

Report of the Specialist Group on the DNA Register/Market Sampling Scheme Approach (SGDNA)

The meeting was held from 7-9 March 2005 at the Southwest Fisheries Science Center (SWFSC), La Jolla, California, USA. The list of participants is given as Annex A.

1. CONVENOR'S OPENING COMMENTS

Perrin (Convenor and Chair) welcomed the participants to the Workshop. The group had been established by the meeting of the RMS (Revised Management Scheme) Working Group at its meeting in Sweden in December 2004. The background to this decision and the Terms of Reference of the SGDNA are given as Annex B. Annex B had recognised that while it was hoped that much work could be undertaken by correspondence, it might be necessary to hold a meeting to develop a comprehensive report. The SGDNA had agreed that holding a meeting was the most efficient way for it to complete its business. For practical reasons (the report has to be available in advance of the next RMS Working Group meeting in Copenhagen in March), it had not been possible to arrange dates at which all nominated members of the SGDNA could attend. Members not attending were Nicky Grandy (Secretariat), Michael Tillman (USA), Koen van Waerebeek (Belgium), and Thomas Lyrholm (Sweden).

It was noted that the task of the SGDNA was to provide technical advice and information to allow the RMS Working Group to progress in its work in drafting text for an RMS. Given that the RMS Working Group had not reached agreement on the final form of any references to DNA registers and market sampling, it was agreed that the SGDNA should provide technical advice to allow text to be developed for options based on three main scenarios: (1) systems under complete national control; (2) systems based on a combination of national control with some degree of international audit/oversight; (3) systems under complete IWC control.

On behalf of the IWC, Donovan thanked the SWFSC for hosting the meeting and the participants for donating their time to this important work.

2. ADOPTION OF AGENDA

The adopted agenda, based on the terms of reference, is given as Annex C.

3. APPOINTMENT OF RAPPORTEURS

It was agreed that Perrin, Morin and Donovan would act as rapporteurs with assistance from others as appropriate. Final editing of the report was carried out by Donovan and Perrin.

4. REVIEW OF DOCUMENTS

A list of new documents is given in Annex D. IWC/N04/RMSWG9 also provided valuable information and suggestions. The Secretariat had circulated relevant background papers to members of the SGDNA in advance of the meeting.

5. SPECIFICATIONS FOR THE ESTABLISHMENT/MAINTENANCE OF DIAGNOSTIC DNA REGISTERS

The SGDNA took as its starting point the existing registers of Norway and Japan. The former has been discussed and approved by the IWC Scientific Committee in the past (e.g. IWC, 1998) and Japan has stated that its register is based on technical specifications similar to those of the Norwegian register. Updated information was provided in IWC/M05/RMSWG/SGDNA2 and 4 with additional information provided by email by Hans Skaug (Norway). The SGDNA was grateful for the cooperation given by Iceland, Japan and Norway with respect to existing and proposed registers and protocols. Unless noted in the report, the guidance here follows the existing protocols. The Scientific Committee has agreed that registers should be diagnostic, i.e. that they should contain DNA profiles of any animals from which products *might* legally appear in the market (e.g. from legal catches, bycatches, ship strikes) on the understanding that products from animals not included in the register(s) would be considered infractions. Information in IWC/M05/RMSWG/SGDNA2 indicates that both the Norwegian and Japanese registers are close to this goal. The most appropriate way to incorporate such specifications into the RMS should be via an operating clause in the *Schedule* with a reference to a dated version of the specifications as discussed earlier by the RMS Working Group.

5.1 Collection of samples

The SGDNA agreed that where possible, samples should be collected by trained personnel. For commercial whaling operations, this should be a nominated individual or individuals – for example, the national inspectors. For whaling undertaken under scientific permits it should be by nominated biologists. The SGDNA recognised that for stranded/bycaught/ship-struck animals it may not always be possible for the samples to be collected by trained personnel. However, written instructions on how to do this should be made widely available; this is already the case for Japan (IWC/M05/RMSWG/SGDNA2); a copy of the Japanese field form was available for inspection. It was noted that in Japan, all bycaught animals must be DNA-registered before products can be sold (IWC/M05/RMSWG/SGDNA2). Information on the regulations and procedures can be found at www.icrwhale.org/pdf/higekujira.pdf.

It was agreed that two samples of skin and muscle of at least 5x5x5mm must be collected from each animal (four if it is decided to establish an IWC tissue archive – see options below). If tissue is collected to be frozen, four muscle samples of 20x20x20mm should be taken and frozen as quickly as possible.

Samples should initially be preserved in 95% ethanol (in at least five times the volume of the sample, due to potential problems of dilution and evaporation) and if practical refrigerated or frozen immediately. If not able to be frozen immediately, the samples should be shipped as soon as possible (preferably within 7 days) to the analysing laboratory. This temporary storage and shipping should be in temperatures <25°C to minimise the possibility of degradation of the sample. Long-term storage of tissue samples should be in 95% ethanol at at least -20°C. In addition to skin and muscle samples in EtOH, if possible four samples of muscle should be collected and frozen in liquid nitrogen; transport should be with dry ice. Long-term storage of frozen tissue samples should be at at least -80°C.

Reliable labelling of the sample is essential. The container should be labelled on both the inside and the outside with a unique identifying code that can be related directly to the biological and other information collected for the individual. The label on the inside must be indelible and insoluble in alcohol to ensure that the number remains legible after storage in ethanol. Similarly the label on the outside must be robust and remain legible if exposed to ethanol or water.

Information collected at the time of sampling must include at least the following: date; sample code; locality; species; body length; sex; name¹ of sampling person.

5.2 Tissue analysis

5.2.1 Extraction of DNA

The SGDNA agreed that extraction of DNA should be carried out using standard methods which have been reviewed and approved by the IWC Scientific Committee. Extracted DNA aliquots should be stored in freezers at at least -80°C. The existing registers use appropriate methods.

5.2.2 Laboratory inventory management

It is important that a suitable inventory management system is developed in order to be able to trace the whereabouts and use of each sample/aliquot over time during storage and analysis.

5.3 Specification of markers and methods of analysis

It was agreed that analysis of the samples should be undertaken without knowledge of the biological and other information available for the whale from which the sample was taken. Samples should be analysed for (at least) mitochondrial DNA, microsatellites and Y chromosomes (sex identification), as is done for the existing registers.

5.3.1 mtDNA

This is primarily used for species identification and population genetic studies in the context of DNA registers. It also increases the statistical power of individual identification by serving as an additional marker. The initial step is for simple discrimination between the species for which there is agreement that the IWC has management responsibility, i.e. the great whales (sperm + baleen), and the other cetacean species (for which there is no general agreement that the IWC has management responsibility). The second step is to identify the sample to species. Details of the present systems for Japan and Norway are given in IWC/M05/RMSWG/SGDNA2 and 4.

¹ plus address if non-nominated person, e.g. in the case of bycatches

The SGDNA agreed that species identification should be accomplished with an approximately 500bp fragment of the 5'-end of the control region. It also agreed that sequencing should occur in both directions. As noted below, it is important that the DNA sequence quality scores and the electropherograms are retained for future reference (see Item 5.4).

5.3.2 *Microsatellites*

At present, microsatellites (or Short Tandem Repeats, STRs) are considered the marker of choice for individual identification (although see Item 10). Typically, microsatellite loci are amplified with fluorescently labelled primers that can be visualized by laser using an automated system. The genotype of an individual at a given microsatellite locus is scored by measuring the size in base pairs (bp) of the two alleles in relationship to an absolute size standard or a relative size standard, such as an allelic ladder (see (7) under Item 5.4) or both. Allele size can be reported as a 'raw' output (in decimals of a bp) or 'binned' into the assumed true sizes, in units of two, three or four bp. Genotypes scored at several microsatellite loci can be combined to establish a 'DNA profile' that has some degree of individual 'distinctiveness', depending on the frequency of the alleles at each locus. The average probability of two individuals having a matching profile by chance (a match by chance) is often expressed as the probability of identity, and is calculated from the number and variability of the microsatellites used to generate the profile. Identity can be further confirmed or excluded using other variable markers, such as sex or the mitochondrial DNA (mtDNA) control region.

Details of the markers currently used for the Norwegian and Japanese registers can be found in IWC/M05/RMSWG/SGDNA2 and 4. The SGDNA noted a number of important features about this approach that must be included and addressed when specifying requirements for registers. It is important to recall that the primary purpose of the registers is to determine whether IWC regulations on catch limits are being obeyed and that total removal levels are not being exceeded. This is partially achieved and confirmed by ascertaining whether products in the market can be matched to individual legal whales found in DNA registers. Matching errors can occur in two ways:

- erroneously failing to match products to an animal in the register when it is actually there – i.e. falsely implying an infraction;
- erroneously matching products to an individual in the register when it is not actually there – i.e. missing an infraction when one has occurred.

It is therefore extremely important that errors in matching are minimised.

The SGDNA agreed that it is important that the number and degree of variability of loci used in DNA registers should be sufficient to allow for an acceptable level of average probability of correctly identifying an individual. This number will vary by species. The existing microsatellite loci used by the Japanese and Norwegian registers should be used as the default loci in any new registers for the species/populations in the Japanese and Norwegian registers; new loci may be required for other species and populations. New loci should be incorporated into all registers as they are validated and added.

Similarly, the SGDNA agreed that laboratory error rates should be sufficiently low to ensure acceptably low level of probability of mismatch due to such errors. It should be recognised that some degree of laboratory 'error' is inevitable, although error levels (which should be estimated) should be minimised by good practice at all stages of the process from tissue collection onwards. Practical guidelines for this are given under Item 5.3, and results from such exercises should be reported to the IWC Scientific Committee for periodic review (see below).

In this regard, the SGDNA recognised the statistical and technical complexity of determining what comprises 'acceptably low' and it noted the interaction between laboratory error rates and the numbers and variability of loci are contributing factors in determining the robustness of matching success (and see Item 10).

Given the importance of this issue, the SGDNA noted that it is important that error rates be estimated in an agreed manner and reported for each locus on a regular basis.

Where more than one laboratory is used to generate a single register or a network of registers, it is critical that calibration of microsatellite genotype scoring (e.g., absolute size or binning) across laboratories is undertaken (and see Item 5.8.2). Considerable experimental variation is encountered in sizing microsatellite alleles, even under standard conditions (Gnosh *et al.*, 1997; Hoffman and Amos, 2005). This calibration should be carried out using a double blind experiment with known individuals. Cloned alleles should be used to construct an allelic ladder for calibration purposes. Methods and results of calibration exercises should be reported for periodic review (see below). The SGDNA agreed that it would be extremely helpful if nations presently holding registers facilitated the initiation

of such calibration studies in the near future. It was noted that progress towards this has been made by both Norway and Japan.

The SGDNA stressed that as occurs at present, current fluorescent techniques that allow electronic records to be kept should be used.

5.3.3 Sex identification

Both the Norwegian and Japanese registers incorporate information on sex. The former uses the method of Palsbøll *et al.* (1992) while the latter use that of Abe *et al.* (2001). The SGDNA agreed that sex identification should be continued, noting that sex is an additional genotype that may prove useful to identify market samples and that it may also serve as a check on field data. Once again, error rates (obtained by comparison with reliable field identification of sex) should be estimated and reported.

5.4 Minimum laboratory requirements

The SGDNA agreed the following guidelines for minimum laboratory requirements, based on those developed by Norway when choosing an appropriate laboratory for its register (IWC, 2001).

(1) The laboratory should adhere to high quality standards (such as those defined by forensic organisations). It may under certain circumstances be appropriate for laboratories to be formally accredited for DNA work or for them to work towards such accreditation. Experience with marine mammal genetic work may be advantageous but should not be considered a requirement.

Quality control and quality assurance features should assure that:

- (a) analysts have acceptable education, training and experience for the task;
- (b) reagents and equipment are properly maintained and monitored;
- (c) procedures used are generally accepted in the field; and
- (d) appropriate controls are used (as specified in procedures).

The laboratory should be available for external evaluation (e.g. by some combination of site visit, inspection, peer review and external audit).

(2) The laboratory should participate regularly in proficiency tests such as double-blind comparisons.

(3) Portions of the tissue samples and DNA extracts should be retained (and stored in an appropriate manner, e.g. in 95% ethanol at at least -20°C - see Item 5.1).

(4) Thorough laboratory records (protocols, notes, worksheets, etc.) should be maintained and archived by the laboratory for possible inspection. It is important that a suitable inventory management system is developed in order to be able to trace the whereabouts and use of each sample/aliquot over time during storage and analysis (see Item 5.2.2).

(5) Changes in equipment and methods should be noted and reported annually to allow ongoing standardisation among registers.

(6) The probability of errors occurring should be estimated and minimised, using standard procedures. DNA data quality/acceptability should be decided in accordance with generally accepted rules and reported annually where possible (e.g. PHRED scores for sequences, SDs of fragment length measurements for microsatellites alleles, means and SDs of peak heights for microsatellites, some evaluation of stutter for each microsatellite locus).

(7) A reference set of samples should be designated for allelic standards and an equimolar allelic ladder should be constructed by cloning and sequencing a range of alleles for each microsatellite locus.

5.5 Format of individual records

At present, DNA profiles in the existing registers are presented as follows:

- (a) as two Excel files - one for the microsatellites and sex profiles, the other for the mtDNA sequence;

- (b) in each file, whales are given unique identifiers that can be cross-referenced back to the biological and associated data for that animal. In the microsatellites/sex file, each whale profile is given one row, with one column for each allele (two columns for each microsatellite marker and the sex locus). In the mtDNA profile file, each profile has one row, and one column for each site where the sequence deviates from the reference sequence.
- (c) Hard copies are also made available.

The SGDNA group endorsed this basic approach but agreed that additional information should be archived, particularly in the context of calibration among laboratories when e.g. microsatellite markers are used. In this regard it agreed that in addition to the above, the following should also be stored:

General information for each sample

- genotyping system
- software system

'Raw' data

- electropherograms
- quality scores
- raw allele sizes
- peak heights
- gel image (depending on platform used)
- number of times the genotype replicated

Summary data on each locus

- error rate and how determined
- allele frequencies in a given population
- deviations from Hardy-Weinberg equilibrium
- evidence of null-alleles, short-allele dominance (or short-allele bias due to preferential amplification) or other artefacts

Although such data can be stored in Excel files, it was agreed that given the different types of information stored, a relational database system (such as Microsoft Access) would be both more flexible and more efficient.

5.6 Database structure

For scenarios *1DNA* and *3DNA* discussed below under Item 5.7, the specifics of the database structure are not especially important, although there are advantages in compatibility. For scenario *2DNA*, the minimum criterion is that the national databases are compatible for the purposes of matching and searching. An example of one possible system for scenario *2DNA* is given in Annex E.

5.7 Matching facility

As noted above, the reliability of matching is the key to the success of the DNA register approach. Different software packages of varying degrees of complexity are and can be used (e.g. see Walløe and Grønvik, 1998). It is important that whatever method is used, its performance is assessed using control samples. Performance needs to be assessed both in terms of erroneous matches and erroneous failures to match.

5.8 Issues relevant to the various potential options

As noted earlier, three general scenarios for register systems have been considered by the RMS Working Group:

Scenario 1DNA - national systems, including a national tissue archive and a national DNA register controlled and maintained by a member nation or under contract to a member nation, with requirement for reporting of infractions to IWC;

Scenario 2DNA - national systems with some level of international oversight

- (a) national systems with conditions for technical audit by IWC (e.g., submission by IWC of samples for double-blind comparisons; see Item 8.1)
- (b) national systems with technical audit by IWC and electronic copies of DNA profiles held by IWC
- (c) national systems with technical audit by IWC, electronic copies of DNA profiles held by IWC and duplicate samples of tissue held by the IWC;

Scenario 3DNA – a centralised, international system (IWC based) with central tissue archive and central register of DNA profiles derived centrally.

The SGDNA made the following observations with respect to some of the implications of adopting the different scenarios.

5.8.1 Sample shipment

Scenario *3DNA* would necessitate the shipment of all samples (from whaling operations, bycatch etc.) to a central laboratory. This has attendant logistical problems with respect to CITES permits and potential loss and/or degradation of samples. Such problems may not be so severe for scenarios *1DNA* and *2DNA*. At present, all Japanese samples are processed within Japan. However, the analysis and storage of the Norwegian samples was carried out in Canada up to 2002 and from 2003 is being carried out in Iceland. Icelandic samples are being analysed in Iceland (at the same laboratory as the Norwegian samples). International audit under scenario *2DNA* could require shipment and CITES permits.

5.8.2 Calibration

Calibration is necessary if microsatellite profiles of samples obtained from more than one laboratory are to be compared. Thus in the case of scenario *3DNA*, calibration of laboratories is unnecessary unless the central laboratory is changed.

The levels of calibration necessary for scenarios *1DNA* and *2DNA* would depend on circumstances, approaches chosen and the degree of co-operation among national governments/registers. A number of situations can be envisaged, including:

- (a) all laboratories involved in DNA registers are fully and successfully calibrated (and also a third party laboratory for analysing suspect' samples, if one is deemed appropriate under Item 8);
- (b) all samples from 'suspect' animals are analysed by all laboratories for comparison with their own registers – this would require no calibration but entail a degree of duplication;
- (c) profiles from exported whales are given to the importing country – this will require calibration if the profiles are to be used by the importing country in e.g. its market sampling, and, if under *2DNA*, it is agreed that a third party laboratory analyses 'suspect' samples;
- (d) information on the individual animal origin of legally exported products (e.g. anonymously but by a unique identifier) is given to the importing country and the importing country then analyses a sample from each animal for incorporation into its own register – no calibration is required unless under scenario *2DNA* it is agreed that a third party laboratory analyses 'suspect' samples.

5.8.3 Expertise/duplication

Scenario *3DNA* may result in the choice of a new laboratory and possibly the need to reanalyse all of the past samples or calibration of the old laboratories. If national registers were continued this would also imply duplication of effort. It was also noted that this might entail a loss of experience and expertise gained by the existing registers.

6. TECHNICAL ASPECTS OF POSSIBLE SYSTEM(S) FOR SUBMISSION TO REGISTER(S) TO AVOID FRAUDULENT CLAIMS

The RMS Working Group has noted that an agreed specified system for submitting samples to the register(s) for 'checking' must be developed to prevent fraudulent claims of illegal products being found. The generally agreed approach by that group was that (a) tissue samples must be submitted via national governments or appropriate

intergovernmental organisations with proof of origin of the samples, and (b) analysis must follow agreed techniques in an IWC-approved laboratory.

The SGDNA agreed with this approach. It noted that submitted samples should be accompanied by an officially-attested documentation of chain of custody from time of collection to submission. This should include location obtained, type of vendor, date, time, label if present and if possible photographs. It would also be necessary for a documented chain of custody to be established for the period between submission to a government or the IWC and provision of analytical results. The analysis would need to be carried out by a calibrated laboratory. For scenario 2DNA the matching would be carried out by the IWC (or other international body) holding the centralised register if the oversight includes holding a central register. Under scenario IDNA the samples (but no associated information) may have to be submitted to all of the national registers (not simply the one for the country where the sample was obtained) in order to facilitate a blind test and to avoid problems arising from any lack or failure of calibration (see Item 5.8.2 above).

7. GENERAL APPROACHES FOR DESIGNING A MARKET SAMPLING SCHEME (MSS)

As for the register system, three alternatives were considered:

Scenario 1MSS - national MSS only, with no international oversight;

Scenario 2MSS - national MSS with international oversight;

Scenario 3MSS - IWC-operated MSS.

The SGDNA did not discuss the implications of the various possible permutations of register and MSS scenarios.

The SGDNA agreed that the necessary data to be collected should include:

- location;
- date;
- time;
- label (or verbal description of nature and origin of product offered by vendor);
- type of source (e.g. wholesale market, shop, dockside etc);
- photograph of product before sub-sampling.
- name and contact information of person collecting (c.f. Item 5.1).

Pastene provided information on the present Japanese scheme and noted that their market surveys are systematic, with an attempt to provide adequate geographical and seasonal coverage (Table 1).

Table 1.

Market sampling surveys carried out or commissioned in Japan by the Government of Japan.

Year	Period	No of market products	Remarks
1995	Mar-May	53	TRAFFIC
1995	Mar-May	175	Japan
1996	Mar-June	353	Japan
1999	Nov 99-Feb00	648	Japan
2000	Nov00-Feb01	978	Japan
2001	Nov-Dec	381	Japan
2002	May-June; Oct-Nov	670	Japan
2003	May-July; Oct-Nov	615	Japan

7.1 Case-specific nature of market sampling

The purpose of market sampling is twofold: to act as a deterrent to illegal activity and to detect whether such activity is occurring. In its initial stage it is not intended to determine the precise number of animals that may be involved. Rather, if illegal products are discovered, a targeted method of detecting the origin of the products and the extent of the illegal operation should be developed. As noted in IWC/N04/RMSWG9, it is not a simple matter to design an

appropriate market sampling strategy and to determine the level of sampling necessary to provide either a deterrent or to estimate the power of a scheme to detect various assumed levels of illegal products. Initially, any such schemes, whilst they might conform to general principles or guidelines, would have to be case-specific in design for different national and regional markets. It would also need to take into account the various levels of success in obtaining DNA profiles from the various types of products available (more heavily processed products are more difficult).

The SGDNA noted that the development of an appropriate MSS would be an iterative process and initially at least be exploratory in nature, as markets are usually complex and poorly documented. This is a challenging technical task and requires methodological development. It is clear that the development of appropriate schemes benefits greatly from as much information as possible being provided by Governments and others on the nature and pathways of the market (which will vary even within a country, for example based on the origin of the product, e.g. from scientific permit catches, commercial catches and bycatches, and possibly by species). It was noted that the exercise being undertaken by the IWC Scientific Committee with respect to determining the value of attempting to estimate bycatches from MSS, whilst different in objectives, may produce some helpful information on sampling design.

7.2 Power to detect infractions, potential levels of coverage

The SGDNA reiterated the difficulty of estimating the likely power of detection of illegal activity in the absence of detailed knowledge of the markets. However, in general it is clear that power will be increased with increased scope of the sampling in terms of temporal (throughout the year) and geographical coverage. It was also agreed that the full range of cetacean products, including putative dolphin and porpoise products, should be sampled, without assumptions being made about accuracy of labelling. The SGDNA also noted the need to carry out appropriate experimental work both in determining the various likelihoods of falsely suggesting illegal activity when there has been none and in missing an infraction when there has been one. Such an effort must include appropriate statistical considerations, recognising that the default position for a diagnostic register is to assume illegal activity if no match is made, and appropriate practical issues such as choice of material when carrying out such a survey (e.g., reliable microsatellite profiles will be difficult to obtain from some highly processed products).

The SGDNA also noted that there will be some level of trade-off between choosing the best approach to act as a deterrent (e.g. sampling is carried out openly and with publicity) and the best approach to detect illegal products (sampling is carried out 'under cover').

7.3 Technical aspects of alternative options for MSS

The difficulties and complexity of designing an appropriate market-sampling scheme are the same irrespective of which option is chosen. In terms of shipment and analysis of samples, the same issues (with respect to CITES permits, shipping loss, degradation of samples and calibration of laboratories) raised in the context of registers are relevant.

8. POTENTIAL MECHANISMS FOR TRANSPARENCY/AUDIT/OVERSIGHT WITH RESPECT TO REGISTER AND MARKET SURVEY SYSTEM(S)

The SGDNA agreed the need for audit and recommended that the technical nature of that audit should apply irrespective of which option for registers or market surveys is chosen. Except in regards to the principle of transparency, the differences between the options thus largely stem from who carries out the audits. The SGDNA also noted that there is a powerful incentive for the holders of national registers to be accurate and fully diagnostic since if a profile from a product is found not to match an animal then the default position is that there has been illegal activity.

8.1 DNA registers

Under Scenario 2DNA, audit/oversight (and by extension transparency) could be accomplished by an international expert group:

- reviewing and approving the initial technical specifications for the national registers (as the IWC Scientific Committee has done for Norway) and any changes to those protocols;
- reviewing annually specific information and statistics formally reported as noted under Item 5;
- undertaking a technical audit including the provision for trials using 'blind' control samples;
- undertaking periodic site visits to examine whether agreed protocols are being followed.

Clearly under Scenario *3DNA*, the same audit/review would be necessary.

Scenario *2DNA* could (alternatively or additionally) also achieve transparency and oversight by having an international body (e.g. the IWC) holding a central master register of profiles supplied by the national registers (see Item 5.5). This list need have no associated information, and a check could be undertaken of a 'suspect' sample (with the appropriate safeguards described under Item 6) and a simple yes/no answer provided. It should be noted that there is a strong incentive for nations to ensure that their registers are accurate and up-to-date, since if a match is not found when tested against a 'suspect' sample, illegal activity is presumed. Given the existing national registers, the level of duplication required is greatest under Scenario *3DNA* and least under *1DNA*. The level of duplication under Scenario *2DNA* depends on the level of oversight required (see Item 5.7).

8.2 Market Sampling Schemes

In all cases a documented chain of custody from collection of the market sample to analysis must be collected and archived.

Under Scenario *2MSS* audit/oversight (and by extension transparency) could be accomplished by an international expert group by:

- either reviewing and approving an MSS submitted by a national government or developing the plan in collaboration with a national government;
- undertaking periodic site visits to ensure that MSS was being correctly implemented.

Under Scenario *3MSS*, the expert group would develop the MSS and publish it for outside scrutiny and it would be important that provision was made for site visits to ensure that the MSS was being correctly implemented. The level of work required is greatest under Scenario *3MSS* and least under *1MSS*. The level of work required under Scenario *2MSS* depends on the level of oversight required (see Item 7.3).

9. TECHNICAL ADVANTAGES AND DISADVANTAGES OF ALTERNATIVES FOR TISSUE ARCHIVE(S)

The Terms of Reference for the SGDNA called for it to: examine the technical advantages and disadvantages of holding a centralised tissue archive and centralised copies of the electronic profiles for national registers versus only having the electronic profiles. The SGDNA welcomed IWC/M05/RMSWG/SGDNA1 that addressed this topic.

A centralised tissue archive would be a repository for pieces of tissue from legally obtained animals that would act as a backup for national tissue archives and could potentially be used to verify genetic analyses reported by the member countries. For convenience, such an archive might also include extracted DNA aliquots provided by the contributing sources.

Establishment of a tissue archive (centralised or otherwise) requires consideration of a number of issues. The SGDNA agreed if it was decided that a centralised archive should be established, it should mirror the conditions for archiving tissue described under Item 5, hold the same associated information and include the same chain of custody records.

The nature of the centralised electronic profiles could range from a simple storage of anonymous profiles to an effective mirror of the national registers (as discussed under Item 5).

The SGDNA made the following observations about four potential alternatives:

- (a) IWC holds centralised profile archive only;
- (b) IWC holds centralised profile archive and complete tissue archive;
- (c) IWC holds centralised profile archive with an agreement that it can request and will be granted access to specific tissue samples;
- (d) IWC holds a centralised profile and a 'sub-set' tissue archive.

(1) A complete centralised tissue archive would comprise a complete back-up to national archives in the event of major malfunctions leading to loss of samples.

(2) There would be a considerable replication of effort to establish and maintain a complete centralised tissue archive. Issues associated with permits and the shipping (and some potential for loss and/or degradation) of samples referred to under Item 5.2 will also apply.

(3) A centralised archive could contribute towards verifiability of international technical audit in that samples can be sent to participating national (or contracted) laboratories for double-blind comparison. This could be achieved with less replication of effort if sub-sets of tissues were archived (as in (d)) or if governments agreed to submit samples on request as in (c) rather than by holding a complete archive (as in (b)).

(4) Apart from the case of a complete back-up, the reasons for a centralised archive are reduced if laboratories are all calibrated under international technical audit.

(5) A centralised tissue archive would allow for completely independent analysis and checking of any sample without recourse to the 'host' government.

(6) Alternative (b) is the most expensive and difficult to implement.

10. OTHER TECHNICAL CONSIDERATIONS

10.1 Efficiency and robustness of testing hypotheses in an MSS

As noted above, the statistical methods for establishing inclusion and exclusion of multiple products subject to multiple laboratory error (such as will be required for market surveys by second or third parties as would occur in scenario *3DNA* and possibly *2DNA*) to a DNA register have not been well developed. These technical and statistical difficulties will be compounded to varying extents if there is expansion of domestic whaling or international trade, requiring matching to multiple DNA registers of different species and populations, depending on options chosen (e.g. see Item 5.8.2).

Statistical development in this area is required to assist in estimating error rates for:

- potentially poor samples as might be the case for some market samples;
- determining the likelihood of missing a match ,i.e. falsely implying an infraction;
- determining the likelihood of falsely making a match, i.e. missing an infraction that has occurred.

Such an analysis must take into account the fact that the default position is that if a match is not made with a register then illegal activity has taken place.

It can be argued that the efficiency and robustness of a compliance mechanism involving a DNA profiling would be enhanced, possibly greatly by product labelling with an individual code linked to the DNA register. In discussing this, the SGDNA did not consider in any detail:

- the ease or likelihood of mislabelling occurring (either accidentally or deliberately);
- the difficulties in labelling for various types of products or markets.

In terms of efficiency, the search for a match would certainly be speeded up, as in the first instance the check will be to the animal indicated on the label rather than to the whole register (or at least all those animals in the register of the same species and sex indicated by the analysis of the sample). However, given the problems of accidental mislabelling, if no match is made, then a test might still need to be made with all animals in the register of the same species and sex.

In terms of robustness, then at least at the initial stages of testing, the technical demands of DNA profiling for identification would be greatly reduced by the much more limited null hypothesis established by the label, i.e., the product is a specific individual of a specific species taken at specific date and location. This would reduce the problems associated with combinatorial analyses (essentially, multiple tests) provided that a match is made. This is more similar to the inclusion/exclusion framework typically applied in human forensic genetics. However, again given the problems of accidental mislabelling, if no match is made, then a test would still need to be made with all

animals in the register of the same species and sex. Statistical evaluation of the benefits of this, both in terms of falsely missing matches and making matches, is important and not trivial. This needs to be examined carefully.

10.2 Alternative genotyping technologies

The present methods of identifying individuals used in both registers involve the use of microsatellite loci. An alternative type of genetic marker, Single Nucleotide Polymorphism (SNP), offers several advantages over microsatellites, including more robust allele scoring based on usually binary character states rather than allele sizes. SNP genotypes are independent of the technology used to generate them, and they can be stored and compared without the need for calibration among labs. SNPs are now the marker of choice for many human genomics studies where large numbers of markers are needed, as they are the most common type of variation in the genome and can be assayed rapidly and efficiently. Because SNPs typically have only 2 alleles, approximately 2-4 times more individual markers are needed to obtain the same level of probability of identity as a set of microsatellites (Chakraborty *et al.*, 1999), but this is likely to be countered by the increase in efficiency of generating the SNP genotype data, for overall increased efficiency and lower costs. One disadvantage of SNPs is that loci are not likely to be useful across species, requiring development of the required number of SNPs for each species in the register. However, the efficiency of this development is like to increase as the discovery of SNPs becomes more routine (Brumfield *et al.*, 2003; Aitken *et al.*, 2004; Morin *et al.*, 2004).

11. ADOPTION OF REPORT

The report was adopted by post.

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Annex A

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Annex B

Draft Terms of Reference for a Specialist Group on the DNA Register/Market Sampling Scheme Approach (SGDNA)

BACKGROUND

The use of a DNA/MSS (DNA register/Market Sampling Scheme) approach to obtain information that will help to ensure that catch limits set under the RMP are not exceeded has been discussed for several years. Such schemes are already in practice in Norway and Japan and discussion of some technical aspects has occurred within the IWC Scientific Committee. Such an approach can be particularly valuable in terms of detecting/deterring IUU operations or unreported bycatch (e.g. IWC/55/COMMS 3). Any wider issues of trade that may be of benefit to individual nations themselves are not of relevance to the IWC.

Chair's proposal

The Chair's proposal stated that DNA registers/market sampling systems should form the major part of the catch verification system. They should have the following attributes:

- National diagnostic DNA register for each whaling country or group of countries (to agreed specifications) to avoid redundancy and additional costs;
- Designed market sampling system (to agreed specifications);
- Some degree of outside audit.

The Chair had noted that further work is needed to adequately specify certain technical details and to consider the level of appropriate transparency that will fulfil the goal that regulations are not only obeyed but seen to be obeyed. He had also noted that an agreed specified system for submitting samples to the register(s) for 'checking' must be developed to prevent fraudulent claims of illegal products being found. Under this system it is proposed that: (1) samples must be submitted via national governments or appropriate intergovernmental organisations with proof of origin of the samples; and (2) analysis must follow agreed techniques in approved laboratories.

There is general (although not exclusive) agreement on this approach in the RMS working group; the primary area from a policy perspective is the level and nature of outside oversight.

ESTABLISHMENT OF A TECHNICAL SPECIALIST GROUP (SGDNA)

In accordance with the Chair's proposal, it has been agreed to establish an SGDNA to provide advice on the technical details related to the DNA/MSS approach. Without making specific recommendations on appropriate levels or who should carry out the outside audit, it would also be useful for the group to provide technical details of potential audit mechanisms for DNA registers and market sampling schemes. This information could then be considered at the next meeting of the RMS Working Group for consideration at both the policy and drafting levels.

Membership

This must be a specialist group and members should be familiar with DNA analysis (particularly with respect to individual identification, ideally in the context of DNA registers), market sampling approaches or both. The USA has agreed to act as Convenor of the group with assistance from the Secretariat. In order to facilitate work, Governments are requested to notify the name and email address of their expert to the Secretariat by 10 December 2004.

Modus operandi

The group should endeavour to complete its business by correspondence. However, it is recognised that with such a complex agenda this may be difficult and the possibility of the need to hold a short meeting (probably immediately prior to the March RMS Working Group meeting) cannot be ruled out. The Commission should consider whether it may be appropriate to provide some funds for participants in this

regard. In either circumstance the report of the group must be available to the next meeting of the RMS Working Group.

Existing documentation

There has been considerable discussion of relevant matters both within the Commission's RMS groups and its Scientific Committee. The Secretariat will compile an electronic reference set of such documents for circulation to the SGDNA.

Terms of reference

Given the above, and taking into account the work already undertaken by Japan, Norway and the Scientific Committee, as well as the various Commission groups, it is agreed that the SGDNA should report on the following technical issues, and, where appropriate develop text for technical specifications, concerning the following:

- (1) specifications for the establishment/maintenance of diagnostic DNA registers (including tissue analysis and specification of markers, minimum laboratory requirements, format of individual records, database structure and search facility);
- (2) technical aspects of possible system(s) for submission to avoid fraudulent claims;
- (3) general approaches for designing MSS including consideration of likely detection rates given assumptions of particular levels of occurrence of infractions and coverage, recognising the case-specific nature of MSS;
- (4) technical aspects of potential mechanisms for transparency/audit/oversight with respect to (1) and (3) above;
- (5) technical advantages and disadvantages of holding a centralised tissue archive and centralised copies of the electronic profiles for national registers versus only having the electronic profiles.

Annex C

Agenda

1. Convenor's opening comments
2. Adoption of agenda
3. Appointment of rapporteurs
4. Review of documents
5. Specifications for the establishment/maintenance of diagnostic DNA registers
 - 5.1 Collection of samples
 - 5.2 Tissue analysis
 - 5.2.1 Extraction of DNA
 - 5.2.2 Laboratory inventory management
 - 5.3 Specification of markers and methods of analysis
 - 5.3.1 mtDNA
 - 5.3.2 Microsatellites
 - 5.3.3 Sex identification
 - 5.4 Minimum laboratory requirements
 - 5.5 Format of individual records
 - 5.6 Database structure
 - 5.7 Matching facility
 - 5.8 Issues relevant to the various potential options
 - 5.8.1 Sample shipment
 - 5.8.2 Calibration
 - 5.8.3 Expertise/duplication
6. Technical aspects of possible system(s) for submission to register(s) to avoid fraudulent claims
7. General approaches for designing a market sampling scheme (MSS)
 - 7.1 Case-specific nature of market sampling
 - 7.2 Power to detect infractions, potential levels of coverage
 - 7.3 Technical aspects of alternative options for MSS
8. Potential mechanisms for transparency/audit/oversight with respect to register and market survey system(s)
 - 8.1 DNA registers
 - 8.2 Market Sampling Schemes
9. Technical advantages and disadvantages of alternatives for tissue archive(s)
10. Other technical considerations
 - 10.1 Efficiency and robustness of testing hypotheses in an MSS
 - 10.2 Alternative genotyping technologies
11. Adoption of report

Annex D

List of documents

IWC/NO5/RMSWG/SGDNA

1. PERRIN, W.F. AND MORIN, P.A. Technical advantages and disadvantages of alternative DNA-register approaches.
2. PASTENE, L.Q. AND GOTO, M. Specifications for the establishment/maintenance of diagnostic DNA registers – text for discussion based on information of Norwegian and Japanese registers.
3. BAKER, C.S. AND GILLESPIE, A. An integrated diagnostic DNA register and catch documentation system for compliance under the ICRW.
4. DANIELSDOTTIR, A.K., THORGILSSON, B., STEFANSSON, RAGNARSDOTTIR, A., JÖRUNSDOTTIR, T.D. AND PAMPOULIE, C. The Norwegian/Icelandic DNA register.

Annex E

One option for the structure of a centralised database based on national registers

Hans Peter Koelewijn

Option 2 of the different scenarios of the DNA register/MSS implies the existence of local databases from which information is regularly updated to a central register. Which information will be available in the central register is up to the judgment of the different contributors and owners of the local databases. For example, the central register might only contain the data that are of importance for detecting infractions. If an infraction is determined, the local databases where the full information is stored have to be consulted. Such a scheme poses different requirements on the structure of the database. The local databases are designed for storage, and all information has to be included in a secure way (transaction based databases). The central database requires easy access and data retrieval and therefore fulfills another purpose: information retrieval instead of storage. These systems are known under the name of Data Warehouses (DWH). These systems are very flexible and allow security and access rules to be defined at different levels. The storage of the data will always be under control of the participating countries, while access to the central register can be granted on a by request basis.

A key concept is the introduction of an ETL layer (Extract, Transform, and Load). This layer translates the data from different databases, formats or flat files (Excel) to one uniform format. This format consists of many indexing variables (sex, age etc) and a few measured variables (the genotype profiles). An ETL profile can be developed for every specific connected data storage system. In the case of the DNA register every country could develop its own storage system, keep it 'in house', have all the rights and access to its own system, and update its own system when corrections have to be made and subsequently upload it to the central DWH such that the changes take effect immediately.

Features:

- Participating countries are in charge of their own register. They only transport selected data to the central repository (DWH)
- Countries are not forced into a common system of storage (ETL will take care of the differences)
- Security and access rules can be defined at different levels
- Updates only necessary when changes have been made in the local database.
- Advanced tools for querying available
- Calibration of the genotypic profiles among participating countries and laboratories is crucial

