

- jusqu'à un maximum de 30 % V/V de dioxyde de carbone et un maximum de 2 % V/V d'oxygène dans les mélanges avec du dioxyde de carbone et de l'argon, de l'azote ou d'autres gaz inertes.

Les temps d'exposition aux mélanges gazeux doivent être suffisants pour assurer que les porcs ne reprennent pas conscience avant la mort induite par saignée ou arrêt cardiaque.

c. Étourdissement des volailles au gaz

L'objectif principal de l'étourdissement au gaz est d'éviter la douleur et les souffrances liées à l'entravement des volailles conscientes dans les systèmes d'étourdissements et de mise à mort à bain d'eau. Aussi, l'étourdissement au gaz doit-il être limité aux oiseaux contenus dans des caisses ou placés sur des convoyeurs. Le mélange gazeux doit être non agressif pour les volailles.

L'étourdissement au gaz de volailles dans les conteneurs de transport évite de manipuler les oiseaux vivants à l'abattoir et supprime tous les problèmes liés à l'étourdissement électrique. L'étourdissement au gaz de volailles sur un convoyeur élimine aussi les problèmes liés à l'étourdissement électrique par bain d'eau.

Les volailles vivantes seront amenées dans les mélanges gazeux dans les caisses de transport ou sur des convoyeurs à bande.

Les mélanges gazeux utilisés pour l'étourdissement des volailles comprennent :

- un minimum de 2 minutes d'exposition à 40 % de dioxyde de carbone, 30 % d'oxygène et 30 % d'azote, puis minimum d'une minute d'exposition à 80 % de dioxyde de carbone dans l'air, ou
- un minimum de 2 minutes d'exposition à tout mélange d'argon, d'azote ou d'autres gaz inertes avec de l'air atmosphérique et du dioxyde de carbone, sous réserve que la concentration de dioxyde de carbone ne dépasse pas 30 % V/V et que la concentration d'oxygène résiduel ne dépasse pas 2 % V/V, ou
- un minimum de 2 minutes d'exposition à l'argon, l'azote ou d'autres gaz inertes ou tout mélange de ces gaz avec de l'air atmosphérique, avec un maximum de 2 % d'oxygène résiduel V/V, ou
- un minimum de 2 minutes d'exposition à au moins 55 % de dioxyde de carbone dans l'air.

Les conditions d'efficacité sont les suivantes :

- les gaz comprimés doivent être vaporisés avant d'être injectés dans la chambre ;
- en aucun cas, on ne fera passer dans la chambre des gaz solides se trouvant à leur température de congélation ;
- les mélanges gazeux doivent être humidifiés ;
- les concentrations gazeuses présentes au niveau des oiseaux à l'intérieur de la chambre doivent être affichées et surveillées en permanence.

En aucun cas, il ne faut laisser reprendre conscience à des oiseaux qui ont été exposés à des mélanges gazeux. La durée d'exposition doit si nécessaire être prolongée.

Article 3.7.5.8.

Récapitulatif des méthodes d’étourdissement acceptables et problèmes de bien-être animal associés

Tableau 2. Méthodes d'étourdissement acceptables

Méthode	**Méthode spécifique**	**Préoccupations de bien-être animal**	**Impératifs majeurs de bien-être animal**	**Espèces**	**Commentaires**
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Gazeuse	Mélange CO_2/air/O_2 Mélange CO_2/gaz inerte	Agressivité des fortes concentrations de CO_2, détresse respiratoire, exposition insuffisante	Concentration, durée d’exposition, conception, entretien et fonctionnement du matériel, gestion de la densité des animaux	Porcs et volailles	Les méthodes gazeuses risquent de ne pas convenir à l’abattage halal
	Gaz inertes	Reprise de conscience	Concentration; durée d’exposition, conception, entretien et fonctionnement du matériel, gestion de la densité des animaux	Porcs et volailles	Les méthodes gazeuses risquent de ne pas convenir à l’abattage halal

Carbon Dioxide Use for Euthanasia of Laboratory Animals

This document reviews the current scientific literature on the use of carbon dioxide (pure and various mixtures) for euthanasia of laboratory animal species. It outlines the various issues that Victorian Animal Ethics Committees should consider when making recommendations to their institutions.

Authors: Dayna Johnson, Kate Blaszak: Bureau of Animal Welfare, DPI Victoria
Date: October 2005

Summary:

The literature on the use of carbon dioxide for inhalation euthanasia of laboratory animals can be considered separately for rodents and poultry. It discusses measurement of aversion to carbon dioxide at different concentrations and with different methods of use, the importance of user training and the variation amongst existing international guidelines. Recent literature recommends the use of halothane for rats and enflurane for mice as the most humane methods of inhalation euthanasia. For chickens a mixture of 60 % argon and 30% carbon dioxide mixture in air is considered to be the most humane method of inhalation euthanasia.

The Victorian Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits states that euthanasia by inhalation for rats and mice using carbon dioxide is an 'acceptable' method. The use of halothane, isoflurane and methoxyflurane is stated as 'acceptable with reservations'. The reservations are based on occupational health and safety considerations that can be overcome with the use of appropriate scavenging equipment. Based on the literature, the promotion of the use of these inhalation agents as the preferred method of euthanasia is advisable.

A table of recommendations of various euthanasia methods for adult rodents and experimental needs based on existing literature is provided. The literature also indicates that gaseous euthanasia in the home cage amongst familiar conspecifics has a measurable welfare benefit.

Introduction

Carbon dioxide is commonly used to euthanase adult rats, mice and chickens in the laboratory setting, the focus species of this review. Its use is a subject of debate within the animal research community. Human studies report that carbon dioxide inhalation is adversive, with a linear relationship between concentration and distress and/or pain sensation at concentrations ranging from 7-100%(HSUS 2002). This has raised concern as to the humaneness of the use of carbon dioxide for animals, which has been the subject of numerous studies and recent literature reviews.

Scientific Literature On The Use and Effects Of Carbon Dioxide

Reviewing the actual physiological mechanism of excessive carbon dioxide helps to understand the associated clinical signs, which may vary between species and individuals to some degree. The primary mechanism of anaesthesia and death is the direct action of carbon dioxide on vital systems (Pritchett et al, 2005). Simplified, the gas molecule essentially diffuses into the blood, body and brain from the lungs. Faced with excessive levels of the gas, the inherent capacity of the blood to buffer for carbon dioxide is overwhelmed and results in acidosis (the lowering of the pH of the blood and associated fluids). Low to moderate concentrations of carbon dioxide (ranging from 5-35%, Conlee et al, 2005) cause mild respiratory acidosis leading to a compensatory increase in depth and rate of respiration in an effort to 'blow off' the excess carbon dioxide (hyperventilation), changes in heart rate and blood pressure. Higher concentrations then lead to more profound respiratory acidosis, suppressing the respiratory centres of the brain leading to a slow, gasping respiratory pattern. Without the buffering capacity of blood, the pH of the cerebrospinal fluid (CSF) drops precipitously which is directly related to anaesthetic depth and subsequent insensibility to pain, stupor and finally unconsciousness and death. In addition, another mechanism is the acidosis-induced depression of heart muscle, precipitating heart arrhythmias and failure.

Of welfare interest, however, is the duration and effect prior to insensibility and unconsciousness (ie. anaesthesia). A number of articles refer not only to the sensation and signs of respiratory distress and asphyxia (including some histological indications of a state similar to conscious drowning) but also to the acidification of mucous membranes (eg. eyes, mouth, respiratory). The particular sensory capacity of nasal mucosa associated with degrees of discomfort and pain is reported in humans and animals (Conlee et al 2005).

The literature on the use of carbon dioxide in rats and mice differs to that of chickens due to behavioural differences between rodents and chickens and also the methods of killing of poultry for food production. Methodological limitations create the tendency to compare various inhalation agents, rather than to compare other methods of euthanasia or aspects of the euthanasia process. The earlier studies involving rodents vary in the methods used to assess welfare whereas the most recent and vigorous studies include exploratory behaviour in order to gain insights into the least aversive agents.

In comparison the literature on chicken euthanasia is more extensive due to their use as a food producing species. The nature of the research is mostly applicable to the food industry but still useful when considering the laboratory setting. This literature has moved beyond behavioural indicators, which indicated that carbon dioxide is aversive. It currently focuses on the effectiveness of less aversive agents and mixtures using spontaneous electroencephalograms (EEGs) and somatosensory evoked potentials (SEPs) as indicators of levels of consciousness and the establishment of death.

Rodents

Rodent studies have investigated behavioural, physiological and histological changes associated with carbon dioxide use. They have examined the effects of different concentrations, adding oxygen and the use of pre-filled chambers versus gradual induction. Observations that may be consistent with pain and/or distress that have been reported with carbon dioxide use include: increased locomotion, excitation and serious agitation, increased rearing, defecation and urination, irritation of mucosal membranes (including frequent washing), hyperventilation, gasping and 'heads turned upwards and backwards'. These signs generally increase in frequency and intensity with the increase in concentration of carbon dioxide from 25-100%, until anaesthesia is induced. Studies considering histological effects are comprehensively reviewed in Conlee et al, 2005.

Recent literature on rats and mice concludes that carbon dioxide is the least preferred inhalation agent when compared to halothane, isoflurane, enflurane, desflurane, sevoflurane and argon. These species show aversion to carbon dioxide at concentrations high enough to cause a loss of consciousness. Variations in use including humidification, mixture with argon or oxygen, the use of pre-filled chambers versus gradual increase in concentration all failed to remove the aversive effects (Leach et al 2002a; Leach et al 2002b). The degree of averseness of the previously mentioned inhalation agents was established by evaluation of the willingness of rodents to enter and dwell in chambers containing these agents. The only agents the authors concluded were non aversive are halothane and sevoflurane for rats. All of these inhalation agents had some degree of aversion in the case of mice. The authors concluded (Leach & Morton 2004):

> 'Therefore, based on the findings of these studies the recommended anaesthetic agent for rats is halothane and for mice is enflurane as at appropriate concentrations they induced a rapid and effective induction with the minimum of distress. The recommended method of euthanasia using a single agent would be argon. However, inducing unconsciousness with a volatile liquid anaesthetic (eg halothane and enflurane) and subsequently rapidly killing with carbon dioxide after the animals are unconscious can be considered more humane than argon alone. As once an animal is unconscious then exposure to carbon dioxide, which is an effective killing agent, is not a welfare issue.'

Although outside the scope of this report, the impact on animal welfare by the manner in which inhalation agents are used is deserving of attention. There is evidence to suggest that the use of the home cage and inclusion of a familiar companion animal may offer a significant welfare advantage during the euthanasia process (Maguire & Arthur 2003). This is consistent with the broader literature on stress in animals. The use of telemetry and new technologies may see increased research specifically investigating these sorts of parameters during the euthanasia process (Williams 2004).

<u>Chickens</u>

The specialised anatomy and physiology of the avian respiratory system, which is highly adapted for efficient gaseous exchange, makes poultry extremely sensitive to inhaled gases and therefore makes it vital that inhalation methods are demonstrated to be humane. Carbon dioxide in older and adult birds is currently still a commonly used technique for the euthanasia of large numbers of poultry, although not permitted in UK slaughterhouses since 1995 (NCCAW 2005). There is substantial recent international review of controlled atmospheric stunning (CAS) techniques, a commercial killing practice of chickens which provides an alternative to the most common process involving electrical stunning or the use of carbon dioxide alone. There are a number of types of gas mixtures that stun birds by different mechanisms involving various combinations of carbon dioxide, inert gases (Argon or Nitrogen) and oxygen or air, and the mixtures must be carefully controlled. Choosing between these mixtures is arguably one of the most contentious questions about CAS (Wathes 2004).

The use of EEG and SEP technology has provided evidence to suggest that carbon dioxide inhalation is more aversive to chickens than argon and argon-carbon dioxide mixes (Raj et al 1998). The use of 90% argon in air results in greater time to isoelectric EEG and cessation of SEPs consistent with loss of consciousness and death compared to 60% argon and 30% carbon dioxide in air. Residual oxygen levels when euthanasing chickens with 90% argon in air are critical as levels of greater than 2% oxygen can increase the time to death and decrease mortality rates substantially (Raj & Whittington 1995). This critical effect of residual oxygen does not occur in the 60% argon and 30% carbon dioxide in air mixture when euthanasing batches of chickens (Raj et al 1992).

There are however, still some differing opinions on the interpretation of some welfare indicators of inhalation euthanasia of chickens. Most experts and recent studies consider specific behaviours such as gasping, head shaking, wing flapping, defecation as indicators of aversion, but some still consider them autonomous (involuntary) responses. Further research results are pending. It is also hoped that a new statistical technique, validated in human anaesthesia may provide further information of the complex EEG wave and its extent to which it indicates level of consciousness (Wathes 2005).

Guidelines, Codes and Scientific Review Bodies

The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes advises that decisions regarding the animal's welfare must be based on the assumption that where pain and distress cannot be easily evaluated in animals, it must be assumed that animals experience pain in a similar manner to humans unless there is evidence to the contrary. This principle is in line with the U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals. In light of these policies and the reviewed body of scientific literature, various international guidelines would appear to be in conflict on the use of carbon dioxide as a sole euthanasia agent as follows:

The 2001 Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART) guidelines on the Euthanasia of Animals for Scientific Purposes recommends the use of carbon dioxide with specialised equipment for rodents, preferring gradual induction but acknowledging and accepting the variation in information and thus methodology of carbon dioxide use. Based on these guidelines, the Australian Veterinary Association (AVA) policy also currently 'approves' such carbon dioxide application. The American Veterinary Medical Association (AVMA) 2000 Panel Report on Euthanasia recommends unreferenced) 70% CO2 in a pre-filled chamber for the euthanasia of rodents, similar to a 1996 European Commission report. The UK Home Office Code of Practice (1997), which is currently being reviewed, indicates that carbon dioxide is appropriate only for rodents, rabbits and birds up to 1.5 kg and recommends exposure to a rising concentration. The UK University Federation of Animal Welfare (UFAW) and Canadian Council for Animal Care (CCAC) acknowledge that neither slow nor swift carbon dioxide induction is stress-free (HSUS 2002). One author comments on behalf of 2 independent scientific review committees (Morton,2005):

> 'the overwhelming consensus of published work in animals including humans shows clear evidence of aversion. This is the view arrived at by both the EU European Food Standards Authority's Scientific Panel on Animal Health and Welfare as well as the UK's Farm Animal Welfare Council'.

McDonald's Animal Welfare Team have also recently considered the scientific literature concerning the existing methods of stunning and slaughter of poultry and is moving towards the use of controlled atmospheres using inert gases (McDonald's 2005). The (Australian) National Consultative Committee for Animal Welfare reviewed methods of poultry euthanasia in 2005. This report acknowledges that there is an increasing discrepancy between the more recent scientific literature and some international policies and recommendations (NCCAW 2005).

Conclusions:

The evidence of distress and aversive responses associated with the use of carbon dioxide as an anaesthetic agent or as a sole agent for the euthanasia of laboratory animals is considered conclusive by a number of independent review bodies and recent scientific studies. Leach et al (2004) specifically conclude that exposing rats and mice to carbon dioxide in any form, either for anaesthesia or for euthanasia, is likely to cause considerable pain and distress and is therefore unacceptable when efficient and more humane alternatives are readily available.

It is recommended that carbon dioxide be coupled with inhalant pre-anaesthetic gases, such as isoflurane, halothane or methoxyflurane if used for euthanasia. The use of other gaseous combinations for euthanasia of rodents or chickens, as discussed, are also preferred over the sole use of carbon dioxide. It should be noted that argon/oxygen/air and various argon/carbon dioxide gas mixtures are commercially available from BOC Gases Ltd.

Proper training of personnel in all techniques involving euthanasia is also fundamental. Importantly the welfare of rodents and chickens undergoing inhalation euthanasia could be further enhanced by drafting institutional recommendations as to what constitutes preferred practical procedure(s).

In summary, it is recommended that the scientific community carefully reconsider the routine use of carbon dioxide as a sole agent for anaesthesia or euthanasia and adopt these more humane practices wherever possible.

……………………………………………………

Table comparing recommendations for various euthanasia methods for rodents, based on existing literature (HSUS 2002, Conlee et al 2005).Note this table does not include the use of injectable anaesthetics or barbiturates for euthanasia which are considered acceptable by the Victorian Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits.

	CO2 alone	*Pre-anaesthetic &Co2	Argon/O2	**Decapitation	Cervical Dislocation
Large numbers surplus rodents	No	Yes	Yes	No	No
Few-moderate numbers of rodents on experiments	No	Yes	Yes	No	Yes***
Few-moderate numbers of rodents where no contamination is permitted	No	No	Maybe	When Justified	Yes***

*Pre-anaesthetic refers to the gaseous agents, as mentioned in the conclusion, with appropriate scavenging equipment.

** Decapitation produces a quicker loss of consciousness (3-6 seconds) than sole carbon dioxide use. With regard to the adverse impact on the animal, however, the entire process from handling to execution must be considered. This method may be considered acceptable to be carried out by trained personnel for a small number of small rodents and chickens where other resources are currently unavailable (Conlee et al, 2005).

*** Authors' note: The original table does not recommend cervical dislocation for any category. There is limited published information on cervical dislocation, but it is accepted that this method does not induce instantaneous unconsciousness and is very reliant on operator technique and skill (Gregory and Wotton, 1990). In combination with this discussion and the Victorian Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits, cervical dislocation may be considered acceptable for small numbers of rodents less than 150 grams conducted by a skilled operator.

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Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals[1]

(Question N° EFSA-Q-2003-093)

Adopted on the 15th of June 2004

SUMMARY OF OPINION

The EFSA Scientific Panel on Animal Health and Welfare was asked by the Commission services to report on the welfare aspects of the main systems of stunning and killing in the main commercial species of animals with consideration of Directive 93/119/EC. Species referred to in the present opinion are: cattle, sheep, pigs, poultry, horses and farmed fish. Welfare aspects of the systems for stunning other species, such as rabbits, deer, ratites or goats, have not been included in the present opinion.

Stunning before slaughter is a statutory requirement in the EU (with exceptions in some Member States for religious slaughter) to induce unconsciousness and insensibility (inability to perceive stimuli) in animals, so that slaughter can be performed without avoidable fear, anxiety, pain, suffering and distress.

This Scientific opinion is a scientific assessment of the welfare during stunning and killing adopted by the EFSA AHAW Panel based on the data of the Scientific Report. In drafting this Scientific Opinion, the panel did not consider ethical, socio-economic, cultural or religious aspects of this topic. Considering the mandate, the present opinion concentrates on the welfare of the animals concerned at the point of application of the stunning and stun / killing techniques and does not consider in detail other preceding or subsequent procedures, although it is recognised that, for instance, transport to the slaughterhouse, lairage conditions, pre-slaughter handling and restraint prior to stunning may cause serious welfare problems. Scientific data on other issues such as food safety, BSE (bovine spongiform encephalopathy), human operator safety, economic impact are not reviewed in this opinion.

This opinion considers the main stunning and stun / killing methods under commercial slaughterhouse and under farm conditions in Europe. Killing of animals without stunning and stun / killing methods for disease control are also considered.

Stunning methods induce temporary loss of consciousness and rely solely on prompt and accurate sticking procedures to facilitate bleeding and to cause death. Sticking involves the severing of major blood vessels e.g. neck cutting or chest sticking. If unbled, even the adequately stunned animal has a potential to regain brain and body functions. Stun / killing methods induce unconsciousness and death either simultaneously or sequentially.

Procedures appropriate to cattle, sheep, pigs, chickens, turkeys, farmed fish and horses and their related minimum requirements such that unconsciousness and insensibility are induced and poor welfare is minimised, are recommended.

[1] For citation purposes: Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals, *The EFSA Journal* (2004), 45, 1-29

An understanding of the states of unconsciousness and insensibility and the measures to assess these permit evaluation of the effectiveness of the different methods applied. Efficient stunning methods disrupt the neurons or neurotransmitter regulatory mechanisms in the brain, causing a long-lasting depolarised neuronal state that renders animals unconscious and insensible. Indeed, most of the known or established stunning methods also induce high degrees of electrical synchronisation in the brain leading to a quiescent or isoelectric electroencephalogram. During and immediately after stunning, depending on the method and species involved, animals show typical behaviour patterns and physical reflexes, which can help to monitor the effectiveness of stunning under commercial conditions. In general, vocalisation in animals during the induction of unconsciousness with any stunning method is indicative of pain or suffering (however, absence of vocalisation does not guarantee absence of pain or suffering). Under practical conditions, eye reflexes and reactions to painful stimuli should always be investigated and evaluated, in combination with the resumption of normal rhythmic breathing and righting reflexes, to assess stunning effectiveness.

The duration of unconsciousness and insensibility varies between methods, species and animals. The stun-stick interval should be sufficiently short to induce death through blood loss before the animal recovers from the stun. Sticking procedures vary between species; however, the supply of oxygenated blood to the brain should be stopped as rapidly as possible.

Stun / killing methods, which induce unconsciousness and death either simultaneously or sequentially, do not rely on bleeding to cause death and therefore should be preferred when available and proven to be effective.

In all the stunning and stun / killing methods (excluding gas mixtures), animals should be restrained appropriately and heads properly presented to the operator for effective application of the procedure(s).

Due to the serious animal welfare concerns associated with slaughter without stunning, pre-cut stunning should always be performed.

As a general rule, each method should be applied only once, i.e. animals should be rendered unconscious and insensible by a stunning or stun / killing method or device applied for the first time. In the event of a failure (unsuccessful stun), the animal should be killed immediately by an appropriate backup killing method.

It is important that all operators involved with stunning and slaughter are competent, properly trained and have a positive attitude towards the welfare of the animals.

All the equipment used for stunning or stun / killing should be maintained in good working conditions and recorded evidence of parameters applied, maintenance and rectified defects should be kept.

There are no ideal methods for the stunning and killing of farm animals for commercial slaughter or disease control purposes and it is therefore necessary to select those procedures whose proper application have most advantages in terms of animal welfare. Bad practice increases the disadvantages of any method.

The penetrating captive bolt, if applied properly, can render sheep and cattle insensible with minimal effects on welfare. Captive bolt usage is appropriate for some pigs, but there can be problems if it is used for boars and old sows. Captive bolt has the disadvantage that there is no automated method for practical use available today and depends essentially on the education and skill of the person who performs the stunning.

Gas stunning has a high potential for humane stunning or stun / killing if non-aversive gases or gas mixtures are used. It requires sophisticated technical equipment. The animals are exposed to a moderate handling stress only.

Electrical stunning can immediately cause unconsciousness and makes the animal insensible. It requires high standards of technical equipment and skilled people to perform and monitor the stun and a system to record the stunning details such as voltage, current and frequency of the current for each individual stun. For automated applications the animal has to be restrained. There is still a lack of knowledge about mechanisms of brain function during application of electrical currents to the head.

There is an urgent need for further detailed investigations of the mechanisms and effects of the different stunning methods, their technical and organisational performance in practice and improved and continuing education of the staff to ensure good animal welfare.

Key words : cattle, sheep, pigs, poultry, horses, fish, animal welfare, stunning, killing, slaughterhouses, disease control, consciousness, mechanical stunning methods, mechanical stun / killing methods, electrical stunning methods, electrical stun / killing methods, gas stunning methods, gas stun / killing methods, controlled atmospheres, waterjet stun / killing method, microwave irradiation, needle bolts, percussive stunning, mechanical spiking, asphyxia, thermal shock, salt bath, ammonia solution, decapitation, exsanguinations, anaesthesia, slow live chilling, shooting, electric harpoon, barbituric acid derivates, T61, chloral hydrate, magnesium sulphate, potassium chloride, biosecurity.

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BACKGROUND

The EFSA Scientific Panel on Animal Health and Welfare (AHAW) was asked by the Commission services to report on the welfare aspects of the main systems of stunning and killing the main commercial species of animals with consideration of Directive 93/119/EC.

The mandate was accepted by the AHAW Panel at the first Plenary meeting, in 2003. It was decided to establish a Working Group of AHAW experts chaired by one Panel member. Therefore the Panel entrusted a Scientific Report to a working group under the Chairmanship of Dr. H. Blokhuis.

This opinion has been adopted by the Plenary Meeting of the AHAW Panel on the 15th of June 2004 and the relevant conclusions and recommendations are based on the Scientific Report separately published on the EFSA web site www.efsa.eu.int, which was drafted by the Working Group and accepted by the Panel.

TERMS OF REFERENCE

The EFSA Panel on Animal Health and Welfare was asked to report on the welfare aspects of the main systems of stunning and killing the main commercial species of animals.

For each stunning and killing method commonly used, the following three areas are covered:

- The minimal conditions by which the method is likely to be efficient from the animal welfare point of view in field conditions,
- The criteria or procedures to check that the stunning and the killing method is properly used,

- The advantages and disadvantages of the method used, taking into account the commercial and field conditions.

Two separate contexts are considered: stunning and killing methods used in slaughterhouses and those used for disease control measures.

Species referred to in the present opinion are: cattle, sheep, pigs, poultry, horses and farmed fish. Welfare aspects of the systems for stunning other species have not been included in the present opinion.

ASSESSMENT

A full assessment can be found in the Scientific Report published in the EFSA web site www.efsa.eu.int, which was drafted by a Working Group set up by the AHAW Panel. The Scientific Report is considered as the basis for the discussion to establish the conclusions and recommendations by the AHAW Panel, as expressed in this opinion.

CONCLUSIONS AND RECOMMENDATIONS

The Scientific Panel on Animal Health and Welfare concludes on the welfare aspects of the main systems of stunning and killing the main commercial species of animals as follows:

1. GENERAL

1.1. CONCLUSIONS

Most animals which are slaughtered in the EU for human consumption are killed by cutting major blood vessels in the neck or thorax so that rapid blood loss occurs. If not stunned, the animal becomes unconscious only after a certain degree of blood loss has occurred whilst after greater blood loss, death will ensue. The animals which are slaughtered have systems for detecting and feeling pain and, as a result of the cut and the blood loss, if not stunned, their welfare will be poor because of pain, fear and other adverse effects. The cuts which are used in order that rapid bleeding occurs involve substantial tissue damage in areas well supplied with pain receptors. The rapid decrease in blood pressure which follows the blood loss is readily detected by the conscious animal and elicits fear and panic. Poor welfare also results when conscious animals inhale blood because of bleeding into the trachea. Without stunning, the time between cutting through the major blood vessels and insensibility, as deduced from behavioural and brain response, is up to 20 seconds in sheep, up to 25 seconds in pigs, up to 2 minutes in cattle, up to $2^1/_2$ or more minutes in poultry, and sometimes 15 minutes or more in fish.

In general, stunning methods induce temporary loss of consciousness and rely on prompt and accurate sticking procedures (bleeding out) to cause death. The duration of unconsciousness and insensibility varies between methods, species and animals.

Effective stun / kill methods on the other hand, which induce unconsciousness and death either simultaneously or sequentially, do not rely on bleeding to cause death.

Restraint of animals, needed to ensure proper application of mechanical or electrical stunning or stun / killing methods, can be one of the most stressful and painful stages of the slaughtering process. Therefore, the ability to move animals in groups with less handling and restraint is an advantage on welfare grounds of all gas stunning or stun / killing systems as compared with mechanical or electrical methods.

While carbon dioxide (CO_2) has many advantages, aversion (a tendency to show behaviour to avoid or withdraw from a situation which is associated with a noxious stimulus) to this gas at some level (usually above 20%) is clearly a welfare problem. Depending on how one interprets an animal's behaviour it is difficult to quote a level from the published work that will apply to all pigs and poultry. However, it is likely that levels above 30% in pigs and turkeys and 25% in chickens are at the very least unpleasant and that higher levels are aversive.

1.2. RECOMMENDATIONS

Due to the serious animal welfare concerns associated with slaughter without stunning, all animals which are slaughtered should be adequately stunned in a humane way, whenever possible, so as to avoid poor welfare in the period before unconsciousness ensues. Effective stun / killing methods, when available and reliable, are preferred from an animal welfare point of view.

In all the stunning and stun / killing methods, animals should be restrained appropriately and heads properly presented to the operator for effective application of procedure(s) (excluding gas mixtures).

The stun-stick interval should be sufficiently short to induce death through blood loss before the animal recovers from the stun.

Sticking procedures vary between species. However, supply of oxygenated blood to the brain should be stopped as rapidly as possible.

No carcass processing or electrical stimulation to improve meat quality should commence until the animal is dead.

All operators involved with stunning and slaughter should be properly trained, their skills and knowledge examined, in particular in the field of welfare, and the person should be certified to be competent and should have a positive attitude towards improving animal welfare. They should also attend retraining courses and their ability to implement new knowledge and acquire new skills should be assessed as new technologies evolve.

All the equipment used for stunning or stun / killing should be maintained in good working conditions. Recorded evidence of maintenance and rectified defects should be kept.

1.2.1. Mechanical methods

When using captive bolt guns, colour codes indicating cartridge strength should be harmonised across manufacturers. Colour codes should be the same for the same species and age group (e.g. red for cows and horses, black for bulls).

Open cartridges should not be used as they can easily absorb moisture and lose their function. All captive bolt equipment, including cartridges, should be stored in appropriate conditions in abattoirs.

Bolt velocity should be measured regularly according to the manufacturers' specifications and appropriate field devices made available to ensure proper use in the field.

1.2.2. Electrical methods

All stunning and stun / kill electrical parameters should be based on sound science.

Electrical stunning tongs should be placed on the head such that they span the brain. Electrical stun / killing tongs (one cycle method) should be placed on the head and body such that they span the brain and the heart.

Electrical stunning and stun / killing devices should supply constant currents and should also be fitted with an acoustic or optic signal to indicate: (a) an interrupted stun, (b) excessively short stun duration or (c) increase in total electrical resistance in the pathway (due to dirt, fleece or carbonisation), which could lead to failure. This would facilitate effective monitoring of electrical stunning and stun / killing methods under commercial conditions. Electrical stunning and stun / kill devices should indicate that the recommended voltage and current have been delivered during the stun or signal if this is not the case. The voltage and current measuring devices should be appropriate to the waveform and frequency of the current used in the stunner. A calibrated volt and/or current meter appropriate to the waveform and frequency of the current should be used to verify the output of the stunner. The sampling rate of the meter needs to be fast enough and appropriate to the electrical parameters.

The details of electrical stunning parameters, such as waveform, frequency and the output voltage and current in appropriate units (average or root mean square) need to be recorded and readily available for internal or external audit and to verify that correct parameters are applied, thus ensuring that a current of sufficient magnitude beyond that recommended to induce generalised epilepsy is applied.

Monitoring of electrical stunning and stun / killing efficiency should be improved by evaluating the stunners in designated laboratories, using established neurophysiological criteria, prior to installation and delivery of a certificate (kite mark).

1.2.3. Gas methods

Gas concentrations and exposure times need to be monitored and records kept. They should be readily available for internal or external audit and to verify that the recommended methods are used.

1.2.4. Backup stunning methods

As a general rule, each method should be applied only once, i.e. animals should be rendered unconscious and insensible by a stunning or stun / killing method or device applied for the first time. In the event of a failure (unsuccessful stun), the animal should be killed immediately by an appropriate backup killing method. Two consecutive failures to stun with any specific device should warrant immediate investigation and the fault should be rectified before starting stunning procedures again.

1.3. FUTURE RESEARCH

1.3.1. High research priorities

a) Restraint systems

For both mechanical and electrical stunning or stun / killing methods, there is an urgent need to develop appropriate restraint systems.

b) Mechanical methods

A field tool to measure the velocity and power of the **penetrating captive bolt** under practical conditions should be developed. Such a device should be available for all captive bolt guns.

The appropriate length, diameter, shape and velocity of the penetrating captive bolt to be used for stunning or killing should be determined for each species to ensure immediate onset of unconsciousness or death.

c) Electrical methods

There is an urgent need to revise and scrutinise the electrical methods. The interactive effects of various electrical parameters on onset and duration of unconsciousness and insensibility (current strength, duration, wave forms and frequency) should be determined for the different species, using neurophysiological evidence (electroencephalogram and evoked potentials) rather than induction of seizures. It is necessary, for welfare reasons, to make sure that the total electrical current which is applied reaches immediately the respective centres of the brain to perform a proper and immediate stun. The timing of sticking techniques should also be incorporated in these investigations.

For control purposes, there is a need to develop monitoring systems to register all relevant electrical variables such as strength of current, voltage and frequency, under practical conditions.

Successful induction of cardiac ventricular fibrillation during electrical stun / killing would depend upon the delivery of sufficient electrical current to the myocardium. The amount of current delivered will depend upon the voltage and total impedance in the pathway (between the electrodes). Research should be carried out to determine the effects of such variables during the induction of cardiac ventricular fibrillation.

d) Gas methods

Aversion to gas mixtures and the mental state of animals during the induction of unconsciousness with gas mixtures need further evaluation to develop humane mixtures and to facilitate better understanding and determination of suffering in animals.

1.3.2. Other future research

Research is needed to reveal the diversity of spinal reflexes and spinal automatisms and the times they occur after the application of stun / killing methods in animals.

Methods to establish times of "sensibility" post stunning or at slaughter in relation to the time of onset of the full effects of "sticking" need to be studied. Development of a chest sticking technique simultaneously cutting skin and vessels would make it easier and more rapid. The differential effects of severing the external jugular veins alone or in conjunction with the common carotid arteries as part of the sticking procedure in relevant species need to be investigated.

The implications for good animal welfare of the most efficient methods for achieving rapid exsanguination should be established.

Systems of killing "fallen animals" that facilitate sampling the brain stem for subsequent testing for TSEs (Transmissible Spongiform Encephalopathies) should be investigated for cattle and sheep.

a) Mechanical methods

The **non-penetrating captive bolt** is considered unreliable and consequently is not currently used in most abattoirs and needs extensive modification if efficiency is to be improved. Non-penetrating captive bolt stunning, which is ineffective in its current form, might be improved by changing shape of the bolt, force of the impact, in relation to skull characteristics (e.g. to avoid bone crushing) and a combination of different stunning methods. The possibility and efficiency of applying a rapid killing method (possible chest sticking) after the non-penetrating stun should be studied

b) Combined methods

Research and development so far have focused on using a single method or procedure to stun / kill animals. Each of them has their own animal welfare advantages and disadvantages. The use of a combination of established or novel methods to stun / kill animals need to be evaluated. When investigating such combinations, their practicability should be kept in mind.

2. METHODS FOR STUNNING AND STUN / KILLING CATTLE

Two main methods exist to stun adult cattle and calves: mechanical stunning (captive bolt) and electrical stunning. In captive bolt stunning of adult cattle and calves, the penetrating captive bolt is the most commonly used method.

2.1. MECHANICAL STUNNING METHODS

2.1.1. Conclusions

Penetrating captive bolt stunning has several animal welfare advantages over non-penetrating captive bolt stunning (success rate, duration of unconsciousness) and, if properly used, results in an effective stun. However, field observations indicate that 4% of stuns can be improper, often due to insufficient head restraint, wrong position of the operator, inadequate maintenance of the gun or bad quality of the cartridges.

Insufficient data are available on the effectiveness of **non-penetrating captive bolt** stunning in different age/weight groups of animals. It is however unlikely that the existing method is suitable for all types of cattle, because of varying characteristics of the skull depending on breed, age and sex (different skull shapes, local deformation of skulls in young calves, inadequate concussion in mature bulls).

From an animal welfare point of view, the best method currently available for stunning cattle and calves is the **penetrating captive bolt**.

2.1.2. Recommendations

The penetrating captive bolt should be used for cattle and calves. The **non-penetrating captive bolt** stunning method available at present is unreliable and should not be used.

2.2. ELECTRICAL STUNNING OR STUN / KILLING METHODS

2.2.1. Conclusions

Electrical stunning can be applied manually to young calves. If the system is properly maintained and used, unconsciousness can be reliably induced. It also can be manually applied to adult cattle that are calm or restrained.

Electrically induced head-only stuns may not last long enough to allow normal killing by bleeding. Cardiac ventricular fibrillation or immediate sticking while the animal is restrained will prevent recovery during bleeding (electrical stun / killing). The heart can resume normal functioning if an animal is manipulated too soon after application of the stun / killing method.

Electrical stunning and stun / killing induces tonic / clonic seizures, making prompt and accurate sticking difficult. In some parts of the world, electro-immobilisation (low voltage spinal discharge) is applied following the stun to prevent the movements. However, electro-immobilisation will mask the signs of consciousness in inadequately and poorly stunned animals and will cause pain to such animals. Alternative systems exist which allow electrical stunning and immediate sticking within the restraining pen before development of tonic / clonic seizures.

2.2.2. Recommendations

Manual application of electrical stunning may be used with very low throughput rates. For less calm animals or at higher throughput rates, automated systems should be used.

For head-only electrical stunning, a minimum current of 1 second, > 1.28 A[2] (200 V[3], 50 Hz[4]) can be used to effectively stun adult cattle, and 1 second, 1.25 A (150 V, 50 Hz) to stun calves (6 months) when applied on the temporal region of the skull.

Either animals have to be chest stuck within 23 seconds (adult cattle) or 12 seconds (calves), or ventricular fibrillation has to be induced.

In adult cattle, ventricular fibrillation can be induced in an automatic stunning system by a head-brisket discharge (5 seconds, 1.5 A (175 V, 50 Hz)) or by placing manually electrodes across the chest (25 seconds, 1.8-2.8 A or 5-10 seconds, 2.3-3.5 A (250 V, 50 Hz)).

In calves, ventricular fibrillation can be induced using withers-to-back (1-2 seconds, current not reported, 600 V, 50 Hz), head-to-back (5 seconds, 0.9 A, 300 V, 50 Hz) or head-to-leg (5 seconds, 0.5-2.0 A, 400 V, 50 Hz) application of electrical current.

Manipulation of the carcasses for hoisting and sticking should be delayed for 30 to 60 seconds to avoid that the heart resumes normal functioning.

2.2.3. High research priorities

More information is needed on electrical parameters (duration of application, current type and strength) and electrode placements to be used to induce loss of consciousness and cardiac fibrillation in adult cattle and calves.

The depth and duration of unconsciousness and insensibility induced with various electrical parameters need to be clearly established in cattle. The return of possible signs of consciousness such as breathing in adult cattle subjected to electrical stun / killing needs further investigation in order to determine whether they do indicate return of consciousness and/or sensitivity. If so, the method should be improved to ensure unconsciousness until death.

There is a need to develop systems allowing sticking in an electrical stunning box to allow immediate sticking after stunning, before clonic convulsions start. As the animals would lose blood very quickly, cardiac ventricular fibrillation or gentle handling after a stun / killing procedure would be unnecessary.

3. METHODS FOR STUNNING AND STUN / KILLING SHEEP

Mechanical methods (penetrating captive-bolt) and electrical methods are the most common methods for stunning sheep under slaughterhouse conditions.

3.1. Mechanical stunning methods

3.1.1. Conclusions

The use of the penetrating captive-bolt leads to an immediate loss of consciousness, when the devices are properly maintained and used in properly restrained animals.

The effectiveness of the **non-penetrating captive bolt** under slaughterhouse conditions is not known.

3.1.2. Recommendations

The penetrating captive-bolt should be used for stunning sheep under slaughterhouse conditions. **Non-penetrating captive bolts** should not be used because no investigations exist for adult sheep to prove that it is suitable for them.

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[2] A : ampere
[3] V : volts
[4] Hz : hertz

It is important to sever both common carotids to facilitate rapid bleeding and shorten time to death, and therefore to reduce the likelihood of recovering consciousness before death.

3.1.3. High research priorities

Methods for restraining single sheep with minimal stress to the animal prior to the use of the penetrating captive bolt should be developed.

3.2. Electrical stunning or stun / killing methods

3.2.1. Conclusions

Electrical resistance in sheep varies according to age, breed, and the extent of wool cover.

Electrical stunning leads to an immediate loss of consciousness when the devices are properly maintained and used in properly restrained animals. Electrical stunning of unrestrained sheep in a pen can cause incomplete stunning or painful electric shocks and thus poor welfare.

Electrical stunning in sheep has the disadvantage that maintenance of good electrical contact is not easy due to the small size of the animal's head and insulation resulting from the wool. Poor electrical contact with consequent carbonizing of electrodes or wool would increase electrical resistance of the stunning electrodes. Electrodes fitted with pins penetrate the wool cover better and help to achieve more effective stunning. Wetting of electrodes or wool at the site of tong placement helps to reduce electrical resistance.

3.2.2. Recommendations

Head-only electrical stunning should be induced using a minimum of 1.0 A (root mean square or average) during a minimum of 2 seconds on restrained sheep only, sticking should then be performed within 8 seconds.

Head-to-back stun / killing (one cycle method) should only be used in a restrainer. For effective use, a minimum of 1.0 A (AC[5] 50 Hz) for a minimum of 3 seconds should be applied. Sticking should then be performed without unnecessary delay.

The two cycle stun / killing method (for killing under disease control situation) should be performed using a minimum of 1.0 A (AC 50Hz) for a minimum of 2 seconds for the first cycle, and 4.0 seconds for the second cycle.

In animals with wool at the site of tong placement, electrodes should be fitted with pins or wetted to reduce electrical resistance.

3.3.3. High research priorities

To improve electrical stunning under slaughterhouse conditions, methods to maintain good electrical contact and low-stress devices for restraining single sheep should be developed. The effect of water used for wetting the fleece on stunning effectiveness is not fully understood.

[5] AC: alternating current

4. METHODS FOR STUNNING AND STUN / KILLING PIGS

Two main methods exist to stun or stun / kill pigs: electrical stunning or stun / killing and gas stunning with CO_2. Electrical stunning or stun / killing is the most commonly used method in Europe, but as many pigs are stunned with electricity as with CO_2.

4.1. MECHANICAL STUNNING METHODS

4.1.1. Conclusions

Only **penetrating captive bolts** can be used on pigs (**non-penetrating bolts** are not used). Stunning of pigs under slaughterhouse conditions with a penetrating captive bolt is mainly restricted to casualty slaughter and is not used routinely. However, it is widely used as a back-up method when other methods fail. When properly used, it leads to an immediate loss of consciousness. The welfare concerns are that accurate shooting is difficult and it may not be effective in mature sows and boars.

4.1.2. Recommendations

The captive bolt should be fired perpendicular to the frontal bone surface.

4.1.3. Other future research

There is a need to develop and evaluate a captive bolt gun that would effectively stun / kill pigs, including breeding sows and boars.

The feasibility and effectiveness of pithing pigs to kill them after shooting with penetrating captive bolt need to be evaluated. If this is not possible, then the effectiveness of administration of a neurotoxin (e.g. potassium chloride) through the bolt wound to chemically destroy the brain should be evaluated.

4.2. ELECTRICAL STUNNING AND STUN / KILLING METHODS

4.2.1. Conclusions

When properly used, electrical stunning leads to an immediate loss of consciousness.

In electrical stunning, serious welfare concerns are related to the introduction of an animal into the restrainer as well as being restrained in poorly designed systems or in high throughput slaughterhouses. Restraint in V-shaped restrainers can be extremely stressful for pigs. Band restrainers appeared to cause less stress.

A major risk with electrical stunning, especially with unrestrained pigs, is improper manual placement of electrodes which can cause incomplete stunning and painful electric shocks and thus result in poor welfare. Automated electrical systems using V-shaped restrainers may fail to induce effective stunning in all animals, due to incorrect electrode placement related to varying animal size or to bad design of the system. Automated electrical stunning systems using chest belt conveyors have a high stunning efficiency due to the use of photo sensors to improve placement of electrodes and accurate positioning of the animals head.

Electrical stun / killing, which induces cardiac ventricular fibrillation, should ensure that no pigs regain consciousness during bleeding. However, impacts during shackling and hoisting could resuscitate the heart.

4.2.2. Recommendations

When electrical stunning is used, guiding and handling equipment should be designed to facilitate introduction of pigs into the restrainer, in order to minimize stress. Low stress restraining devices should be used to reduce movements caused by stress. Band restrainers should preferably be used. .

Correct placement of the electrodes should be ensured in all the electrical methods. In the automated systems, the placement of the electrodes should be adapted to the size of the pigs.

For **electrical head-only stunning**, a minimum current of 1.3 A (root mean square or average) should be applied across the brain for at least 1 second to induce immediate loss of consciousness. Sticking should then be performed within 15 seconds after end of the stun.

For **the one cycle electrical head-to-back stun / killing method**, a minimum current of 1.3 A (root mean square or average) using 50 Hz sine wave AC should be applied for at least 1 second to induce immediate loss of consciousness and cardiac ventricular fibrillation.

For the **two cycle electrical stun / killing method**, a minimum current of 1.3 A (root mean square or average) should be applied across the brain for at least 1 second to induce immediate loss of consciousness, followed by a minimum current of 1 A (root mean square or average) using 50 Hz sine wave AC spanning the heart for at least 1 second to induce cardiac ventricular fibrillation. When using this method for manual stun / killing, the recommended minimum currents should be applied for at least 3 seconds. The method should ensure that the current reaches the brain before or at the same time as it reaches the heart, lest the conscious animal be killed by cardiac arrest, an inhumane method.

Following electrical stun / killing of pigs, the stun-stick interval is not critical, providing that subsequent forceful handling of the animal is avoided as this could resuscitate the heart before sticking or during bleeding.

4.2.3. High research priorities

The technical reference data for electrical stunning such as 1.3 A are either rather old or worked out under experimental laboratory conditions. There is a need to verify these data under commercial conditions.

Contradictory results in the duration of an electrical stun using different frequencies and waveform currents have been found in pigs. The depth and duration of unconsciousness and insensibility induced with various electrical waveforms, frequencies and amount of current need to be clearly established using well defined criteria such as quantitative electroencephalogram and/or evoked potentials in the brain.

Stunning of pigs with an electric current is expected to increase the brain extra cellular levels of GABA (gamma amino butyric acid), as happens in sheep. It is not known how long the GABA level remains elevated and what are its animal welfare implications. This needs to be evaluated using a combination of neurochemical and neurophysiological techniques.

4.3. GAS STUNNING AND STUN / KILLING METHODS

4.3.1. Conclusions

In CO_2 stunning, loss of sensibility and consciousness is not immediate but immersion of pigs into 80 to 90% CO_2 usually leads to the induction of unconsciousness within 30 seconds. At a given high concentration of CO_2 (80% by volume in air) and using increasing exposure times, the duration of unconsciousness increases and the stun-stick interval can be increased proportionally without animals recovering consciousness. However, at concentrations above 30% CO_2, the gas is known to be aversive and cause hyperventilation and irritation of the mucous membranes that can be painful, and elicits hyperventilation and gasping before loss of consciousness.

Hypoxic stunning induced with 90% argon in air is less aversive than hypercapnic hypoxia induced with 30% CO_2 in argon or nitrogen or stunning with 80-90% CO_2 in air.

At the exposure time of 3 minutes, the duration of unconsciousness induced with 30% CO_2 and 60% argon in air is short (<50 seconds). Exposure times of 7 minutes will be necessary to stun / kill pigs with this gas mixture.

At the exposure time of 3 minutes, the duration of unconsciousness induced with 90% argon or nitrogen in air is short (<50 seconds). Exposure times of longer than 7 minutes will be necessary to stun / kill pigs with argon-induced hypoxia, but it is not known how long an exposure time is needed to achieve killing of all pigs.

To overcome practical problems due to this short stun-stick interval, inducing cardiac ventricular fibrillation with an electric current might be a viable option.

4.3.2. Recommendations

Ideally, gas stunning and stun / killing systems should incorporate some general animal welfare principles. Pigs should be maintained in a stable social group with the minimum of restraint (group stunning).

Pre-slaughter handling facilities used for loading animals into cradles should be designed to minimise stress.

All pigs should be rendered rapidly unconscious in the gas. An irreversible state of unconsciousness should be reached in all pigs prior to sticking.

There should be adequate monitoring of gas concentrations of the system and efficient evacuation in the event of any system failure.

The gas used to induce unconsciousness should be non-aversive. In this regard, the use of argon, nitrogen or mixtures of these gases seems to have animal welfare advantages, because hypoxia induced with these gas mixtures is not aversive to pigs.

Stunning in gas mixtures containing low oxygen concentrations should be done with: (a) a mixture of 30% CO_2 and 60% argon or nitrogen in air, or (b) with 90% argon or nitrogen (or other inert gas) in air. In both cases, the maximum residual concentration of oxygen should be 2% by volume. Pigs should be immersed into these recommended gas concentrations within 10 seconds from leaving the atmospheric air and they should be exposed to gas mixtures for a minimum of 3 minutes under situations where death will be achieved through bleeding.

After stunning with hypoxic gas mixtures, pigs should be bled out as fast as possible. Considering the duration of unconsciousness given above (see 4.2.1.), the recommended stun-to-stick interval are:

- After exposure to 30% CO_2 and 60% argon or 90% argon in air:
    - for 3 minutes exposure: the stun-to-stick interval should not exceed 25 seconds,
    - for 5 minutes exposure: the stun-to-stick interval should not exceed 45 seconds.
- After exposure to 30% CO_2 and 60% argon for 7 minutes: the stun-to-stick interval is not critical (as all the pigs are dead).
- After exposure to 90% argon for 7 minutes: the stun-to-stick interval should be kept short i.e. less than 60 seconds.

However, the prolonged exposure times and relatively short stun-to-stick intervals mentioned above may not be commercially feasible where high throughput rates are required. Therefore, potential alternatives have been proposed. For example, stunning of pigs with 30% CO_2 and 60% argon in air or with 90% argon in air, and then inducing cardiac ventricular fibrillation with an electric current to kill them prior to shackling, hoisting and bleeding. In this regard, exposure of pigs to the novel and humane gas mixtures has been reported to induce isoelectric electroencephalograms within 90 seconds. Therefore, induction of ventricular fibrillation immediately (e.g. within 10 seconds) after 90 seconds exposure could be an option; but it may not be practically possible to induce ventricular fibrillation in all the pigs under group stunning situations (e.g. five pigs). Under these circumstances, exposure times should be prolonged such that the last animal within a group will remain unconscious until the induction of ventricular fibrillation.

The times recommended previously for exposure to novel and humane gas mixtures and stun-to-stick intervals could be used to provide guidelines. For example, ventricular fibrillation should be induced within 25 and 45 seconds after 3 and 5 minutes exposure, respectively, to these gas mixtures.

4.3.3. Future research

a) High research priorities

Further research is needed to develop humane gas mixtures and to determine stress levels of pigs during the induction stage of gas stunning before loss of consciousness.

Further research and development should aim to evaluate and, if relevant, to develop stun / killing systems based on the induction of hypoxia.

In humans, inhalation of high concentration of CO_2 for a short time or prolonged inhalation of a low concentration of CO_2 induces breathlessness. Breathlessness can be determined from the increases in intrathoracic / diaphragmatic pressures. Similar techniques could be employed to ascertain whether pigs experience breathlessness during stunning with this gas.

b) Other future research

Exposure of pigs to CO_2 has been reported to increase the extra cellular levels of GABA in the brain. Since GABA is an inhibitory amino acid neurotransmitter and hence would prevent induction of epilepsy in the brain, it is believed that electrical stunning may not be appropriate to re-stun pigs showing signs of recovery of consciousness after CO_2 stunning. This needs to be scientifically investigated.

4.4. Other methods

4.4.1. Conclusions

The use of the **waterjet stun / kill method** and **microwave irradiation** have been tested experimentally and have not been further developed due to disadvantages on animal welfare, operative health and safety grounds.

4.4.2. Other future research

Combined methods needs to be evaluated as it may be possible to develop equipment for pigs to induce unconsciousness and insensibility with non-aversive gas mixtures and then to subsequently kill them with electric current.

4.5. Other future research for all methods where pigs are stuck

There appears to be a potential conflict between the size of the sticking wound and chances of carcass contamination during scalding (welfare vs. hygiene), which needs to be evaluated and resolved.

5. METHODS FOR STUNNING AND STUN / KILLING POULTRY SPECIES (CHICKENS AND TURKEYS)

Electrical and gas methods are the most common methods for stunning and stun /killing poultry under slaughterhouse conditions.

Since welfare is poor when the shackling line and water bath electrical stunning method is used, and birds are occasionally not stunned before slaughter, the method should be replaced as soon as possible. At present, the inert gas stun / killing method is the best alternative.

5.1. MECHANICAL STUN / KILLING METHODS

5.1.1. Conclusions

Captive bolts are normally used for stun / killing birds and as a backup method when other methods fail. Shooting of poultry with existing captive bolts, both penetrating and non-penetrating guns, results in severe skull fractures and structural damage to the brain leading to death. A commercially available captive bolt (Humane Poultry Killer) fitted with a plastic percussive head is widely used to kill poultry on farm and as a backup method in processing plants. When penetrating captive bolts are used, the bolt diameter, velocity and penetration depth are critical to achieving a humane stun / kill.

During restraint of birds using shackles before mechanical stun / killing, there is a potential in a significant number of animals for dislocations and fractures to occur before being stunned.

5.1.2. Recommendations

Birds should be restrained to facilitate accurate placement and effective shooting. The methods for shackling birds should be such that it minimises the potential for joint dislocations and fractures through careful handling and good shackle design.

Captive bolts should be fired perpendicular to the frontal bone surface.

The bolt diameter should be a minimum of 6mm and deliver an impact energy of 21 Joules and, in any case, appropriate to the species of poultry to destroy the skull and brain.

5.1.3. High research priorities

Research is needed to develop better restraining mechanisms.

5.2. ELECTRICAL STUNNING AND STUN / KILLING METHODS

5.2.1. Conclusions

Stunning methods used for poultry are head-only electrical stunning and water baths electrical stunning (involving high frequency (>100Hz) electric currents). The duration of unconsciousness decreases with increasing frequency, e.g. above 200Hz, of the stunning current applied head-only or in a water bath.

Electrical water bath involving 50 to 60Hz sine wave alternating currents is used as a **stun / killing method.**

Electrical stunning and electrical stun / killing using water baths require extremely stressful handling and shackling of live poultry. The pain and distress associated with inversion (hanging upside down) and shackling (compression of metatarsal bones) induces wing flapping in the majority of birds, and there is a potential in a significant number of animals for dislocations and fractures to occur.

Allowing certain time intervals (e.g. 12 and 20 seconds in chicken and turkey, respectively) between shackling and water bath stunning or stun / killing, provision of breast comforters up to the entrance to the water baths and dim light in the area of shackling and stunning or stun / killing have a calming effect on birds and reduces the prevalence and duration of wing flapping.

Wing flapping at the entrance to the water baths predisposes birds to receive electric shocks prior to be stunned, those pre-stun electric shocks are extremely painful and distressing to the birds. Owing to large wingspans in turkeys, their wings hang lower than their heads and therefore the leading wings normally make contact with the electrified water baths before the heads are fully immersed.

The amount of current delivered to individual birds in a multiple-bird water bath stunning or stun / killing system varies according to the electrical resistance or impedance of individual birds in the bath and cannot be controlled without the implementation of constant current stunners.

Electrical stun / killing technique (experimental), using dry electrodes, performed on poultry restrained in conveyors avoids the poor welfare that results from shackling when using electrical stun / kill in a water bath. However, the minimum currents necessary to effectively stun / kill have been established only for chickens and need to be determined for turkeys.

Electrical stun / killing methods involving dry electrodes or water baths are better than electrical stunning on bird welfare grounds because the stun-to-neck cutting interval and the blood vessels severed at slaughter are not critical, and also because in water bath stunning systems, (a) delivery of effective current to all the birds could not be guaranteed due to the reason that current flow through individual birds in a multiple bird water bath stunner varies inversely according to the electrical impedance of birds (in the circuit or pathway), (b) effectiveness of stunning decreases with increasing electrical frequencies that do not induce ventricular fibrillation, (c) mechanical neck cutting may not be effective in severing all the major blood vessels in the necks of all birds to prevent return of consciousness during bleeding, and (d) under commercial conditions, birds showing signs of consciousness during bleeding could not be accessed safely and swiftly to apply a backup stunning or killing method.

5.2.2. Recommendations

Recommendation of one effective minimum current for all the electrical waveforms and frequencies used under commercial conditions is not feasible and it could not possibly ensure good welfare in the birds. Therefore, based on the available scientific information (in chickens), certain minimum currents are recommended for different ranges of electrical frequencies.

Stunning procedures should be followed within 20 seconds by severance of all the major blood vessels in the neck, including both common carotid arteries, to avoid recovery of consciousness during bleeding and all the birds should be dead when entering scald tanks.

For head-only electrical stunning, the size, shape and design should be appropriate to the species such that they facilitate effective application of the stun and deliver recommended currents within a second of stun application. Birds should be restrained suitably to facilitate uninterrupted and effective application of the stun. Head-only electrical stunning electrodes should be placed on either side of the head such that they span the brain. Minimum root mean square or average currents of 240 and 400 mA should be applied for a minimum of seven seconds to chickens and turkeys, respectively, when using a constant voltage stunner supplied with 50 to 60 Hz sine wave AC. When using constant current stunners delivering sine wave AC, the following minimum currents should be applied for a minimum of one second.

Table 1. Minimum currents to apply for a minimum of one second when using constant current stunners delivering sine wave AC

Sine wave AC frequency (Hz)	Minimum root mean square current (mA)
50	100
> 50 and up to 400	150
> 400 and up to 1500	200

To facilitate effective monitoring and auditing, **electrical water bath** stunners should be fitted with visible and audible alarm systems to warn when the supply voltage falls below the levels that would be necessary to deliver the minimum recommended currents.
The size and shape of the metal shackles should be appropriate to the size of legs of poultry, such that secure electrical contact is provided without causing avoidable pain. Wetting shackles prior to hanging live birds reduce electrical resistance and improve contact between legs and shackle.

The methods of shackling birds should be such that it minimises the potential for joint dislocation and fractures through careful handling and good shackle design. Shackle lines should not have bends and dips that induce wing flapping. There should be a sufficient delay between shackling and stunning to provide time for the birds to stop wing flapping. The minimum shackle duration should be 12 and 20 seconds in chickens and turkeys, respectively. Poultry should be hung on the shackle line by both legs for a time as short as possible. The maximum time interval between shackling and stunning should not exceed one minute.

Runts (smaller than average birds), which are likely to miss the water bath stunner, and injured birds that are in pain should not be shackled. Instead, they should be killed using an appropriate killing method (e.g. captive bolt).

Lighting conditions during shackling of live poultry should be controlled to reduce wing flapping. Breast comforting plates that help to calm the birds should be used from the point of shackling until the birds enter the water bath stunner.

Pre-stun electric shocks occurring at the entrance to the electrified water bath should be avoided by providing an electrically insulated entry ramp to the bath and avoiding overflow of water at the entrance, for example.

The height of the water bath should be adjusted according to the size of poultry to ensure at least complete immersion of the birds' heads in the water or, preferably, immersion of the birds up to the base of the wings. Food-grade salt, at least 0.1% weight / volume, should be added to the fresh water bath to improve electrical conductivity, where appropriate. The electrodes in water bath stunners should extend to the full length of the water bath.

During stunning, there should be secure and uninterrupted contact between the shackle and the earth (rubbing) bar.

Electrical water baths for stunning or stun / killing poultry should be supplied with constant current, rather than constant voltage, source and each bird in the water bath should receive the recommended minimum amount of current.

The voltage supplied to the water bath stunning systems should be sufficient to deliver the following minimum recommended root mean square or average currents (mA) to each of the birds in the water bath (table 2).

Table 2. Minimum recommended root mean square or average currents (mA) delivered to birds in water bath stunning systems

Frequency (Hz)	Chickens	Turkeys
Up to 200 Hz	100	250
200 to 400 Hz	150	400
400 to 1500 Hz	200	400

When using pulsed DC the mark:space ratio (which is the duration for which current remains ON and OFF within each cycle (Hz)) should be 1:1.

In the event of line breakdown or a delay in stunning the birds, access should be available to unshackle the birds that have not reached the water-bath and have not been stunned, and bleed those birds that have been stunned and remain in the water-bath.

Recommendations for electrical stunning in water baths apply also to electrical stun / killing in water baths, except for the minimum currents. Minimum root mean square or average currents of 150 and 250mA delivered with a 50 to 60Hz sine wave AC should be applied for a minimum of one second to chickens and turkeys, respectively.

Since stunning and stun / killing using a shackling line and water bath causes very poor welfare in the birds, these systems should be replaced as soon as possible by a system causing less stress and pain such as those using non-aversive gases. If this is not feasible, application of electrical stun / killing technique to poultry restrained in conveyors should be considered.

However **Electrical stun / killing using dry electrodes** is not used commercially, the technique is available for processors (especially small and on farm processors) and its use should be encouraged.

Birds should be restrained suitably to facilitate uninterrupted application of the stunning and killing current cycles. Birds should be stunned head-only first, immediately followed by a head-to-body current application. Good electrical contact should be maintained during stunning and killing. The body electrodes should span the heart.

When using a constant voltage stunner, a minimum root mean square current of 240mA of 50Hz sine wave AC should be applied for at least 5 seconds across the head to stun and another one seconds across the body to stun / kill chickens uninterruptedly. When using a constant current stunner, a minimum root mean square current of 150mA of 50 Hz sine wave AC should be applied for at least one second across the head to stun and one additional second across the body to kill chickens uninterruptedly.

No birds shall survive the application of any electrical stun / killing method or show signs of recovery of consciousness during bleeding.

5.2.3. Future research

a) High research priorities

If the use of shackles is to continue, research is needed to develop better shackling mechanisms for birds and to develop alternative systems of restraint.

Evaluation and development of electrical stun/ kill techniques that do not involve shackling of conscious turkeys are needed. In that scope, devices that stun / kill turkeys restrained in conveyors using dry electrodes need to be investigated. This will eliminate the need to shackle conscious birds, especially heavy turkeys, and avoid the associated pain and distress.

The minimum currents necessary to achieve effective stunning and killing need to be established. The impact of electrical stunning (head-only or water bath) current waveform, frequency and the amount of current on the depth and duration of unconsciousness induced in poultry need to be clearly established using neuro-physiological parameters to understand the effect of these variables.

The time to cessation of wing flapping from the moment of hanging turkeys on a moving shackle line under commercial conditions needs to be determined to specify a minimum shackling duration for this species (there is only one survey study available for turkeys comparing to broilers for which many reports exist).

The prevalence of pre-stun electric shocks occurring prior to electrical water bath stunning of chickens under commercial conditions is not known and needs to be determined.

The prevalence of alive and conscious poultry (chickens and turkeys) entering scald tanks under commercial processing conditions needs to be determined and methods found to prevent it.

b) Other future research

The previously reported times to onset of brain death after cutting common carotid arteries and external jugular veins (or combination of blood vessels) in the necks of turkeys and chickens, as determined from the loss of visual evoked potentials in anesthetised and mechanically ventilated birds, appear to be longer than the time normally needed to reach the scald tanks under commercial processing conditions. Poultry are known to defecate as they enter scald tanks and it is not certain whether this potential hygiene problem is an indicator of a serious welfare problem, i.e. birds entering scald tanks before the onset of brain death and hence defecating in the scald tanks.

Further investigations involving electrical stunning and slaughter procedures are needed to establish the cumulative impact of stunning and slaughter on the time to onset of brain death and recommend bleed out times that are appropriate to the species of bird.

5.3. Gas stunning or stun / killing methods

5.3.1. Conclusions

The balance of evidence suggests that controlled atmospheres containing concentrations of more than 30% CO_2 are aversive and may cause pain and respiratory distress before loss of consciousness. Hypoxia induced with argon and / or nitrogen with less than 2% by volume of oxygen is not aversive to poultry.

A controlled atmosphere containing 30% or less by volume of CO_2 in argon and / or nitrogen with less than 2% by volume of oxygen seems to be adequate for stun / killing poultry. In addition an alternative system anaesthetising birds with an atmosphere of 30 to 40% CO_2 (with O_2 and N_2) for 1 minute, followed by 2 minutes in 80% CO_2 or more to cause death, is being evaluated.

The exposure times necessary to effectively stun poultry with any gas mixtures, without killing some birds and / or inadequately stunning some other birds, are not known. The duration of unconsciousness induced with the known gas mixtures are very short and therefore, it will be difficult to avoid return of consciousness either prior to or during bleeding. Birds showing signs of consciousness following stunning need to be effectively re-stunned, preferably using captive bolts, instead of neck dislocation.

5.3.2. Recommendations

In the absence of sound scientific evidence concerning the depth and duration of unconsciousness induced with gas mixtures, minimum conditions **for stunning** poultry could not be defined.

When using gas mixtures **for stun / killing**, live poultry should only be conveyed into the gas mixtures either in transport crates or on conveyor belts. Birds should reach the recommended gas mixtures within 10 seconds of leaving atmospheric air.

Under no circumstances should gases at freezing temperatures enter the chamber. Appropriate gas concentrations should be monitored continuously at bird level inside the chamber.

The recommended gas mixtures are: (a) a minimum of 2 minutes exposure to argon, nitrogen or other inert gases, or any mixture of these gases, in atmospheric air with a maximum of 2% oxygen by volume; (b) a minimum of 2 minutes exposure to any mixture of argon, nitrogen, or other inert gases with atmospheric air and CO_2 provided that the CO_2 concentration does not exceed 30% by volume and the oxygen concentration does not exceed 2% by volume.

All the birds should be killed by the gas mixtures and under no circumstances should they show signs of recovery of consciousness once they had been through the chamber.

Stun / killing poultry in transport crates using hypoxia and shackling of relaxed carcasses would result in best welfare since it would not only eliminate live bird handling and shackling at the processing plants but also effectively kill all the birds. In this regard, the use of hypoxia (less than 2% by volume of oxygen) induced by argon, nitrogen, inert gases or mixtures of these may be the best option from an animal welfare point of view.

5.3.3. High research priorities

Stunning of poultry with gas mixtures needs further investigation to determine more humane gas mixtures as well as the duration of unconsciousness, appropriate stun-to-neck cutting interval, blood vessels to be severed and the time to onset of brain death.

Gas stunning mixtures should be improved and validated so they can be quickly and widely used in slaughterhouse, thus decreasing distress and pain due to shackling.

Bleeding techniques that do not need shackling of gas-stunned birds need to be evaluated and developed.

5.4. Other methods

5.4.1. Conclusions

The use of **needle bolts** for poultry or **microwave irradiation** have been tested experimentally but have not been further developed due to disadvantages on animal welfare and operative health and safety grounds.

5.4.2. Recommendation

Needle bolts or **microwave irradiation** should not be used for poultry.

6. METHODS FOR STUNNING AND STUN / KILLING HORSES

Penetrating captive bolt stunning is the most common used method in European abattoirs. When performed correctly, captive bolt and free bullet can be effective methods for respectively stunning and killing horses and for both methods, loss of consciousness is immediate.

6.1. Penetrating captive bolt stunning

6.1.1. Conclusions

Although scientific investigations in mechanisms and effectiveness of captive bolt stunning in horses were not available for the scientific report, practical information and experience indicates that the method may work well under slaughterhouse conditions, if the equipment is well maintained.

6.1.2. Other future research

Pre-slaughter handling, mechanisms and effectiveness of captive bolt stunning and bleeding in horses should be scientifically evaluated.

6.2. FREE BULLETS FOR KILLING

6.2.1. Conclusions

The use of free bullets can be necessary in excited and uncontrollable horses.

6.2.2. Recommendations

This method cannot be used in confined spaces and requires personnel trained in the use of firearms.

7. METHODS FOR STUNNING AND STUN / KILLING FARMED FISH

7.1. CONCLUSIONS

Many existing commercial killing methods expose fish to substantial suffering over a prolonged period of time. For some species, existing methods, whilst capable of killing fish humanely, are not doing so because operators don't have the knowledge to evaluate them.

In practice, **percussive stunning** tends to be a stun / killing method. It has the capacity to cause a humane death in many moderately sized species (0.2 to 14kg) if correctly applied.

Only **mechanical spiking methods** can be considered humane. **Non mechanical methods** are difficult to standardise and even mechanical methods require considerable skill to apply.

Electrical methods can be stunning or stun / killing methods, depending on the electrical parameters applied. Evidence indicates that electrical stunning systems do not induce a sufficiently long period of insensibility to ensure the fish dies before recovery of consciousness. Electrical stun / killing systems can be humane if the correct parameters are used but might cause substantial suffering when incorrectly applied.

Fish find **CO_2 narcosis** very aversive. It can be a stunning or a stun / killing method. But in commercial practice, it is usually a sedation method only because of the short exposure times used.

Shooting and electric harpoon for farmed tuna, **hydraulic shock and hypoxic stunning** have poor welfare implications.

Asphyxia, asphyxia in ice / thermal shock, salt bath, ammonia solution, electro-immobilisation / electrostimulation / physical exhaustion using electrical shocks, decapitation and **bleeding out / exsanguination** are not humane methods for killing fish

Sedation / anaesthesia prior to slaughter reduces the stress associated with handling if used correctly but it is not possible to use currently available anaesthetic or sedative for any fish that might enter the food chain. Pre-slaughter sedation by slow live chilling is not a humane method to sedate or kill fish.

7.2. RECOMMENDATIONS

Many fish killing process are designed for commercial efficiency rather than welfare priorities. Criteria for humane application of percussive stunning, spiking and electrical stunning should be made available to the industry.

For **percussive stunning and spiking**, the species for which the method is appropriate, the force that should be applied and the correct target area should be specified. Mechanical percussive stunning devices should be regularly tested for correct function and efficiency.

For **electrical stunning**, species for which the method is appropriate, the stunning current, voltage and exposure time and method that ensures immediate and sustained insensibility should be specified. With development, electrical stun / kill systems are likely to be the most appropriate method for humanely killing many small farmed species of fish for which there is currently no other satisfactory method available.

CO_2 Narcosis, asphyxia, asphyxia in ice/thermal shock, salt bath, ammonia solution, electro-immobilisation/electrostimulation/physical exhaustion using electrical shocks and decapitation should not be used to kill fish because they cause avoidable suffering before death.

Fish should not be **cooled on ice in air or water** as a means of removing muscular activity or killing.

In all cases, it should be mandatory that a stunning / killing step is incorporated before exsanguinations or any processing of fish commences e.g. gutting, desliming, etc

7.3. Future research

There is a major lack of knowledge of many aspects of stunning and killing in fish.

There is a need to develop (a) techniques to facilitate handling and restraint of fish, (b) methods that ensure immediate unconsciousness in all species of fish currently farmed and, (c) for all stunning methods, to evaluate methods for killing fish before recovery from the stun.

Existing equipment and procedures should be evaluated and certified to ensure that they effectively and humanely stun or stun / kill fish.

For many species, there is not a commercially acceptable method that can kill fish humanely. The development of humane slaughter methods should be an integral part of developing any new species for fish farming.

7.3.1. High research priorities

The parameters required to stun and/or stun / kill fish by means of electric current should be investigated in all appropriate farmed species (<10kg). The relationship between required exposure duration, required electric field strength, electrical frequency and water conductivity should be identified for the induction of both insensibility and death. The mechanism of death in fish exposed to electrical stunning parameters should be identified. The relationship between the above electrical parameters and carcass quality should be examined. Apparatus for electrical stunning of smaller farmed sea fish (sea bass, sea bream) should be developed.

The welfare implications of electric harpoon or shooting in the case of farmed tuna should be investigated.

The welfare implications of hydraulic shock and hypoxic stunning should be assessed by measurement of brain function in combination with observation of behaviour.

7.3.2. Other future research

Mechanical devices such as captive bolt pistols should be developed for all species for which they would be of value. The humaneness of percussive killing should be investigated in each farmed species of fish. Mechanical devices for percussive killing of fish larger than those currently killed by this method should be investigated.

There is also a need to investigate ways to humanely kill fish taken from the water with a hook, a line or a net.

8. KILLING FOR DISEASE CONTROL

Previous conclusions for various stun / kill methods and species of animals should also be considered in this section.

For disease control purpose, a stun / kill rather than a "stun and bleed" method is normally required on the grounds of biosecurity, efficiency of operation and disposal of potentially infective materials.

There is a need to survey the common on farm killing practices during disease outbreaks in European countries, assessing practicability and animal welfare implications. Research to define optimal practices for emergency killing on farm as well as animals' handling and restraint systems for the various killing methods is urgently required to improve animal welfare, while maintaining biosecurity aspects. On farm non-invasive killing methods should be developed, especially if samples of CNS (central nervous system) tissue are required.

8.1. MECHANICAL METHODS

8.1.1. Conclusions

Free bullet fired to the brain is effective for on-farm killing of cattle, sheep, pigs and horses, due to massive brain destruction that causes immediate unconsciousness and death. It is suitable for animals that are difficult to handle and restrain.

Penetrating captive bolt are used to kill on farm cattle, calves, sheep, pigs, horses and poultry. It induces death in poultry but may only stun rather than kill large animals. Pithing should be performed immediately after shooting to ensure death, except where a pneumatic gun that injects air into the brain is used. Disposable pithing rods that would plug into the bolthole are commercially available however their effectiveness has not been validated. Adequate restraint of the animal is necessary to enable accurate delivery of the shot and this may limit its practical application on farms.

Percussive blow applied manually to the head is only suitable as a killing method for small number of poultry, piglets and lambs and should not be used on calves. It may not always cause death. Operator fatigue is an important factor in this method. Restraint of the animal is necessary and may be stressful.

Neck dislocation may not concuss poultry and it is therefore uncertain whether it causes immediate unconsciousness.

Mechanical maceration of chicks up to 72 hours old and embryonated eggs in a high-speed grinder fitted with rotating blades (6000 or more revolution per minute) results in immediate death.

8.1.2. Recommendations

Free bullets are only recommended to kill on farm cattle, calves, sheep, pigs and horses when other methods cannot be applied. The cartridge, calibre and type of bullet should be appropriate for the species and age. An animal should be killed by a single shot to a recommended anatomical position. While with increasing distance the chance of failure to shoot accurately with free bullet increases, a telescopic device and/or infrared targeting systems would help to improve precision. The ammunition should be appropriate to the species of the animals and distance of shooting.

The animals should be restrained wherever possible and/or sedated if necessary prior to killing with **penetrating captive bolts**. Pithing should be performed in large animals after shooting to ensure death. Precautions should be taken to avoid the spread the body fluids and tissues, e.g. brain tissue and blood, that has leaked from the hole.

A **percussive blow** to the head of piglets and lambs should not be used because it is not always effective.

Cervical dislocation or decapitation should be preformed after the birds have been stunned by some other means.

8.1.3. Future research

a) High research priorities

Requirements to induce death of all the animals by using penetrating captive bolt should be determined (kinetic energy, bolt diameter, penetration depth, type of pistol).

The susceptibility to infection in relation to the body fluids and brain tissues coming from the cranial hole after the use of free bullets or penetrating captive bolt should be determined.

b) Other future research

The minimal velocity of the impact to induce effective non-penetrating captive bolt stunning in each type of animal should be determined, taking into account the type of captive bolt. The duration of unconsciousness after such stunning methods should be specified.

8.2. ELECTRICAL METHODS

8.2.1. Conclusions

Electrical stun / kill methods are effective and non-invasive to kill large animals and poultry on-farm (various mobile systems are available for poultry). However, it is worth noting that induction of ventricular fibrillation in piglets and lambs may not be successful; these two species need prolonged current application across the heart.

Particular restraint of animal is needed to facilitate proper application of the electrodes, which can be distressing.

8.2.2. Recommendations

Electrical stun / killing methods are recommended, as they are non-invasive, to kill on farm calves, sheep, pigs and poultry.

In large animals, the appropriate current regarding species and ages should be applied for at least 10 seconds on the head and 45 seconds across the heart in order to ensure the death of all animals. The animals should be suitably restrained to ensure the correct placement of the tongs. Electrical methods are not recommended for killing piglets and lambs, as they may not cause ventricular fibrillation.

For poultry, the length of the water bath should be sufficient to provide a minimum of 10 seconds current application. Birds should be monitored during the following 10 minutes to ensure that death has occurred.

8.3. GAS METHODS

8.3.1. Conclusions

Killing of poultry and piglets with **controlled atmospheres** are non-invasive methods that permit the euthanasia of animals in groups, minimising stress caused by handling and restraint procedures.

Killing of all pigs in a group can be achieved with exposure to 90% CO_2 for 5.5 minutes although this gas is very aversive it may be the most practical. Exposure times of 7 minutes will be necessary to kill pigs in a mixture of 30% CO_2 and 60% argon. Exposure times of longer than 7 minutes will be necessary to kill pigs in 90% argon, but it is not known how long exposure time is needed to achieve killing of all pigs

Carbon monoxide is suitable for killing piglets and poultry, inducing a rapid loss of consciousness and death, provided that the gas source is pure and not obtained from outlet gases.

Hydrogen cyanide gas is not suitable for killing poultry because it may cause respiratory distress and convulsions prior to loss of consciousness.

8.3.2. Recommendations

Controlled atmospheres for killing can be used in poultry and piglets. Animals should be introduced into the chamber only after it has been filled with the required gas (mixture) concentration and they should remain in this atmosphere until they are dead. Chambers should not be overcrowded and measures should be taken to avoid animals climbing on top of each other when entering the chamber. Poultry should not be thrown in the chamber, bedding should also be added. Compressed gases, especially CO_2, should be vaporised prior to filling the chamber.

Only **carbon monoxide** administered from a pure source (not from outlet gases) should be used to kill piglets. Animals should be exposed to between 4% and 6% of CO by volume in air for a minimum of 6 minutes. Carbon monoxide should however be administered at a low flow rate as high flow rates induce severe convulsions, which can occur before loss of consciousness. As carbon monoxide is extremely noxious, personnel should be informed of the danger and suitably protected.

Exposure to **hydrogen cyanide** is not recommended to kill poultry as it causes respiratory difficulties before the onset of unconsciousness and is dangerous to humans.

8.3.3. High research priorities

Optimal gas euthanasia concentration and exposure time should be defined.

8.4. Other methods

8.4.1. Conclusions

Intravenous administration of barbituric acid derivates overdose, usually results in death. Restraint is necessary for intravenous administration which could be distressing. It passes the placental barrier, killing the unborn foetus. Other routes of administration can be painful and/or difficult to achieve or take longer time to induce unconsciousness and death.

When barbiturates cannot be used, **T61** may be a suitable substitute. T61 is a mixture of three drugs: hypnotic agent (Embutramide, 200mg/ml), curariform drug (Mebezonium iodide, 50mg/ml) and local anaesthetic (Tetracaine hydrochloride, 5mg/ml). However, accidental injection of T61 outside the vein or intravenous injection at a rapid rate have been known to cause signs of pain in some animals. T61 does not cross the placental barrier. As T61 contains a neuromuscular blocking agent, there is a possibility that an animal may not be unconscious at the time of muscle paralysis causing some fear.

Killing of live fish in emergency is best achieved by euthanasia using overdose of anaesthetic. A variety of anaesthetics is used, but the most common would be methane tricaine sulphonate or benzocaine. Euthanasia is not a medicinal function so, medicines legislation would not apply, but welfare and environmental requirements still have to be observed and no fish killed by chemical euthanasia can be allowed to enter the food chain. Under emergency circumstances, such as slaughter under notifiable disease legislation, when stocks slaughtered may be allowed to enter the food chain, **CO_2 narcosis**, followed by slaughter under strict containment, while highly aversive, is used.

8.4.2. Recommendations

Intravenous lethal injection of barbiturates may be recommended to kill large animals and poultry. It should be the first choice for pregnant animals, since the drug crosses the placenta and will also kill the foetus.

T61, chloral hydrate, magnesium sulphate and potassium chloride are not recommended for killing conscious animals.

Killing of fish in emergency should be achieved by euthanasia using overdose of anaesthetic. Appropriate methods of electrical killing may also be used. When possible however, in such circumstances, careful transport of fish for slaughter in a processing plant under strict containment would be the preferred option.

8.4.3. Future research

The perception of the foetus to adverse effects during killing of pregnant animals may need to be studied further.

DOCUMENTATION PROVIDED TO EFSA

Letter, 19 May 2003 with ref. SANCO.C.2 RT/mcd D(2003) 112-320517, from Mr Robert J. COLEMAN from the Health & Consumer Protection Directorate-General transferring pending scientific questions to the European Food Safety Authority.

REFERENCES

All references are available in the Scientific Report on welfare aspects of the main systems of stunning and killing the main commercial species of animals.

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The EFSA Journal (2005) 292, 1-46 - Opinion on the "Aspects of the biology and welfare of animals used for experimental and other scientific purposes"

Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to

"Aspects of the biology and welfare of animals used for experimental and other scientific purposes"

EFSA-Q-2004-105

Adopted by the AHAW Panel on 14 November 2005

Summary

EFSA was invited by the EU Commission to produce a scientific opinion concerning the "Revision of the Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes".

This scientific opinion was adopted by written procedure on the 14th November 2005, by the Scientific Panel on Animal Health and Welfare (AHAW) after its Plenary Meeting held on the 12th and 13th of October.

According to the mandate of EFSA, ethical, socio-economic, cultural and religious aspects are outside the scope of this opinion.

Summary of the Scientific Opinion for each of the three parts of the Mandate from the Commission:

1. <u>Summary of the need for protection for invertebrates and fetuses and the criteria used (Questions 1 & 2)</u>

   The Panel was asked to consider the scientific evidence for the sentience and capacity of all invertebrate species used for experimental purposes and of fetal and embryonic forms to "experience pain, suffering, distress or lasting harm". Indicators of an animal's capacity to experience suffering include long-term memory, plasticity of behaviour, complex learning and the possibility of experiencing pain. Some invertebrate species: (i) possess short and long term memory, (ii) exhibit complex learning such as social learning, conditioned suppression, discrimination and generalisation, reversal learning, (iii) show spatial awareness and form cognitive maps, (iv) show deception, (v) perform appropriately in operant studies to gain reinforcement or avoid punishment, (vi) possess receptors sensitive to noxious stimuli connected by nervous pathways to a central nervous system and brain centres, (vii) possess receptors for opioid substances, (viii) modify their responses to stimuli that would be painful for a human after having had analgesics, (ix) respond to stimuli that would be painful for a human in a manner so as to avoid or minimise damage to the body, (x) show an unwillingness to resubmit themselves to a painful procedure indicating that they can learn to associate apparently non-painful with apparently painful events. At a certain stage of development within an egg or the mother, the characteristics listed above may appear. Such information has been used in coming to conclusions about sentience.

   Cyclostomes (lampreys and hagfish) have a pain system similar to that of other fish and brains that do not differ much from those of some other fish. There is evidence that cephalopods have adrenal and pain systems, a relatively complex brain similar to many vertebrates, significant cognitive ability including good learning ability and memory retention especially in octopuses, individual temperaments, elaborate signalling and communication systems, especially in cuttlefish and squid that can show rapid emotional colour changes, may live in social groups and have complex social relationships. Nautiloids have many characters similar to those of other cephalopods, they can track other individuals, live for a long time and are active pelagic animals. The largest of decapod crustaceans are complex in behaviour and appear to have some degree of awareness. They have a pain system and considerable

learning ability. As a consequence of this evidence, it is concluded that cyclostomes, all Cephalopoda and decapod crustaceans fall into the same category of animals as those that are at present protected. Using similar arguments, the dramatic evidence of the sensory processing, analytical and prediction ability of salticid spiders provides evidence for awareness greater than in any other invertebrates except cephalopods but we have little evidence of a pain system so do not at present put these spiders in that same category. Free-swimming tunicates are also in this borderline area and social insects and amphioxus are close to it.

Whenever there is a significant risk that a mammalian fetus, or the fetus or embryo of an oviparous animal such as a bird, reptile, amphibian, fish or cephalopod, is for any length of time sufficiently aware that it will suffer or otherwise have poor welfare when a procedure is carried out on it within the uterus or egg, or after removal therefrom, such animals should be included in the list of protected animals. The stage of development at which this risk is sufficient for protection to be necessary is that at which the normal locomotion and sensory functioning of an individual independent of the egg or mother can occur. For air-breathing animals this time will not generally be later than that at which the fetus could survive unassisted outside the uterus or egg. For most vertebrate animals, the stage of development at which there is a risk of poor welfare when a procedure is carried out on them is the beginning of the last third of development within the egg or mother. For a fish, amphibian, cephalopod, or decapod it is when it is capable of feeding independently rather than being dependent on the food supply from the egg.

Precocial oviparous species, some of which are breathing at the time of hatching present much evidence of being aware before hatching and during the last days before hatching,

Even though the mammalian fetus can show physical responses to external stimuli, in some species perhaps for as much as the last third of their development, the weight of present evidence suggests that consciousness is inhibited in the fetus until it starts to breathe air. It is possible that in a mammalian fetus there might be transient episodes of increased oxygenation above the threshold required to support some aspects of consciousness. It is clear that there is a risk, perhaps a small risk, that any mammalian fetus may on occasion be affected by some experimental procedures in such a way that their welfare is poor, sometimes because they are suffering pain. If a mammalian fetus is removed from the mother and starts to breathe, its level of awareness will change to that typical of such animals after parturition. In addition, protection may need to be given against emotional states in pregnant mothers to safeguard subsequent behavioural modification and welfare of the offspring.

When a procedure is performed on a fetus that is likely to produce pain in the newborn or newly-hatched of that species, adequate anaesthesia and analgesia should be given provided that the agents used do not significantly increase the likelihood of fetal mortality. In the circumstance where no suitable anaesthetic or analgesic agents are available, procedures should not be carried out on such fetuses. When the procedure might cause a lasting inflammatory response that persists post-natally, protection should be given against pain and suffering. A schedule of anaesthetics and analgesics that are suitable for use in pregnant animals, and fetuses should be prepared.

2. Summary of the need for purpose breeding of animals and the criteria used (Question 3)

Species listed in Annex I to Directive 86/609/EEC are those that must be 'purpose bred' when used in experiments (unless a specific exemption has been obtained). The criteria for inclusion of species in Annex I have not been clearly defined and hence no information is available on why they were originally included. Therefore, the Commission has asked the EFSA to issue a scientific opinion on the scientific criteria that could be used to determine in which cases animals to be used in experiments should be purpose-bred and, based on these criteria, determine which species currently used in experiments meet these criteria.

It is the opinion of the AHAW panel that including a species as "purpose-bred" within Annex I will confer a considerable degree of assurance that animals of that species will be provided with suitable accommodation, welfare and care practices. As a consequence of health and colony management within breeding establishments, there can be improved confidence in the quality of the animal, resulting in improved science and a reduction in animal numbers required. Taking these factors in isolation, for the great majority of scientific investigations, there would be welfare and scientific merit in recommending that all animals used in scientific procedures be purpose-bred. However, before making such a recommendation, there are a number of other important factors that have to be considered. The consequences of inclusion of all species could, for example, result in loss of genetic diversity, the generation of large numbers of surplus animals and significant delays in scientific progress. A risk assessment approach has therefore been taken to this issue, with the group analysing the potential benefits for and the adverse consequences of the inclusion of each species in Annex I. Two issues have been considered: animal welfare and scientific quality. For each, three steps have been followed: identification of the hazards, exposure assessment and consequence assessment.

The criteria suggested by the Technical Expert Working Group (TEWG) organised by DG ENV (2003) have been considered and incorporated into an assessment process against which the inclusion of each of the commonly used laboratory species was reviewed. The criteria considered by the AHAW panel have been whether legislation already exists to protect animal welfare, genetically altered animals, health and genetic quality of animals, demand, extrapolation of results to farming or to wild populations and capture from the wild.

It is recommended that, wherever possible, animals used should be of a uniform standard so that there is good and effective control over the animals' genetic fidelity, microbial status, nutrition, socialisation to humans and other animals (e.g. ferrets, dogs and even rodents) and environment. Ideally all animals should be purpose bred but, in practice, some exceptions will be necessary. Exceptions should be made to purpose breeding when it is necessary for the research that a particular strain or breed is used, or that scientific progress would be unduly delayed providing that the scientific data resulting from such research were considered likely to be of good quality, i.e. the competent authorities should consider the potential adverse consequences for research should an exemption for the use of non-purpose bred animals be refused (86/609/EEC: Article 19(4)). Genetically altered animals (of all species) should be added to Annex I. The review of all the commonly used laboratory species has concluded that with the exception of quail (*Coturnix coturnix*) all the other species listed should continue to be purpose-bred and some further species should be added, namely: Chinese hamster (*Cricetus griseus*), Mongolian gerbils (*Meriones unguiculatus*), two *Xenopus* species (*X. laevis* and *X. tropicalis*) and two species of *Rana* (*R. temporaria* and *R. pipiens*).

3. Summary of humane methods of killing animals (Question 4)

Nearly all animals are killed at the end of a research project and it is important that this is done humanely i.e. causing as little suffering as possible for the animals concerned. The majority (85-90%) of animals used in research are small rodents however, of necessity (as we are trying to cover all methods for all animals), much of the Report deals with the methods for large animals. The Opinion of the scientific panel on AHAW is based on the Report annexed to this Opinion that presented recent data building on the three earlier authoritative reports on the humane killing of animals i.e.: 1) the Scientific Report related to welfare aspects of animal stunning and killing methods of the main commercial species of animals (EFSA, 2004, http://www.efsa.eu.int); 2) Close *et al.* 1996/1997 (endorsed by the EU for the humane killing of laboratory animals); and 3) the AVMA Report (2000) dealing with methods for all animals. The Opinion does not repeat what is already dealt with in detail in those reports but we have included a section dealing with new data for each method where applicable, and some conclusions and recommendations are retained. The Scientific Report and Opinion deal with the various technical ways of killing animals starting with electrical and mechanical methods, followed by gaseous and then injectable methods. The section on the use of gaseous agents is in some considerable detail as it is the subject of much new data, with more than 20 new papers in the past 10 years, many of them dealing with the commonest laboratory animals. The interpretation of this data has been varied. The recommended methods for each species are given in Tables 1 to 8 at the end of this section but, in general, we have adopted the recommendations given in the existing EU Guidance (Close *et al.*, 1996/97) except where stated. The AHAW panel suggested that these methods could be varied but only with a scientific justification and appropriate authority, i.e. the recommended methods represent the default position. We also address more general issues including ensuring death, training of personnel, killing animals for their tissues and oversight, the choice of method and when this might affect the scientific outcomes, and gathering information on methods used as well as their efficiency and effectiveness.

Key words

Animal research, experimental animals, animal welfare, invertebrate sentience, fetal sentience, purpose breeding, euthanasia.

Table of Contents

1. Terms of Reference

1.1. Background

Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes provides for controls of the use of laboratory animals, it sets minimum standards for housing and care as well as for the training of personnel handling animals and supervising the experiments.

Since 1986, important progress has been made in science and new techniques are now available, such as use of transgenic animals, xenotransplantation and cloning. These require specific attention, which the current Directive does not provide. Nor is the use of animals with a higher degree of neurophysiological sensitivity such as non-human primates specifically regulated. Therefore, Directorate-General Environment (DG ENV) has started revising the Directive.

The revision addresses issues such as compulsory authorisation of all experiments, inspections, severity classification, harm-benefit analysis and compulsory ethical review. Also specific problems relating to the use and acquisition of non-human primates will be tackled.

In 2002, as part of the preparatory work for the revision, DG ENV requested the opinion of the Scientific Committee on Animal Health and Animal Welfare, SCAHAW, on the welfare of non-human primates used in experiments. This Opinion, adopted by SCAHAW on 17 December 2002, was made available to the TEWG. The Opinion already provides some information especially concerning the requirements for purpose-bred animals and the question on gestation for non-human primates.

In 2003, DG ENV organised a Technical Expert Working Group (TEWG) to collect scientific and technical background information for the revision. The experts from Member States, Acceding Countries (which are now the new Member States), industry, science and academia as well as from animal welfare organisations worked through a set of questions prepared by DG ENV. The results of the TEWG provide an important input for the revision of the Directive. However, the TEWG highlighted four specific questions requiring further scientific input. These questions are detailed below. The final reports of the TEWG are provided as background documents.

1.2. Mandate

1.2.1. *Question 1 on the sentience of invertebrate species, and fetal and embryonic forms of both vertebrate and invertebrate species*

1.2.1.1. Detailed background on invertebrate species

The following definitions are applied in the current Directive:

“'animal' unless otherwise qualified, means any live non-human vertebrate, including free-living larval and/or reproducing larval forms…”

“'experiment' means any use of an animal for experimental or other scientific purposes which may cause it pain, suffering, distress or lasting harm, including any course of action intended, or liable, to result in the birth of an animal in any such condition, but excluding the least painful methods accepted in modern practice (*i.e.* 'humane' methods) of killing or marking an animal"

The TEWGs and other experts recommended to enlarge the scope to include **invertebrate species** provided there is sufficient scientific evidence as to their sentience and capacity to "experience pain, suffering, distress or lasting harm". Certain species of invertebrates are already included in the national legislation of some countries, both within and outside the EU (*e.g.* UK, some Scandinavian countries, Australia Capital Territories, New Zealand). The UK currently only includes *Octopus vulgaris* in its national legislation but is considering the inclusion of additional cephalopod species.

1.2.1.2. Terms of reference of question 1

In view of the above, the Commission asks the European Food Safety Authority to issue a scientific opinion on:

- the sentience and capacity to "experience pain, suffering, distress or lasting harm" of all invertebrate species used for experimental purposes.

1.2.2. *Question 2 on fetal and embryonic forms*

1.2.2.1. Detailed background on fetal and embryonic forms

The definition of 'animal' in the current Directive excludes fetal or embryonic forms.

According to TEWG and other experts, fetal and embryonic forms should be brought under the scope of the Directive in case there is enough scientific evidence on their capacity to "experience pain, distress or lasting harm".

Some Member States have included in their national legislation such forms beyond a certain stage of pregnancy. A criterion for determining the appropriate stage of pregnancy may be the development of the cerebral cortex and when it reaches a stage at which it can register sensory experiences.

The view of several members of the TEWG was that a time limit of half way through the gestation period should be used, at least for all large mammalian species other than rodents. This was based on data relating to sheep and non-human primates whilst providing for a 'safety margin' with regard to the ability of fetuses/embryos of these species to feel pain. However, the TEWG could not reach a consensus on when a rodent fetus or new-born may be capable of suffering, although they suggested that the final 20% of pregnancy may be appropriate for rodent and poultry species.

1.2.2.2. Terms of reference of question 2

In view of the above, the Commission asks the European Food Safety Authority to issue a scientific opinion on:

- The stage of gestation after which the fetus/embryo of the species in question is assumed to be capable of "experiencing pain, suffering, distress or lasting harm",

- whether a generic rule for a cut-off point for the advancement of gestation can be indicated for those species where insufficient scientific data exist to establish a species-specific cut-off point.

1.2.3. *Question 3 on purpose-bred animals*

1.2.3.1. Detailed background on purpose-bred animals

Species listed in Annex I to Directive 86/609/EEC are those that must be 'purpose bred' when used in experiments (unless a specific exemption has been obtained). The criteria for inclusion of species in Annex I have not been clearly defined and no information is available on why the various species were originally included.

For example, mini-pigs which have become a widely-used laboratory species, obtained from commercial suppliers where they are bred in a controlled environment similar to that to be experienced at user facilities. According to the TEWG, their inclusion in Annex I would therefore appear logical and in the interest of sound principles of scientific research and welfare. Other species to be considered for inclusion could be ferrets and some hamster species in addition to *Mesocricetus auratus*. Conversely, the current inclusion of quail (*Coturnix coturnix*) should be re-considered.

The TEWG proposed multiple criteria as a basis for species inclusion into Annex I, such as:

- numbers of animals required for procedures;
- the type of procedures (*e.g.* farm animal studies/population studies);
- animal welfare aspects;
- practical and commercial aspects of establishing breeding;
- disease-free requirements;
- specific animal welfare aspects such as social deprivation, confinement and other aspects of sudden involuntary changes of living environment (use of pet or stray animals as experimental animals.)

1.2.3.2. Terms of reference of question 3

In view of the above, the Commission asks the European Food Safety Authority to issue a scientific opinion on:

- the scientific criteria that could be used to determine in which cases animals to be used in experiments should be purpose-bred, in order to safeguard *inter alia* animal welfare, using the proposal of the TEWG. The proposed criteria should also take into account other factors such as current and future needs, practicability or any specific scientific requirements.

- Based on these criteria, determine which species currently used in experiments meet these criteria.

1.2.4. *Question 4 on humane methods of euthanasia*

1.2.4.1. Detailed background on humane methods of euthanasia

Some experimental animals are only bred to be euthanised for the purpose of using their tissues and/or organs, e.g. in the development and application of *in vitro* methods. To ensure highest possible animal welfare standards in the EU, it needs to be defined which methods of killing are scientifically the most humane and appropriate for different species of experimental animals.

1.2.4.2. Terms of reference of question 4

In view of the above, the Commission asks the European Food Safety Authority to issue a scientific opinion on:

- the methods of euthanasia which could, on the basis of current scientific knowledge and respecting good animal welfare, be justified as being the most appropriate per type of species.

- To specify these methods and their suitability for different species most commonly used in experiments.

1.3. Approach

This Scientific opinion is a scientific assessment of the needs for a revision of the Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. It has been based on the Scientific Report accepted by the EFSA AHAW Panel. In drafting this Scientific Opinion, the panel did not take into consideration any ethical, socio-economic, human safety, cultural or religious aspect of the topic, the emphasis has been to look at the scientific evidence and to interpret that in the light of the terms of reference.

The three working groups (WGs) were set up to address these questions with relevant experts being appointed as members.

This scientific opinion comprises 3 parts / Chapters in response to the 4 questions posed by the Commission (see Section 1.2). Questions 1 and 2 overlapped in scope essentially dealing with sentience of both fetal forms and invertebrates, and are addressed in *Chapter 2*. Questions 3 and 4 remain separate and as they are given in the mandate. They cover purpose breeding of animals (*Chapter 3*), and euthanasia of the commonly used species (*Chapter 4*). It was decided that if in Chapter 2, some species were to be recommended to receive protection, then the report and opinion should also address the question of whether they should be purpose bred in Chapter 3, and how they could be humanely killed in Chapter 4.

A full assessment and the risk profiles can be found in the Scientific Report, published on the EFSA web site, which were drafted by three Working Groups set up by the AHAW Panel.

The Tables 1-8, at the end of the Opinion are taken from Close *et al.* 1996, 1997 and have been modified according to the Scientific Report and update the EU recommendations on humane methods of killing protected animals.

As part of the approach by EFSA two Stakeholders consultation meetings were held on 18th February and the 31st August 2005. At the first meeting Stakeholders were asked to comment on the mandate from the Commission and on the proposed method working. Stakeholders were asked to propose scientific experts, not organisational representatives, that EFSA could call on for help in the working groups (WGs), and to provide any background scientific papers that the WGs might find useful. The suggestions made were very helpful. The scientific experts were selected by EFSA on the basis that they had made a significant contribution to the topic under review in the past 5 years or, where there was no or little scientific data, that they had relevant and appropriate experience. A draft of the Scientific Report (including the proposed recommendations) was sent out on the 28th July for the Stakeholders to seek comments from their members in time for the meeting on the 31st August. At that meeting views were sought from the Stakeholders on the draft Report and the WG's conclusions and recommendations. After Aug 31st Stakeholders were given another 7 days to reconsider their views in the light of the responses from other Stakeholders to make a written response to EFSA on their final views. These views were then considered by the WGs in their preparation of their final Report.

2. QUESTION ON THE SENTIENCE OF INVERTEBRATE SPECIES, AND ON FETAL AND EMBRYONIC FORMS OF BOTH VERTEBRATE AND INVERTEBRATE SPECIES.

All invertebrate animals were considered and our recommendations propose some groups as "protected animals".

2.1. Memory and Learning in Invertebrates

Conclusion: The memory and learning of invertebrates has been widely investigated. It has been shown that invertebrates are capable of learning in several ways very similar to vertebrates: for example, slugs are capable of first- and second-order conditioning, blocking, one-trial associative learning and appetitive learning (Yamada *et al.*, 1992). In a comprehensive review of invertebrate learning and memory, Carew and Sahley (1986, p. 473) were so impressed by the learning capabilities of invertebrates they were moved to write -

"In fact, the higher-order features of learning seen in some invertebrates (notably bees and *Limax*) rivals that commonly observed in such star performers in the vertebrate laboratory as pigeons, rats, and rabbits."

2.2. Nociception and Pain in Invertebrates

Summary: In respect to brain and nervous complexity, there is no doubt that invertebrates have simpler nervous systems than vertebrates, but does this mean they are unable to suffer? The cerebral cortex is thought to be the seat of consciousness in humans (Smith and Boyd 1991). In fact, pain and suffering are sometimes defined in terms of neural activity in the cerebrum, which makes it a rather circular argument to then dismiss the possibility of invertebrates being capable of suffering because they lack such a structure. It is possible that other structures, as yet undetermined, within the brain or elsewhere fulfil a similar function to the cerebrum in terms of processing information related to suffering. Analogous yet disparate structures have evolved throughout the animal kingdom. For example, the compound eye of some invertebrates is strikingly different in form from the mammalian eye, yet they both achieve the same function - they allow the animal to perceive light. Parts of the nervous system of invertebrates that are not the anterior brain are capable of controlling breathing, movement and learning (e.g. octopuses, cockroaches). Possibly, areas of invertebrate nervous tissue have evolved abilities analogous to the cerebrum of mammals and give these animals the capacity to suffer. Above all, we should remember that absence of evidence is not evidence of absence.

Conclusion 1: It is often suggested that indicators of an animal's capacity to experience suffering include long-term memory, plasticity of behaviour, and 'higher' learning. Many invertebrate species:

- Possess short and long term memory;
- Exhibit higher learning such as social learning, conditioned suppression, discrimination and generalisation, reversal learning;

- Show great spatial awareness and form cognitive maps (possibly indicating self-awareness);
- Appear to show deception (possibly indicating they possess a theory of mind);
- Perform appropriately in operant studies to operate a manipulandum or change the environment in some way to gain reinforcement or avoid punishment.

Conclusion 2: Regarding the possibility of invertebrates experiencing pain, many invertebrate species:

- possess receptors sensitive to noxious stimuli connected by nervous pathways to a central nervous system;
- possess brain centres;
- possess nervous pathways connecting the nociceptive system to the brain centres;
- possess receptors for opioid substances;
- after having had analgesics, modify their responses to stimuli that would be painful for a human;
- respond to stimuli that would be painful for a human in a functionally similar manner (that is, respond so as to avoid or minimise damage to the body);
- show behavioural responses that persist and show an unwillingness to resubmit to a painful procedure; they can learn to associate apparently non-painful with apparently painful events.

2.3. Non-vertebrate groups

2.3.1. *Cyclostomes (lampreys and hagfish).*

Conclusion: Cyclostomes have a pain system similar to that of other fish and brains which do not differ much from those of some other fish.

Recommendation: Cyclostomes should be in Category 1 (see Section 2.5) and so receive protection.

2.3.2. *Amphioxus*

Conclusion: In general, insufficient is known about whether amphioxus are able to experience pain and distress

Recommendation: Given our present state of knowledge amphioxus should be in Category 3 (see Section 2.5) and not receive protection at present.

2.3.3. *Tunicate*

Conclusion: Free swimming larval forms and pelagic adult tunicates show responses which may indicate complex processing of stimuli but little information on this topic is available. The free-swimming adult and larval tunicates are similar in form and in some aspects of behaviour to amphibian tadpoles but most are smaller.

Recommendation: Given our present state of knowledge tunicates should be in Category 3 (see Section 2.5) and not receive protection at present.

2.3.4. *Hemichordata such as Balanoglossus*

Conclusion: *Balanoglossus,* the acorn worm, lives on the bottom in marine environments. There is no indication from its behaviour that it has any sophisticated brain function.

Recommendation: Given our present state of knowledge *Balanoglossus* should be in Category 2 (see Section 2.5) and not receive protection.

2.3.5. *Cephalopods (octopods, squid, cuttlefish, nautiloids)*

Conclusion: There is evidence that cephalopods have a nervous system and relatively complex brain similar to many vertebrates, and sufficient in structure and functioning for them to experience pain. Notably, they release adrenal hormones in response to situations that would elicit pain and distress in humans, they can experience and learn to avoid pain and distress such as avoiding electric shocks, they have nociceptors in their skin, they have significant cognitive ability including good learning ability and memory retention, and they display individual temperaments since some individuals can be consistently inclined towards avoidance rather than active involvement. Most work on learning ability has been carried out in the non-social but visually very competent *Octopus vulgaris*. All squid, cuttlefish and octopods (coleoid cephalopods) studied have a similar ability to sense and learn to avoid painful stimuli, and many are more complex and more likely to experience pain and distress than *O. vulgaris*. Learning is involved in most signalling and the most elaborate signalling and communication systems occur in cuttlefish and squid that can show rapid emotional colour changes and responses to these. Indeed many of these animals live in social groups and hence may have levels of cognitive ability like those of vertebrates that have complex social relationships. Nautiloids have less complex behaviour than coleoid cephalopods and much less is known about their learning ability. They use odour discrimination to find mates and respond to and track other individuals of their own species (Basil 2001, 2002) but little is known about their pain system and it is not clear whether they are as capable of suffering as other cephalopods. However, they live for a long time and are active pelagic animals so we cannot be sure about their level of awareness.

Recommendation: All cephalopods should be in Category 1 (see Section 2.5) and so receive protection.

2.3.6. *Land gastropods*

Conclusion: Snails and slugs can show quite complex learning but the relatively slow locomotion of most of them does not enable them to show rapid escape responses, except for localised movements like eye withdrawal. The case for a substantial degree of awareness would appear to be weak.

Recommendation: Given our present state of knowledge land gastropods should be in Category 2 (see Section 2.5) and not receive protection

2.3.7. *Tectibranch and nudibranch molluscs*

Conclusion: The most active marine gastropod molluscs are the tectibranchs, such as *Aplysia* and some of the nudibranchs (sea slugs). Much research has been carried out on the nervous system of *Aplysia* and it relatives. Evidence of learning and flexibility of behaviour is considerable but there are also studies showing very rigid responses. Nudibranchs appear to be less flexible than some tectibranchs.

Recommendation: Given our present state of knowledge tectibranch and nudibranch molluscs should be in Category 2 (see Section 2.5) and not receive protection.

2.3.8. *Social insects*

Conclusion: The social ants and bees, and to a lesser extent the wasps and termites, show considerable learning ability and complex social behaviour. There is evidence of inflexibility in their behaviour but the trend in recent research has been to find more flexibility. The small size of the brain does not mean poor function as the nerve cells are very small. A case might be made for some bees and ants to be as complex as much larger animals. They might be aware to some extent but we have little evidence of a pain system.

Recommendation: Given our present state of knowledge social insects should be in Category 3 (see Section 2.5) and not receive protection

2.3.9. *Other insects*

Conclusion: There is a difference in complexity of behaviour between the social and non-social insects. However, learning is clearly possible in these animals. There is little evidence of awareness but few people have looked for it.

Recommendation: Given our present state of knowledge other insects should be in Category 2 (see Section 2.5) and not receive protection.

2.3.10. *Spiders, especially jumping spiders*

Conclusion: In recent years, dramatic evidence has been produced of the sensory processing, analytical and prediction ability of salticid spiders. The eyes are large and complex and although the brain is composed of a relatively small number of cells, the level of processing is considerable and sophisticated, if rather slow. Evidence for awareness is greater than in any other invertebrates except cephalopods but we have little evidence of a pain system.

Recommendation: Given our present state of knowledge spiders should be in Category 3 (see Section 2.5) and not receive protection at present.

2.3.11. *Decapod crustaceans (lobsters, crabs, prawns etc.)*

Conclusion: The largest of these animals are complex in behaviour and appear to have some degree of awareness. They have a pain system and considerable learning ability. Little evidence is available for many decapods, especially small species. However, where sub-groups of the decapods, such as the prawns, have large species which have been studied in detail they seem to have a similar level of complexity to those described for crabs and lobsters.

Recommendation: All decapods should be in Category 1 (see Section 2.5) and so receive protection.

2.3.12. ***Isopods (woodlice and marine species)***

Conclusion: Learning is clearly possible in these animals and some of them live socially. The degree of complexity of functioning is lower than that of the larger decapods or many insects and spiders.

Recommendation: Given our present state of knowledge isopods should be in Category 2 (see Section 2.5) and not receive protection.

2.3.13. ***Other phyla (e.g. Annelida, Echinodermata, Platyhelminthes, and Nematoda)*** not described above, as well as other Classes, have been considered but are not thought to need protection and therefore have all been placed in Category 2

2.4. Fetal and embryonic animals which might be protected

Summary: Even though the mammalian fetus can show physical responses to external stimuli, the weight of present evidence suggests that consciousness does not occur in the fetus until it is delivered and starts to breathe air. However, events *in utero* can influence the behaviour of the individual once it is born, and some of those effects could be important to its subsequent welfare. Precocial oviparous species present much evidence of being conscious at hatching, and during the last days before hatching.

Conclusions

1. Precocial oviparous species, some of which are breathing at the time of hatching present much evidence of being aware before hatching and during the last days before hatching, perhaps for as much as the last third of their development. They are often capable of independent life if removed from the egg during the last few days before hatching. Altricial oviparous species and species with larval forms do not develop awareness until a later age. For all oviparous species and especially for the many precocial species there is a high risk that fetuses in the egg during the last part of incubation will be affected by some experimental procedures in such a way that their welfare is poor, sometimes because they are suffering pain.

2. Even though the mammalian fetus can show physical responses to external stimuli, the weight of present evidence suggests that consciousness is not the normal state in the fetus until it is delivered and starts to breathe air.

3. It is possible that in a mammalian fetus there might be transient episodes of increased oxygenation above the threshold required to support some aspects of consciousness. We have insufficient knowledge to conclude whether or not this occurs in all, or even any, fetuses. It is clear that there is a risk, perhaps a small risk, that any mammalian

fetus may on occasion be affected by some experimental procedures in such a way that their welfare is poor, sometimes because they are suffering pain.

4. If a mammalian fetus is removed from the mother and starts to breathe, its level of awareness will change to that typical of such animals after parturition.

5. Emotional stresses experienced by a pregnant mother mammal can influence the behaviour of the offspring after it is born and some of those effects could be important to the offspring's subsequent welfare. It may be that the effects are mediated via nutrition or other means from the mother or it may be that the fetus experiences these effects directly.

6. The fetus in oviparous species, especially those which are precocial, can react to and learn from experiences received during the last few days of incubation.

7. For most vertebrate animals and cephalopods, the stage of development at which there is little risk of poor welfare when a procedure is carried out on them is the beginning of the last third of development during incubation or pregnancy. Before that time the risk to animal welfare is not thought to be significant. For some species this may be earlier but we have not been able to compile a database of species and fetal forms at which some form of protection was assessed as being necessary.

8. For fish, amphibians and cephalopods which develop in water, functioning has many similarities to that of adult fish once they start to feed independently rather than being dependent on the food supply from the egg.

9. The protection of the animals recommended to be included as a protected animal in Chapter 2 poses practical problems during the early stages of their development when they will be microscopic.

Recommendations

1. Whenever there is a significant risk that a mammalian fetus or the fetus or embryo of an oviparous animal such as a bird, reptile, amphibian, fish or cephalopod is for any length of time sufficiently aware that it will suffer or otherwise have poor welfare when a procedure is carried out on it within the uterus or egg, such animals should receive protection. The stage of development at which this risk is sufficient for protection to be necessary is that at which the normal locomotion and sensory functioning of an individual independent of the egg or mother can occur. For air-breathing animals this time will not generally be later than that at which the fetus could survive unassisted outside the uterus or egg.

2. Once a fetus is removed from the uterus or egg, if it is capable of breathing such animals should receive protection.

3. As a guideline, and because of the risk that even mammals in utero may sometimes be aware at times before parturition, when a procedure is performed on a fetus that is likely to produce pain in the newborn of that species, adequate anaesthesia and analgesia should be given provided that the agents used do not significantly increase the likelihood of fetal mortality. In the circumstance where no suitable anaesthetic or analgesic agents are available, procedures should not be carried out on such fetuses.

When the procedure might cause a lasting inflammatory response that persists post-natally, protection should be given against pain and suffering.

4. A schedule of anaesthetics and analgesics that are suitable for use in pregnant animals, oxygenated fetuses and newborn animals should be prepared.

5. Protection against pain and distress during any procedures that might cause these, should be given to any precocial birds or reptiles, for example domestic chicks, that are breathing before hatching.

6. In order to avoid the risk that a fetus, whether it is developing in the mother or in an egg outside the mother, will be affected by some experimental procedures in such a way that its welfare is poor, sometimes because it is suffering pain, it should receive protection if it is in the last third of its development during incubation or pregnancy. This recommendation should be taken together with those above in order that any species at an appropriate stage of development will be protected.

7. Protection may need to be given against emotional states in pregnant mothers to safeguard subsequent behavioural modification and welfare of the offspring. This needs to be considered on a case-by-case basis.

8. In order to avoid the risk that a fish, amphibians, cephalopods or decapods will be affected by some experimental procedures in such a way that its welfare is poor, sometimes because it is suffering pain, it should be included in the list of protected animals receive protection if it is capable of feeding independently rather than being dependent on the food supply from the egg. This food supply is carried around by young fish etc. after emerging from the egg but the young animal is not independent of it for some time. The point of development at which complex function is possible is predicted well by independent feeding.

2.5. Implications for the definition of a “protected animal”

While the principal reason for the existence of legislation is to harmonise the implementation of the Three Rs of Replacement, Reduction and Refinement. This would imply that it is important to define the term “protected animal” and other animal forms which are to be protected during experimental and other research work.

When experiments are carried out in vivo (literally meaning scientific procedures involving a living animal with its whole body systems intact) a key point is whether the animal is able to experience pain and distress and other forms of suffering. The inclusion, therefore, of invertebrates and fetal forms from certain stages of gestation, as well as vertebrates, based on the information given in Chapter 2, is essential information for risk management. The WG have tried to give guidance on that issue with the criteria used to do so. The use of terms such as free-living, capable of independent feeding etc are fraught with difficulties as they do not allow all animals forms at all stages of development to be clearly distinguished on the basis if their ability to experience pain, distress etc. There are however, some worthwhile analogies that can be made, so that more complex forms are more likely to be sentient than simple forms i.e. independent feeders are more likely to be sentient than sessile free living forms,

The WG is proposing therefore, that three categories be established.

Category 1 - The scientific evidence clearly indicates that those groups of animals are able to experience pain and distress, or the evidence, either directly or by analogy with animals in the same taxonomic group(s), are able to experience pain and distress.

Category 2 - The scientific evidence clearly indicates that those groups of animals are NOT able to experience pain and distress, or the evidence, either directly or by analogy with animals in the same taxonomic group(s), are unable to experience pain and distress.

Category 3 - Some scientific evidence exists that those groups of animals are able to experience pain and distress, either directly or by analogy with animals in the same taxonomic group(s), but it is not enough to make a reasonable risk assessment on their sentience to place them in either Category 1 or 2.

Any such categorisation of animals and their forms will need updating as scientific knowledge accumulates.

3. QUESTION ON PURPOSE-BRED ANIMALS

Including a species as "purpose-bred" within Annex I will confer a considerable degree of assurance that animals of that species will be provided with suitable accommodation, welfare and care practices. As a consequence of health and colony management within breeding establishments, there can be improved confidence in the quality of the animal, resulting in improved science and a reduction in animal numbers required. Taking these factors in isolation, for the great majority of scientific investigations, there would be welfare and scientific merit in recommending that all animals used in scientific procedures be purpose-bred. Before making such a recommendation, there are a number of other important factors that have to be considered and there will have to be exceptions to this in some areas of research e.g. studies into the normal biology of a species, commercial strains and veterinary clinical research. The consequences of inclusion of all species could, for example, result in loss of genetic diversity, the generation of large numbers of surplus animals and significant delays in scientific progress, breeding wild animals in captivity could be detrimental to their health and welfare.

A risk assessment approach has therefore been taken to this issue, with the group analysing the potential benefits and adverse consequences of inclusion of each species in Annex I.

3.1. Key criteria to be considered for being purpose bred and inclusion in Annex I:

1. <u>Other legislation already protecting animal welfare</u> - Absence of any relevant animal welfare legislation is a reasonable criterion for inclusion into Annex I.

2. <u>Genetically altered animals</u> - Welfare requirements for GAA are more likely to be met if purpose bred.

3. <u>Health and genetic fidelity of animals</u> - Animals that are purpose bred are likely to be of high health status and genetic fidelity.

4. <u>Demand</u> - Species with low or widely fluctuating demands are reasons for not including in the Annex I.

5. <u>Extrapolation of results to farming or to wild populations</u> - Species primarily used in studies where the data are extrapolated, for example, to commercial farming production, or ecological studies in wild animals, is a reason for not including them in Annex I.

6. <u>Capture from the wild</u> - Capturing a species from the wild for use in a laboratory is a major welfare concern and is, therefore, an important criterion for inclusion of the species in Annex I. Purpose breeding primates may in some cases be the only alternative source to capture in the wild.

3.2. Conclusions and Recommendations

Specific conclusions and recommendations with regard to species where changes might be made to their particular purpose bred status are given in the Tables from the Scientific Report (Appendices 1 - 7). See below.

Conclusion 1: Purpose-breeding is considered to be an important measure of producing high quality animals for research, to minimise inter-animal variability thus reducing the overall number required, and to promote improved welfare for the animals as well as the scientific outcomes. Therefore, the most appropriate animals in most cases will be purpose bred.

Recommendation 1: For most areas of research it is appropriate that the animals used should be of a uniform standard so that there is good and effective controls over the animals' genetic fidelity, microbial status, nutrition, socialisation to humans and other animals (e.g. ferrets, dogs and even rodents) and environment. The most appropriate animals should be used for research. In most cases, these will be purpose bred. The use of non-purpose breed animals will require appropriate justification.

Conclusion 2: Purpose breeding some species of animals that are not frequently used, or that are needed for a narrow area of research, or whose demand fluctuates widely, or that are protected by other legislation, or that have long gestation periods, could all result in difficulties in obtaining suitable animals for research programmes. At best this could delay scientific progress and could result in the abandonment of some research programmes.

Recommendation 2: Exceptions should be made to purpose breeding when it is necessary for the research that a particular strain or breed is used, or that scientific progress would be unduly delayed providing that the scientific data resulting from such research was of good quality, i.e. the competent authorities should consider the potential adverse consequences for research should an exemption for the use of non-purpose bred animals be refused (Council Directive 86/609/EEC: Article 19(4)).

Conclusion 3: Welfare requirements for genetically altered animals are more likely to be met if they are purpose bred.

Recommendation 3: Genetically altered animals should be purpose-bred unless an exemption is authorised by the Competent Authority. An exemption should only be approved where good evidence is provided that any genetic alteration does not cause the animals pain, suffering, distress or lasting harm, and is unlikely to cause such suffering in subsequent generations.

Conclusion 4: The process of genetic alteration can produce, either intentional adverse effects, or as an unexpected consequence of the alteration produce unexpected adverse effects, both of which require that animals are provided with specialist husbandry and care. Failure to provide appropriate accommodation and care practices could adversely affect animal welfare and scientific outcomes.

Recommendation 4: Genetically altered animals of all protected species and forms should be added to Annex I but can be exempted if it is shown that there are, or likely to be, no serious adverse effects on the animals in their future environment and the way they are used (e.g. future breeding programmes).

Conclusion 5: Because the welfare of the animals and the scientific validity of the data are inextricably linked with good quality care and husbandry of animals it is important that all those who come into contact with the animals are adequately educated, trained and skilled on an ongoing basis. This is more likely to happen when animals are purpose bred.

Recommendation 5: In registered breeding and supplying establishments personnel should be properly trained and only competent staff should be given responsibility for the care and husbandry of animals.

Conclusion 6: Inclusion of a species in Annex I requires that animals will be purpose-bred for research purposes. The inclusion of such an Annex is considered to have welfare and scientific benefits. The review of all the commonly used laboratory species has concluded that with the exception of quail (*Coturnix coturnix*) all the other species listed should continue to be purpose-bred. The review also concluded that some further species should be added.

Recommendation 6: The criteria for purpose bred animals and the current guidelines on accommodation and care included in Annex II (and any revision) which is expected in the future to be revised to reflect the revised Appendix A of Council of Europe Convention (1986) ETS 123 should apply irrespective of the origin of the experimental animals. In making this recommendation it is appreciated that in practice not all establishments will at present meet these criteria, but nonetheless all establishments should be strongly encouraged to make progress towards these in a timely manner.

Conclusions in relation to specific species used in research

Hamsters

Conclusion 7: Syrian hamsters are the most commonly used of all the 'hamster types' and, at present, are included in Annex I. However, from an analysis of scientific papers through PUBMED, Chinese hamsters are also commonly used, and only very few European and Djungarian hamsters.

Arguments against inclusion of all hamster species: The small numbers of European and Djungarian hamsters used would make difficulties to match supply and demand leading to delays in scientific programmes

Arguments for inclusion of all hamster species: It would be likely that there would be an improved and more uniform health quality. Moreover no other welfare legislation exists.

Recommendation 7: Retain Syrian hamsters and include Chinese hamsters. No compelling need to include any other hamster species.

Gerbils

Conclusion 8: The commonest gerbil used in research is the Mongolian *(Meriones unguiculatus)* which is not in Annex I.

Arguments against inclusion: Difficulties to match supply and demand that may lead to some delays in scientific programmes;

Arguments for inclusion: Better and more uniform health quality; improved accommodation leading to reduced behavioural abnormalities; no other suitable welfare legislation

Recommendation 8: To include Mongolian gerbils in Annex I *(Meriones unguiculatus*).

Quail

Conclusion 9:

<u>*Arguments for inclusion*</u>: There may possibly be better protection for quail if listed in Annex I, through improved accommodation and care practices.

<u>*Arguments against inclusion*</u>: Small numbers of *Coturnix coturnix* used. Few breeding establishments – difficult to match supply and demand.

Recommendation 9: There is no compelling need to retain *Coturnix coturnix*, nor to include any other species of quail.

Xenopus species (laevis and tropicalis), Rana species (temporaria and pipiens)

Conclusion 10:

<u>*Arguments against inclusion:*</u> Wide range of species but for many species only small numbers are used. Production of the less commonly used species, *e.g.* newts, salamanders (including axolotls) may not be practicably viable due to the very small numbers used. The purpose breeding of *Xenopus laevis* and *tropicalis* may prove to have economies of scale that make it viable. Potentially high cull rates, difficulties to match supply and demand leading to delays in scientific programmes, lack of information on husbandry and care practices.

<u>*Arguments for inclusion:*</u> better and more uniform health quality, increasing numbers of some species, no other welfare legislation, elimination of zoonotic diseases, no animals taken from wild.

Recommendation 10: *Xenopus* species (*laevis* and *tropicalis*) and *Rana* (*Rana temporaria* and *R. pipiens*) should be purpose bred.

Invertebrates such as cephalopods, cyclostomes, decapods.

Conclusion 11: The recommendation from Chapter 2 is for these phyla to receive protection during experimental work due to their potential to experience pain and distress.

Recommendation 11*:* If the recommendations put forward in Chapter 2 are accepted, there is no compelling need to include any of these invertebrate species, at the moment, in those to be purpose bred.

4. QUESTION ON HUMANE METHODS OF EUTHANASIA

4.1. Reasons for euthanasia:

The reasons for killing animals have also to be considered, as some methods may cause more pain and distress than others. For example, breeding more animals than are required simply to have them available on demand, and then killing those that have not been used. This is especially true for animals that have a painful harmful defect caused for example by a genetic alteration. Sometimes killing of surplus is inevitable as in the breeding of some transgenic or mutant animals as only a particular genotype is wanted, and uses cannot be found for the surplus animals. On other occasions, breeding strategies can avoid having to kill such large numbers, but can also increase the numbers that have to be killed due to a balance between inducing adverse effects in all animals as opposed to just some. Archiving (freezing down) rodent strains that are currently unwanted is a way of reducing the number of animals to be culled, as is accurately forecasting the number of animals to be used.

Recommendation: One way in which any poor welfare during euthanasia could be avoided is to not have to kill animals in the first place. Therefore, the production of animals should be carefully considered so that an avoidable surplus is not generated.

4.1.1. *Scientific reasons*

Occasionally, after considering all available methods, animals may have to be killed using methods that do not meet the animal welfare criteria set out for a humane method of killing for scientific reasons e.g. using some of the recognised methods may interfere with the scientific outcome. In a choice between two or more methods of humane killing, pilot studies may be carried out to determine the method that is most suitable for the scientific purpose and for the animals concerned. This may not always be the traditional method as new methods come along, or more information is gained on old methods questioning its humaneness, or its impact on the animal, its scientific validity and, therefore, its suitability. If animals are killed using less than ideal methods then that should be justified and taken into account when carrying out the harm (cost) benefit analysis. Some methods are listed in the report that cannot be considered humane, and are identified as such. For others, where there is a lack of information, that is addressed in future research.

Because the numbers of animal killed at any one time can range from one to several hundred, the method should be appropriate to dealing with both ends of the scale, again with the minimum distress to the animals as well as to the human operators.

Recommendation 1: In a choice between two or more methods of humane killing, the scientist should choose the most appropriate and humane but where this is not known pilot studies should be carried out.

As all methods have a margin of error it is important that death is confirmed, and if necessary ensured by the use of a method, such as exsanguination, freezing, or some physical insult that results in an irreversible destruction of the brain or central nervous system, or permanent cessation of the heart.

Recommendation 2: The death of an animal should be confirmed by a method that results in an irreversible destruction of the brain or permanent cessation of the heart.

4.2. Education, training and competence of those carrying out humane killing:

It is important that those carrying out such methods of killing are suitably trained and are deemed competent in that method (Council of Europe 1993). As nearly all methods require an element of restraint, it is equally important that they are competent in handling animals humanely.

The attitude of persons carrying out humane killing is important as over-sensitivity or a lack of care is more likely to result in poor welfare for the animals concerned. Killing animals in research establishments has been described as a kind of “initiation right” for animal care staff, and appropriate help and guidance should be available to guide young persons who are asked to do it (Arluke 1993, 1996). If senior staff members treat animals without sufficient respect, habits which lead to poor welfare may be formed in younger staff members. No-one should be coerced to kill animals, so scientists and others should be sensitive to the fact that those looking after animals did not enter this area of work to kill them; it is seen as an unavoidable, unpleasant aspect of animal care in research.

Recommendation 1: The humane killing of animals for *in vitro* and *ex vivo* research should be addressed so that persons carrying out such work are trained and competent.

Recommendation 2: A training plan should be drawn up, particularly for the use of physical methods that require a measure of manual skill, such as cervical dislocation or concussion, should incorporate a progression from the use of freshly killed animals, to anaesthetised animals, before going on to kill conscious animals. In that way there is less chance of poor welfare and poor scientific outcome due to poor technique.

4.3. Killing animals for their tissues:

Killing animals to retrieve tissues for *in vitro* work is outside the existing EU Directive (86/609/EEC), but such a use of animals is included in some countries (e.g. The Netherlands, Germany), and the number of animals used is counted giving an indication of the level of *in vitro* research by the scientific community. By including those animals killed for their tissues, the total annual number of animals used in research in those countries increased by 10 to 15%. Even though this use of animals is outside the Directive, there is EU and other national guidance on the ways by which animals should be humanely killed under laboratory conditions. Consequently, at present, research work involving killing animals by a recognised and approved method would permit, for example, researchers to kill 100 chimpanzees or dogs for a research purpose, without a licence, without oversight, and without any ethical or scientific approval. As death can be considered to be a lasting harm, it is debatable as to what level of licensing and scrutiny is required, and whether killing should be classified as a regulated procedure. In that case, animals killed for their tissues would receive the same level of care during euthanasia as an experimental animal and the staff would receive appropriate training and be certified competence as for any regulated procedure. Killing sick or injured stock animals could be exempted or encompassed.

Opinion: The humane killing of animals for *in vitro* and *ex vivo* research that, at present, is outside the Directive could cause public concern in regard to the species, the numbers and the competence of those carrying out the killing.

4.4. Gathering information

In order to know how often poor welfare occurs during euthanasia, we need to have quality control procedures and document when things go wrong and why, and what measures have been taken to stop it happening again. It is also important to know how often the method is used successfully so that an overall picture can be gained. This will then inform future risk assessments. At present this sort of information is not available, as it is in abattoirs in some countries.

Recommendation: Information should be collected on methods of euthanasia, e.g. their success rate in terms of an efficient and effective kill and the reasons for failure.

4.5. Methods of euthanasia

<u>General comments applying to all methods</u>:

The WG suggested that the recommended methods can be varied but only with a scientific justification and appropriate authority, i.e. the recommended methods represent the default position.

When pregnant animals are killed, the fetuses should be allowed to die *in utero* before being removed, unless they are required for scientific reasons, in which case they should be considered as neonates and killed by another method that is appropriate for the species and that causes a minimum of pain and distress.

4.5.1. ***Electrical stunning***

Conclusions: Electrical methods, at present, are only used for farm animal species.

Equipment needs to be well maintained to function well.

The outcome depends on many variables including the equipment and the current delivered and also on the particular physical characteristics of the animal that might affect the effectiveness of the method.

Recommendations: Head-only electrical stunning and-head-body killing can be recommended for the following adult species: rabbits, horses, donkeys and cross-bred equidae, pigs, goats, sheep, cattle and birds. Head-body stunning is recommended for fish. After electrical stunning an animal may recover with the consequence that it needs to be exsanguinated to be killed (or another method e.g. cooling down for fish). The unborn fetus will be killed by exsanguination or the cessation of blood supply due to heart failure of the pregnant dam.

Future Research: At present, there is considerable interest in the development in the electrical stunning of fish species. Since electrical techniques are easy to apply it may be worthwhile developing these methods for reptiles and amphibians.

The criteria used to determine a loss of consciousness in amphibia, reptiles, some fish species, and possible some invertebrates are not well known and should be investigated.

4.5.2. *Mechanical stunning methods*

Conclusions: The penetrating captive bolt is an effective method of euthanasia for use in slaughterhouses and in research given adequate facilities in those species of animals in which the captive bolt has been specifically designed.

The equipment needs to be well maintained to function well.

Percussion stunning can be used for several species, however, there may be some doubts about effective stunning and killing in some animals. When correctly performed a concussive blow is very effective for smaller animals with ossified skulls, but it requires skill, confidence and practice (EFSA 2004).

Handling and restraint for concussive methods will cause some distress as the animal will be restrained in an unnatural position.

Recommendations: Concussive methods should not be used on animals with skulls that are not completely ossified or the sutures have not fused.

Future Research (probably depends on species): Water jet and air jet techniques and may be adaptable for many species.

4.5.3. *Mechanical disruption of tissues (Neck dislocation, decapitation, maceration)*

Conclusions:

1. Handling and restraint for neck dislocation and decapitation will cause some distress as the animal will be restrained in an unnatural position and will not be free to escape. Anaesthetising the animal first may reduce this distress.

2. After neck dislocation and decapitation electrical activity of the brain may persist for 13 s during which time animals may feel pain due to afferent stimuli from the trigeminal nerve. Cutting of the skin and tissues of the neck may cause some pain for a short period (less than one second).

3. After cervical dislocation, convulsions only occur when separation is made cranial to the fifth thoracic vertebra, while severance caudal to this location results in paralysis in conscious animals.

4. Mouse fetuses *in utero* are not killed within 20 min when the dam has been killed by cervical dislocation or decapitation. The heads of fetal rodents after decapitation may show signs of consciousness and this would be of welfare concern if the fetus had breathed (see Section 2.4).

5. After decapitation signs of consciousness may persist for some time e.g. 13 min in the heads of eels, and hours in reptiles.

6. If the macerator is overloaded animals may be not be humanely killed.

7. All these mechanical disruption techniques are aesthetically controversial. The interpretation of the electrical activity in the brain after neck dislocation and decapitation is controversial as to what feeling remains, and is still a matter of debate.

8. Anaesthetising animals before decapitation or cervical dislocation will minimise distress and any subsequent pain. This may be required in some cases of maceration where the animal may escape the blades.

9. Tissue damage to the CNS or induced neuronal discharge may affect neuropeptide levels and brain histology.

10. Severance of the spinal cord using a knife does not render the animal immediately unconscious and so it may suffer for some short time.

Recommendations:

1. When using these techniques, cervical dislocation and decapitation, the necessary handling and restraint can be stressful for the animal and anaesthetising them first will minimise distress and eliminate any subsequent pain.

2. A purpose built mechanical device with a sharp blade should be used for decapitation.

3. When pregnant females are killed the fetal forms should be allowed to die *in utero* before being removed, unless they are required for scientific reasons, in which case they should be killed by another method as quickly as possible.

4. Severance of the spinal cord using a knife should not be used.

5. For efficient and effective killing the macerator should not be overloaded.

Future Research: Since there are doubts that some species may not be immediately unconscious after neck-dislocation, alternative techniques should be developed.

4.5.4. *Physical methods*

Conclusions: Focal irradiation of the heads (brain) of restrained small animals with microwaves of 2450 MHz for 1s suggests a rapid loss of consciousness.

Focal heating of the brain by irradiation can only be applied by using a specially and constructed designed microwave oven specific for the species.

Hypothermia is not considered an acceptable method of euthanasia because it prolongs the period of consciousness and does not reduce the ability to feel pain.

Recommendations: Heating the brain focally with appropriately designed microwaves is accepted for use in adult rats and mice by trained operators and can be used for other animals such as guinea-pigs and hamsters when they are less than 300g.

Cooling down should not be used for any species.

Future Research: For many years, techniques using microwaves have been used for local damage of cells in cancer therapy. These techniques could be adapted to locally damage of brain tissue in a variety of species.

4.5.5. *Gaseous methods*

4.5.5.1. Exposure to carbon dioxide mixtures

Conclusions: CO2 is aversive to all vertebrates used in research that have been tested. Some species find even low (10-20% by volume in air) concentrations aversive, regardless of any additions. It cannot be recommended as a sole method of humane killing for any species. CO2 may be used as a secondary euthanasia procedure on unconscious animals.

Mouse fetuses *in utero* are not killed within 20 min even though the mother has been killed with CO2, but it is possible to kill neonatal forms with CO2.

Recommendation: Carbon dioxide should not be used as a sole agent in any euthanasia procedure unless the animal has first been rendered unconscious, i.e. it should be phased out as soon as possible. It is important that equally effective and non-aversive methods that are already partially developed, be developed further from a practical viewpoint, and that users are given time to change to those more humane gas mixtures.

It would be inappropriate to place a fully conscious animal in a known noxious gaseous environment from which it would be unable to escape.

Future Research: Research on euthanasia of animals should follow the guidelines set out by the International Association for the Study of Pain.

New methods of humane killing of animals using gas mixtures other than those containing CO2 need urgently to be developed.

The time to onset of unconsciousness has usually been determined on the basis of behaviour (e.g. ataxia) but needs to be established more clearly using defined neurophysiological criteria.

An objective method of measuring breathlessness is needed to demonstrate and quantify breathlessness in laboratory animals (especially rodents), which would enable quantification of duration and severity of distress in animals exposed to any gas mixture.

4.5.5.2. Argon and Nitrogen as inert hypoxia inducing gases

Conclusions: It is suggested that the use of anoxia as a method of killing is humane for pigs and poultry, and probably rodents, although more practical experience is needed. Because of the high affinity for oxygen of haemoglobin in fetal and neonatal animals it may take longer than in mature animals of the same species to kill. However, no studies on time taken or welfare seem to have been carried out. More research is needed on nitrogen.

Recommendations: Research into hypoxic gas mixtures should be carried out as a matter of urgency, especially practical methods for small animals, such as rodents.

Future Research: Investigation is needed into the humaneness of killing with hypoxic and anoxic gas mixtures.

4.5.5.3. Nitrous oxide

Conclusions: Owing to human health and safety concern, nitrous oxide is not suitable for euthanasia.

Recommendations: (see Tables 1- 8)

Future Research: (probably species driven)

4.5.5.4. Carbon monoxide

Conclusions: Owing to human health and safety concern, carbon monoxide has a high risk for killing humans.

Recommendations: Under controlled conditions carbon monoxide can be used for dogs, cats and mink, however it is not recommended due to concerns for human health and safety, and also animal welfare.

4.5.5.5. Overdose of inhalation anaesthetic gases

Conclusion: Overdose of an established inhalational anaesthetic agent at a suitable concentration may cause minor distress in some species, but all such gases may be aversive at high concentrations. However, they have the advantage that restraint for administration is unnecessary.

Mouse fetuses *in utero* are not killed within 20 min even though the dam has been killed with an overdose, but neonatal forms (1-7 do) are killed.

Recommendation: Overdose of an inhalation anaesthetic agent should be considered as a humane way of killing animals providing some of the caveats relating to aversion and concentration are taken into consideration.

Future Research: Aversion testing may need to be carried out in some species for some agents (e.g. ferrets).

4.5.5.6. Overdose of injectable anaesthetic agents

Conclusion 1: Overdose of any anaesthetic agent may well be acceptable but all agents have some drawbacks in terms of irritancy and necessary restraint for administration. Suitable for mouse neonates (8-14 do) but not fetuses *in utero*.

Conclusion 2: In some member states some chemicals for euthanasia that cause a minimum of pain and distress may not be available.

Recommendation 1: Overdose of an injectable anaesthetic agent should be considered as a humane way of killing animals providing some of the caveats relating to aversion, irritancy and restraint are taken into consideration.

Recommendation 2: Member states should try to ensure that suitable chemicals for euthanasia are available.

4.5.5.7. Lethal injection of non-anaesthetising chemicals including: Neuromuscular blocking agents; Magnesium sulphate; Potassium chloride; Exposure to Hydrogen cyanide (HCN) gas; Ketamine; T-61

Conclusion: the administration of a non-anaesthetising chemical is potentially a major welfare problem.

Recommendation: Lethal injection of non-anaesthetising chemicals should only be administered in unconscious animals.

4.6. Humane killing of cephalopods, cyclostomes, decapods (if accepted)

Decapods include several kinds of crabs, lobsters and crayfish. Neither the number of crustaceans or cephalopods used in research is known and nor the methods of killing them are known. Although humane killing of crustaceans for food is not a statutory requirement in Europe, animal welfare organisations have provided some guidelines, for example, UFAW, RSPCA). In some countries, for example New Zealand, humane killing of some species of crustaceans is covered under the Animal Welfare Act 1999.

Recommendations:

The following methods cause a minimum of pain and distress:

- Chilling in air
- Chilling in ice/water slurry
- Immersion in a clove oil bath
- Electrical methods

The following methods are likely to cause pain and distress:

- Any procedure involving the separation of the abdomen (tailpiece) from the thorax (tailing) or removal of tissue, flesh or limbs while the crustacean is still alive and fully conscious (including when in a chilled state).
- Placing crustaceans in cold water and heating the water to boiling point.
- Placing live crustaceans into hot or boiling water.
- Placing live marine crustaceans in fresh water.
- Unfocussed microwaves to body as opposed to focal application to the head.

5. Tables with the recommended methods for the humane killing of animals in the laboratory.

Adapted and modified Tables from Close et al. (1996/1997)

The following tables have been taken from the previous EU Report on euthanasia, and form the basis for methods of killing laboratory animals that involve a minimum level of pain and distress. The data have been largely retained and only a few recommendations have been changed. (These tables in the scientific report are numbered as 7 to 14)

Table 1 - Characteristics of methods for euthanasia of fish

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
MS-222	++	++	++	++	++	5	Acceptable
Benzocaine	++	++	++	++	++	5	Acceptable
Etomidate	++	++	++	++	++	5	Acceptable
Metomidate	++	++	++	++	++	5	Acceptable
Electrical	++	+	+	+	++	4	Acceptable for some species
Maceration	++	++	++	++	+	4	Only for fish less than 2 cm in length
Quinaldine	++	++	++	+	++	4	Difficult to obtain in Europe
Concussion	++	+	+	++	-	3 *	Death to be confirmed Acceptable for use by experienced personnel
Sodium pentobarbitone	++	++	-	+	++	3	May be useful for large fish, intraperitoneal injection
Cervical dislocation	++	++	+	++	-	3	Not in large fish. To be followed by destruction of the brain
Halothane	+	+	++	++	++	2	Other methods preferable. Death to be confirmed

Changed from Close et al. * was 4

The following methods may only be used on unconscious fish: pithing, decapitation and exsanguinations

The following methods are not to be used for killing fish: removal from water, whole body crushing, hypothermia, hyperthermia, 2-phenoxyethanol, carbon dioxide, diethyl ether, secobarbital, amobarbital, urethane, chloral hydrate, tertiary amyl alcohol, tribromoethanol, chlorobutanol, methyl pentynol, pyridines, electrical stunning only for some species.

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value**: ++ good aesthetically, +acceptable for most people, - unacceptable for many people**. Rating:** 1-5 with 5 as highly recommended

Table 2 - Characteristics of methods for euthanasia of amphibians

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
MS-222	++	++	++	++	++	5	Acceptable
Benzocaine	++	++	++	++	++	5	Acceptable
Sodium pentobarbitone	+	++	-	+	+	4	Involves handling and intravenous or intraperitoneal injection
Concussion	++	++	+	++	- *	3 **	Acceptable for use by experienced personnel
T-61	+	++	-	+	+	3	Involves handling and intravenous injection
Microwave	++	++	-	+	++	3	Only for small amphibians. Not a routine procedure
Electrical stunning	+	+	+	-	-	2	To be followed immediately by destruction of the brain

Changed from Close et al. * was +, ** was 4

The following methods are only to be used on unconscious amphibians: pithing and decapitation

The following methods are not to be used for killing amphibians: hypothermia, hyperthermia, exsanguination, strangulation, carbon dioxide, diethyl ether, chloroform, volatile inhalational anaesthetics, chloral hydrate, ketamine hydrochloride, chlorbutanol, methylpentynol, 2-phenoxyethanol, tertiary amyl alcohol, tribromoethanol and urethane

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Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value**: ++ good aesthetically, +acceptable for most people, - unacceptable for many people**. Rating:** 1-5 with 5 as highly recommended

Table 3- Characteristics of methods for euthanasia of reptiles

Agent	**Rapidity**	**Efficacy**	**Ease of use**	**Operator safety**	**Aesthetic value**	**Overall rating (1-5)**	**Remarks**
Sodium pentobarbitone	++	++	++	+	++	5	Acceptable, but involves handling
Captive bolt	++	++	++	+	+	5	Acceptable for large reptiles
Shooting	++	++	++	-	+	4	Acceptable only in field conditions
Concussion	+	+	+	++	-	3**	Acceptable for use by experienced personnel To be followed by destruction of the brain

Changed from Close et al. * was +; was 4

The following methods are to be used on unconscious reptiles only: pithing and decapitation

The following methods are to be used on unconscious reptiles only: pithing and decapitation
The following methods are not to be used for killing reptiles: spinal cord severance, hypothermia, hyperthermia, exsanguination, chloroform, MS-222, ether, halothane, methoxyflurane, isoflurane, enflurane, carbon dioxide, neuromuscular blocking agents, ketamine hydrochloride, chloral hydrate and procaine

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Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value**: ++ good aesthetically, +acceptable for most people, - unacceptable for many people**. Rating:** 1-5 with 5 as highly recommended

Table 4 - Characteristics of methods for euthanasia of birds

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
Sodium pentobarbitone	++	++	+	+	++	5	Acceptable
T-61	++	++	+	+	++	4	Requires expertise: acceptable for small birds only (<250 g)
Inert gases (Ar, N2)	++	++	++	++	+	4	Acceptable. But more research needed for nitrogen
Halothane, enflurane, isoflurane	++	++	++	+	++	4	Acceptable
Maceration	++	++	++	++	-	4	Acceptable for chicks up to 72 h
*Cervical dislocation decapitation	++	++	-	++	- *	3 **	Acceptable for small and young birds (<250 g) if followed by destruction of the brain
Microwave	++	++	-	++	+	3	To be used by experienced personnel only and specific equipment. Not a routine procedure
Concussion	++	++	-	++	-	3	Acceptable
Electrocution	++	++	+	-	-	3	Danger to operator. Use of special equipment Other methods Preferable
Carbon monoxide	+	+	++	-	-	1	Danger to operator

Changed from Close et al. * was +; was 4

The following methods may only be used on unconscious birds: decapitation, pithing, nitrogen, potassium chloride.

The following methods are not to be used for killing birds: neck crushing, decompression, exsanguination, carbon dioxide, nitrous oxide, diethyl ether, chloroform, cyclopropane, hydrogen cyanide gas, trichlorethylene, methoxyflurane, chloral hydrate, strychnine, nicotine, magnesium sulphate, ketamine and neuromuscular blocking agents

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value**: ++ good aesthetically, +acceptable for most people, - unacceptable for many people**. Rating:** 1-5 with 5 as highly recommended

Table 5 - Characteristics of methods for euthanasia of rodents

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
Halothane, enflurane, isoflurane	++	++	++	+	++	5	Acceptable
Sodium pentobarbitone	++	++	+	+	++	5	Acceptable
T-61	++	++	-	+	++	4	Only to be injected intravenously
*Inert gases (Ar)	++	+	++	+	+	4	Acceptable
Concussion	++	++	+	++	-	3	Other methods preferred; Acceptable for rodents under 1 kg. Death to be confirmed by cessation of circulation
Cervical dislocation	++	++	+	++	-	3	Other methods preferred; Acceptable for rodents under 150g Death to be confirmed by cessation of circulation
Microwave	++	++	-	++	+	3	To be used by experienced personnel only. Not a routine procedure
Decapitation	+	+	+	++	-	2	Other methods preferred
*Carbon dioxide	+	++	++	+	++	1 if sole agent 5 if animal unconscious	To be used when animal unconscious i.e. overall rating then based on the method to induce unconsciouness
Carbon monoxide	+	+	+	-	++	1	Danger to operator
Rapid freezing	-	+	++	++	-	0	Not acceptable

** Changed from Close et al.*

The following methods may only be used on unconscious rodents: rapid freezing, exsanguination, air embolism, potassium chloride and ethanol

The following methods are not to be used for killing rodents: carbon dioxide (when sole agent, but urgent research need for a replacement), hypothermia, decompression, strangulation, asphyxiation, drowning, nitrogen, nitrous oxide, cyclopropane, diethyl ether, chloroform, methoxyflurane, hydrogen cyanide gas, trichlorethylene, strychnine, nicotine, chloral hydrate, magnesium sulphate and neuromuscular blocking agents

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Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value**: ++ good aesthetically, +acceptable for most people, - unacceptable for many people**. Rating:** 1-5 with 5 as highly recommended

Table 6 - Characteristics of methods for euthanasia of rabbits

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
Sodium pentobarbitone	++	++	++	+	++	5	Acceptable
T-61	++	++	-	+	++	4	Acceptable. Intravenous injection only
Captive bolt	++	++	-	+	+	4	Requires skill. Death to be confirmed by another method
Cervical dislocation	++	++	-	++	-	3	Acceptable for rabbits under 1 kg. Sedation prior to dislocation. Death to be confirmed by cessation of circulation
Concussion	++	+	-	++	-	3	Expertise required. Death to be ensured by another method
Electrical stunning	++	+	++	-	+	3	Death to be confirmed by another method
Microwave	++	++	-	++	+	3	To be used by experienced personnel only on small rabbits. Not a routine procedure
Decapitation	+	+	+	-	-	2	Acceptable for rabbits under 1 kg if other methods not possible
Halothane, enflurane, isoflurane	++	++	++	+	-	2	Rabbits show signs of distress
Carbon monoxide	+	+	++	-	++	1	Danger to operator
Rapid freezing	+	+	++	++	+	1	Only in fetuses under 4 kg. Other methods preferred

Changed from Close et al.: CO2 deleted

The following methods are only to be used on unconscious rabbits: exsanguination, nitrogen, potassium chloride and air embolism.

The following methods are not to be used for killing rabbits: carbon dioxide, hypothermia, decompression, strangulation, asphyxiation, drowning, nitrous oxide, cyclopropane, diethyl ether, chloroform, trichlorethylene, hydrogen cyanide gas, methoxyflurane, chloral hydrate, strychnine, nicotine, magnesium sulphate, hydrocyanic acid, ketamine hydrochloride and neuro-muscular blocking agents.

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value**: ++ good aesthetically, +acceptable for most people, - unacceptable for many people**. Rating:** 1-5 with 5 as highly recommended

Table 7 - Characteristics of methods for euthanasia of dogs, cats, ferrets, foxes

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
Sodium pentobarbitone	++	++	-	+	++	5	Acceptable. Intravenous injection
T-61	++	++	-	+	+	4	Acceptable but only by slow intravenous Injectioninjection under sedation
Secobarbital/ dibucaine	++	++	-	+	++	4	Acceptable. Intravenous injection
Halothane, isoflurane, enflurane	++	++	+	+	++	4	Acceptable
*Shooting with a free bullet with appropriate rifles and guns.	++	++	-	-	-	4 *	Acceptable only in field conditions by specialized marksmen when other methods not possible
Captive bolt	++	++	-	++	+	3	To be followed by exsanguination
Electrocution	++	++	-	-	-	3	Use only special equipment.To be followed by exsanguination
Concussion	++	++	+	++	-	2	Only to be used on neonates.To be followed by exsanguination

Changed from Close et al. * was 1

The following methods can be used for unconscious carnivores: exsanguination, neck dislocation and potassium chloride , in order to minimise pain and distress.

The following methods are not to be used for killing carnivores: decompression, decapitation, drowning, strangulation, asphyxiation, inert gases, nitrogen, air embolism, striking chest of cats, carbonmonoxide, carbon dioxide, methoxyflurane, nitrous oxide, trichlorethylene, hydrocyanic acid, diethyl ether, chloroform, hydrogen cyanide gas, cyclopropane, chloral hydrate, strychnine, nicotine, magnesium sulphate and neuromuscular blocking agents

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Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value**: ++ good aesthetically, +acceptable for most people, - unacceptable for many people**. Rating:** 1-5 with 5 as highly recommended

Table 8 - Characteristics of methods for euthanasia of large mammals

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
Sodium pentobarbitone	++	++	-	+	++	5	Acceptable by intravenous injection (all species including primates
Quinalbarbitone/ Nupercaine	++	++	-	+	++	5	Effective for horses intravenously
Captive bolt	++	++	+	+	+	5	To be followed by exsanguination
Free bullet using e.g. appropriate ammunition, rifles and guns	++	++	+	-	+	4 *	Experienced marksman. May need a method to ensure death. In field conditions only.
T-61	++	++	-	+	++	4	Acceptable by intravenous injection
**Inert gases (Ar)	++	++	+	+	+	4	Acceptable for pigs only
Electrical stunning	++	++	+	-	-	4	Use only specialised equipment. To be followed immediately by exsanguination
Concussion	++	+	-	+	+	2	To be followed immediately by exsanguination
Halothane, isoflurane, enflurane	+	+	+	+	+	2	Recommended for lambs and kids

Changed from Close et al. CO2 deleted, * was 5, ** introduced, CO2 deleted

The following methods can be used only on unconscious large mammals: exsanguination, chloral hydrate and potassium chloride, in order to minimise pain and distress.

The following methods are not to be used for killing large mammals: carbon dioxide, carbon monoxide, methoxyflurane, trichlorethylene, strychnine, nicotine, magnesium sulphate, thiopentone sodium, ketamine hydrochloride, neuromuscular blocking agents

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Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value**: ++ good aesthetically, +acceptable for most people, - unacceptable for many people**. Rating:** 1-5 with 5 as highly recommended

6. DOCUMENTATION PROVIDED TO EFSA

Letter sent on the 23/07/2004 with ref. DG ENV. C JV/jm D (04) 430238, from Mr Jos Delbeke, from the Directorate-General Environment, Directorate C - Air and Chemicals

Supportive Documents

- The Commission sent, as background information, the EU reference on approved methods for euthanasia (Close *et al*., 1996, 1997).

6.1. REFERENCES

All references are available in the scientific report.

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8. ACKNOWLEDGEMENTS

The working group drafted the scientific risk assessment, which was then reviewed and adopted by the AHAW Panel. The working group was chaired by David Morton on behalf of the AHAW Panel. The members of the working group were:

Questions 1&2 - Chairman Prof. Donald Broom**:** Dr Chris Sherwin, Prof. Neville Gregory and Dr Roddy Williamson

Question 3 – Chairman Dr Xavier Manteca: Prof Stefano Cinotti, Dr David Anderson and Prof. Timo Nevalainen

Question 4 - Chairman Prof David Morton: Dr Mohan Raj and Dr Bert Lambooij

The declarations of conflicts of interest of all participants in this working group will be available on internet, in the EFSA web site (http://www.efsa.eu.int)