JNCC Report
No 459

A study to identify the suitability of Nonatec™ mini-microchips and Alpha•Dots™ as methods of uniquely marking juvenile Testudinid species (tortoises)

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February 2012

ISSN 0963 8091

CITY OF LONDON

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This report should be cited as:

Walter, O., Quest, R., Bradfield, T & Thornton, S.M. 2009.
A study to identify the suitability of Nonatec™ mini-microchips and Alpha•Dots™ as methods of uniquely marking juvenile Testudinids (tortoises). *JNCC report*, No. 459

Acknowledgements

This project was commissioned jointly by the Joint Nature Conservation Committee (JNCC; also the UK CITES Scientific Authority for animals) and the Department of the Environment, Food and Rural Affairs (Defra; also the UK CITES Management Authority), but does not necessarily reflect the views of either. Alison Littlewood and Nichola Burnett of JNCC initiated the idea for this study and were also responsible for editing the final report.

Thank you to P. Cusdin, N. Blay and A. Greenwood for working on the initial stages of this project, as published in Blay *et al* 2008 and to N. Masters (IZVG) for acting as an independent reviewer and for his advice.

Thank you too to Mathieu Ratard, of Lutronic International, the suppliers of the Nonatec ™ mini microchip and to the staff at the Animal Reception Centre, Heathrow for the day to day management of the tortoises.

Summary

This report presents the findings of a study to determine the suitability of Nonatec™ microchip transponders as a permanent marker for juvenile tortoises, and AlphaDots™ for use as temporary markers until they attain a plastron length of 100mm[1]. Both marking methods were selected after an initial scoping exercise of all known available methods of marking animals and were tested for ease of application, readability and durability. In addition, the microchips were tested to ensure they didn't compromise the welfare of the tortoises, either physically or behaviourally.

European regulations require that live specimens of species listed on Annex A of the European Wildlife Trade Regulations (including 12 Testudinidae spp.), being used for commercial purposes, are permanently and uniquely marked.

Whilst there is general agreement throughout the European Union (EU) that juvenile tortoises cannot be safely fitted with a standard microchip (such as the Trovan ID162 or AVID MUSICC chip); alternative marking requirements and conditions have not been applied uniformly, with the majority of juvenile tortoises being traded without any form of permanent marking. Failure to find an acceptable marking method makes regulation of the trade difficult and has led to high levels of non-compliance.

This study concluded that the Nonatec™ microchip transponders were suitable for uniquely and permanently marking juvenile tortoises; whilst the AlphaDots™ were not sufficiently robust to recommend them as a unique semi-permanent marker. The Nonatec™ microchips were found to have no effect on the physiology or behaviour of the tortoises, irrespective of their size or species, and were found to be as reliable as a standard microchip transponder. However, Nonatec™ microchips do not comply with ISO standards 11784 and 11785, currently a requirement of Regulation (EC) No.865/2006; potentially meaning that tortoises would need to be microchipped again with an ISO compliant chip once they attained a size considered safe for insertion, or requiring a change to the Regulation.

[1] Advice provided by veterinary experts in the UK in 1998 recommended that tortoises with a plastron length of under 100mmm were too small to be safely fitted with a microchip transponder. In teh absence of an acceptable alternative form of making, the UK policy has been that Annex A tortoises in trade, under 100mmm plastron length, are not required to be marked.

Contents

1 Introduction

For many years there has been a growing interest in keeping reptiles as pets in the United Kingdom and the keeping of tortoises in particular. This has influenced the trade, encouraging breeding and importation, as well as illegal trafficking. Even with a responsible, largely law-abiding pet trade, a significant amount of illegal trade in tortoises still occurs in the UK and consumers are unwittingly supporting this detrimental and criminal trade.

All Testudinid species are regulated by the Convention on International Trade in Endangered Species of wild fauna and flora (CITES). The Convention regulates the international trade in approximately 30,000 species to ensure that exploitation for the international trade does not threaten their survival. Member States of the European Union implement CITES through the European Wildlife Trade Regulations (Council Regulation (EC) No. 338/97 and Commission Regulation (EC) No. 865/06). Species are listed in one of four Annexes (A – D) according to the level of protection they need. Annex A contains species, commercial trade in which from, to and within the Community is as a general rule prohibited. Twelve Testudinid species are currently listed in Annex A (see Table 1).

Table 1 List of Testudinid species that are listed in Annex A of the EC Wildlife Trade Regulation (Regulations (EC) No. 709/2010

Radiated tortoise	*Astrochelys radiata*
Angonoka	*Astrochelys yniphora*
Galapagos giant tortoise	*Chelonoidis nigra*
Bolson tortoise	*Gopherus flavomarginatus*
Pancake tortoise	*Malacochersus tornieri*
Geometric tortoise	*Psammobates geometricus*
Madagascar spider tortoise	*Pyxis arachnoides*
Madagascar flat-shelled tortoise	*Pyxis planicauda*
Spur-thighed tortoise	*Testudo graeca*
Egyptian tortoise	*Testudo kleinmanni*
Hermann's tortoise	*Testudo hermanni*
Marginated tortoise	*Testudo marginata*

EC regulations require that live Annex A vertebrates being used for commercial purposes, other than captive born and bred birds, be marked by a uniquely numbered unalterable microchip transponder, or, where this method is not appropriate because of the physical or behavioural properties of the specimen/species, the specimen shall be marked by means of a uniquely numbered ring, band, tag, tattoo or similar means, or be made identifiable by any other appropriate means.

Microchips must be compliant to ISO Standards 11784 and 11785 which means they have an unchangeable unique number and can be read by any reader that reads the ISO prescribed frequencies. However, current ISO compliant microchips are deemed too large for Testudinids with a plastron length under 100mm (Knapp & Affre 2007) and proper regard to humane care, well-being and natural behaviour of the specimen concerned (Art. 67 of Reg. (EC) No. 865/2006) for juveniles cannot be guaranteed. In these cases, the Management Authorities will recognise alternative methods or procedures.

In the absence of an acceptable alternative form of marking, the UK policy has been that Annex A tortoises in trade, with a plastron length under 100mm, are not required to be marked. Whilst there has been general agreement throughout the EU that juvenile tortoises cannot be safely fitted with a standard microchip, alternative marking requirements and

conditions have not been applied uniformly. Until an alternative form of permanently and uniquely marking juvenile tortoises is found and accepted by all Member States enforcement authorities will continue to have difficulties enforcing the regulations and illegal trade is likely to continue, with populations of some species facing severe declines in the wild.

In order to address this problem the JNCC commissioned a study to identify a method of uniquely and permanently marking tortoises (that are too small to be safely fitted with a microchip transponder), which satisfies the requirements of Article 66(3) of Regulation (EC) No.865/2006 and could be adopted by all EC Member States as the preferred method of marking tortoises in trade.

A review of all current and potential marking methods was undertaken by Blay *et al* (2008). A summary of the methods reviewed can be found Appended to this report. Each form of marking was assessed against criteria developed by JNCC. Blay *et al* concluded that Nonatec™ mini-microchips should be tested as a permanent marker and Alpha•Dots™ should be tested for use as temporary markers until the Testudinids were of sufficient size to be microchipped with a standard microchip. The two methods were subsequently tested on live animals under controlled conditions.

This report presents the findings of trials using Nonatec™ mini-microchips and Alpha•Dots™. Both marking methods were tested for ease of application, readability, and durability. In addition, the chips were tested to ensure they did not compromise the welfare of the tortoises, either physically or behaviourally.

1.1 Nonatec™ mini microchips

The Nonatec™ microchip transponder is the smallest chip currently available in the market at 6mm x 1mm and is most commonly used to identify laboratory animals as small as newborn mice. The chips transmit a 16 digit unique identification code with the added possibility of programming in additional information, such as breeders details, hatch date or ISO country code of the country in which the specimen was bred. The chip is implanted using an 18-gauge (1.2mm) needle, which is significantly smaller than the needles used for either the AVID MUSICC chip (12-gauge) (Avid Plc.) or one of the traditionally used transponders such as the Trovan ID162 (13-gauge) (Pet Chip Company Ltd.). Any insertion of a needle carries the risk of causing haemorrhage through damage to superficial blood vessels. The needle used to insert the Nonatec™ chip carries less risk than the standard chip needle due to its smaller size.

Figure 1 Nonatec™ micro transponder

The small size of the chip and needle and current practise in laboratories to monitor very young animals with minimum stress indicates that it would be suitable for even the smallest hatchling tortoise.

The Nonatec chip operates at the High Frequency 13.56 MHz and therefore cannot be read using AVID or TROVAN readers. A Lutronic reader is required to read a Nonatec microchip. A start up cost of €1380+VAT will cover an arm reader with split antenna, a separate case

with LCD display and battery. The microchips come in sterilised syringes and cost €6.44+VAT each. All are supplied by Lutronic International Ltd.

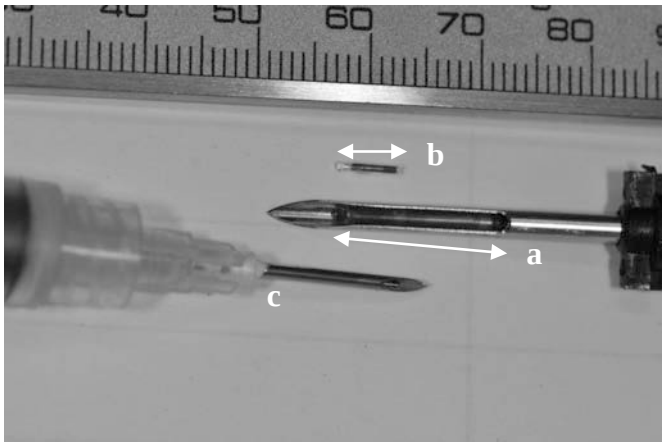

Figure 2 Demonstrates the size difference between a standard ISO compliant chip (a) in its needle and a Nonatec™ mini-microchip (b) and its needle (c)

1.2 Alpha•Dots™

Alpha•Dots™ are an anti-theft marking system composed of 1mm dots suspended in a clear lacquer. The dots are printed with a unique identification number and a phone number allowing the reader it to trace the owner or origin of the marked item. The dots are used by auto manufacturers such as Volvo and Suzuki motorbikes to identify parts and are highly recommended by the UK police force as a security measure (Alpha•Dot Security Ltd n.d.).

Each tube of Alpha•Dots™ has a unique number allocated to it; and all the Alpha•Dots™ in that tube bear the same identical number. The dots are brushed on to the required surface with the brush that comes with each tube. A 'pocket microscope', such as those used by stamp collectors, is used to read the dots.

Although not a permanent form of marking, Alpha•Dots™ could potentially be used from birth until the tortoises are 100mm long. They are inexpensive, easy to implement, and do not affect the animal physically or behaviourally. They are also considered safe to use on juvenile tortoises. The trial tested whether the dots would adhere to a tortoise's carapace and/or plastron for the length of the trial, were tamper proof, and how the alpha dots responded to general wear and tear. A one off purchase of a pocket microscope costs between £5 and £20. The cost for one tube of dots suspended in lacquer is £15.

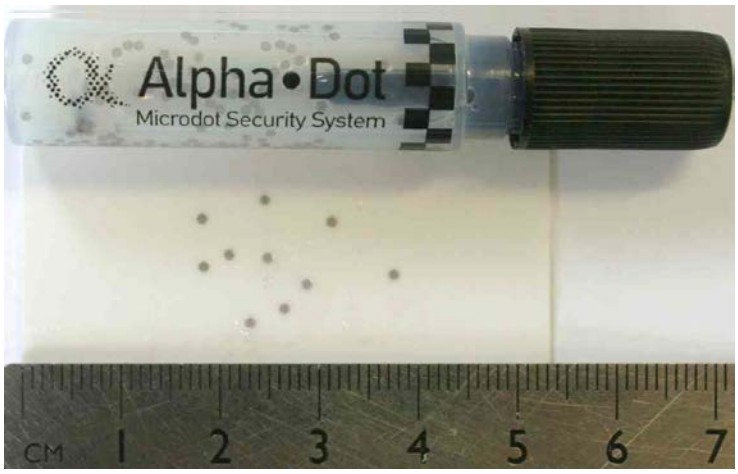

Figure 3 Alpha•Dots™ to scale

2 Methodology

2.1 Subjects and conditions

The specimens used in the trial originated through confiscations by HM Revenue & Customs: one hundred and ninety two tortoises were held at the Animal Reception Centre (ARC), Heathrow, and a further 20 at the SeaLife Centre (SLC), Weymouth. The species used in the trial were determined by those made available by Customs at the start of the project. Six species of tortoise were used in the trial allowing the marking methods to be tested in a variety of different husbandry conditions. See Table 2 for a list of species used, along with details of weight and plastron length (measured using callipers – see Figure 3) and the sample size.

Table 2 Species and size of specimens that were used in the trial

No	English name	Scientific name	Plastron length (mm)	Weight (g)
95	Bell's hinged tortoise	*Kinixys belliana*	82-128	160-558
67	Leopard tortoise	*Stigmochelys pardalis*	48-58, 74-92[1]	52-71,148-330[1]
12	African spurred tortoise	*Geochelone sulcata*	50-57	42-64
8	Home's hinged tortoise	*Kinixys homeana*	66-88; 129-168[2]	80-168, 462-902[2]
4	Spur-thighed tortoise	*Testudo graeca*	60-98	98-280
3	Red-eared terrapin	*Trechemys scripta elegans*	39-46	20-28
3	Horsfield's tortoise	*Testudo horsfieldii*	106-109	444-518
20	Map turtles	*Graptemys pseudogeographica*	Unavailable	unavailable

[1] There were two groups of Leopard tortoises held, both of different sizes. Weight and length ranges for both groups are given
[2] There were two groups of Home's hinged tortoises held, both of different sizes. Weight and length ranges for both groups are given

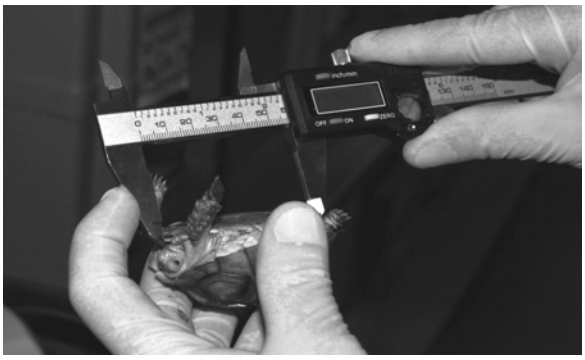

Figure 4 Plastron length being measured by callipers

Of the 192 tortoises used for the trial at ARC, 126 were marked, and 66 were left unmarked as the control group. Specimens were marked with both the Nonatec™ chips and the Alpha-

dots. Tortoises with plastron lengths greater than 100mm were included in the trial in order to test whether the chips were readable in larger animals.

At the ARC, four rooms were allocated to hold the specimens used in the trial. In rooms B9, B10 and B11, specimens were housed on the floor. Heat lamps, water baths and areas of shelter (hay and cardboard boxes) were provided. The Bell's hinged tortoises were held in rooms B10 and B11. These rooms were sprayed with water twice daily, increasing to four times daily after chipping when it was observed that their skin was too dry. These tortoises were used in the comparative behavioural studies.

The fourth room (known as the Export room) housed tortoises in moulded vivaria with glass front sliding doors. Each vivaria was furnished with a newspaper floor, water bath, fluorescent lighting, heat mat and a thermostatically controlled infra red heat source. See Table 3 for species split between these rooms.

Table 3 Species allocation to each room and number of marked and unmarked tortoises in each

Room	Species	Number marked	Number unmarked
B9	Leopard tortoises	35	26
B10	Bell's hinged tortoises	24	16
B11	Bell's hinged tortoises	31	24
Export	Leopard tortoise	6	0
Export	African spurred tortoise	12	0
Export	Home's hinged tortoise	8	0
Export	Spur-thighed tortoise	4	0
Export	Red-eared terrapin	3	0
Export	Horsfield's tortoise	3	0

In order to test the durability of the Alpha•Dots™ in aquatic species conditions, 20 map turtles *Graptemys pseudogeographica* were marked at the ARC and sent to SLC. Specimens were housed in natural enclosures typical of those found in zoos, containing a deep water pool with natural rock surround. Heat lamps provided suitable basking areas.

2.2 Methods of marking

The Nonatec™ micochips were inserted into the right thigh of the specimen to be marked. The Alpha•Dots™ were 'painted' on to predetermined scutes on both the plastron and carapace (see Figure 4). White numbers were painted on the carapace of every tortoise with water based Tipp-Ex™ (*Tipp-Ex*) to allow all specimens to be easily identifiable and to allow identification should other methods fail. In addition, a 2cm blob of Tipp-Ex was also painted on to the marked tortoises in order to distinguish them from the unmarked specimens (see Figure 5). These were repainted weekly, without handling the tortoises.

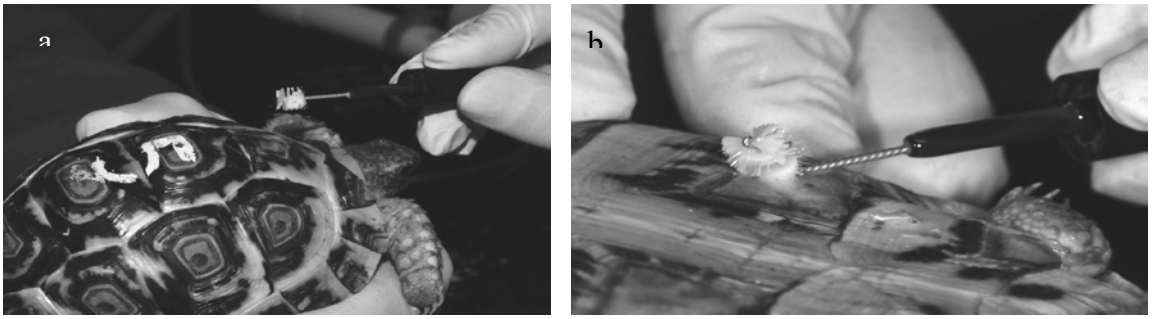

Figure 5 Application of the Alpha•Dots™ to the carapace (a) and the plastron (b)

Figure 6 White spots and numbers applied using water based Tippex
White spots identify those specimens that are marked

Testudinids are normally microchipped in the left hind leg. The Nonatec™ microchips were inserted into the right hind leg, thus avoiding the risk that a specimen may have two microchips inserted into the same leg should it need to be chipped again when 10cm long. All chips were inserted lateral to a superficial vein that runs over the stifle joint. The site of insertion was prepared using Betadine™ antiseptic scrub (Molnlycke) using a cotton bud. The site was then sprayed with surgical spirit. The skin was moved sideways before the needle was inserted so the chip sits to one side of the hole in the skin. This ensures the skin covers the chip and so made it less likely to fall out. The level of elasticity and mobility of the skin may vary between species. The needle was inserted under the skin until just past the bevel of the needle, then pressure applied to the applicator to drive the chip through the subcutis. The chip itself drives the hole through the tissue. This is unlike chipping a mammal and has been found over many years by S.M. Thornton to be a safer method of microchipping reptiles and also reduces the risk of haemorrhage. The hole was sealed using a cynoacrylate glue (SuperGlue™). Figures 6 to 10 show the procedure.

In rooms B9, B10 and B11, two thirds of the tortoises held were marked. All the tortoises held in the Export room were marked (see Table 3). The unmarked tortoises were used as controls.

2.3 Recording effectiveness of the marking methods

All the tortoises were caught and their microchips read every 14 days for ten consecutive weeks. Where possible the chips were scanned through their shell (Figure 11), although some chips needed to be scanned directly on the leg (Figure 12). All unmarked tortoises were handled in a similar fashion in order that all tortoises received identical treatment.

At the same time, the Alpha•Dots™ were checked to see whether they were still in place. The unique numbers on the Alpha•Dots™ were read using a Lumagny 50x illuminated pocket microscope at the beginning and end of the trial (see Figure 13).

At the end of the ten week trial, all specimens whose microchips could not be read were radiographed to determine if the chip was still in place.

Figure 7 The insertion site was prepared on the right leg using antiseptic scrub before being sprayed with surgical spirit
Figure 8 Cotton buds are used to move the skin sideways before inserting the needle
Figure 9 A microchip is inserted into a *Kinixys*
Figure 10 A microchip is inserted into a 40mm red-eared terrapin
Figure 11 The puncture hole was sealed with a tissue adhesive
Figure 12 The chip is read on a leopard tortoise through its shell
Figure 13 The leg was pulled out of *Kinixys* tortoises as the chips would not read through their shells

Figure 14 Reading the Alpha•Dots™ with a pocket microscope

2.4 Recording behavioural effects

The behaviour of the tortoises in Rooms B9, B10 and B11 was recorded to determine whether the marking methods had an effect on tortoise behaviour. Prior to the implantation of the transponders, all behaviour patterns were recorded by the staff at the ARC for between nine and 18 days, allowing a comparison with post implantation tortoise behaviour. The time frame recorded accounts for the fact that the insertion of the chips into the specimens was staggered for logistical reasons. Behavioural data continued to be recorded for nine weeks post implantation.

Scan sampling was used to determine activity budgets. All behaviours are mutually exclusive; feeding, bathing, moving, basking or sleeping. The proportion of marked and unmarked tortoises performing each behaviour was recorded at each observation. Tortoises were watched for a further three minutes to note any additional behaviour such as limping or limited movement. This was repeated at regular intervals, for a minimum of 10 times a day, seven days a week. The timing of the scan sampling varied slightly from day to day in relation to the work load of the staff at the ARC.

To ensure all tortoises were treated identically, all unmarked tortoises went through the process of having their ‘chips’ scanned.

2.5 Regular health checks

All tortoises were weighed at the time their marking was checked (Picture 13). Weights were monitored throughout the trial for significant changes. As many specimens defecated when picked up, faecal and urine samples were weighed. Sudden weight losses or gains could be explained by a difference in gut load.

A vet was available for consultation throughout if any negative behaviour or physiological problems occurred.

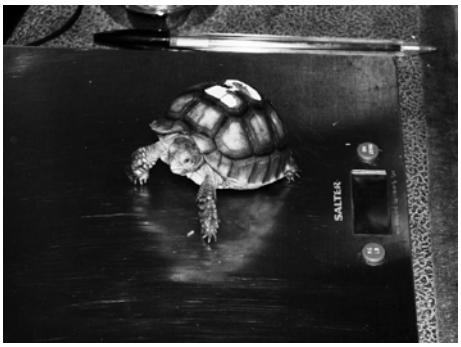

Figure 15 Tortoises are weighed when their marking is

2.6 Data analysis

The data were analysed to test the following hypothesis: Marking juvenile Testudinid species with Nonatec™ mini-microchips will not affect their behaviour or physiology and so indicate that their welfare is not compromised.

To determine whether the marking methods affected the physiology of the tortoises, net weight gain was calculated by subtracting the first weight recorded from the last. Plastron growth was determined in the same way. As the data were normally distributed, t-tests were performed to determine whether the net weight gain and plastron length gain differed between marked and unmarked tortoises in each room (B9, B10, B11).

To determine whether the marking methods affected the behaviour of the tortoises, activity budgets were calculated. A change in activity budget could be caused by a change in behavioural patterns and indicate a possible compromise to the welfare of the specimens.

A G-test (Fowler *et al,* 1998) was used to determine the significance of differences in activity budgets. Data from each room was analysed separately as environmental and species differences meant activity budgets were slightly different between the rooms ($P>0.01$, $d.f.=6$).

The following hypotheses were tested using datasets for each room:

A Marking tortoises has no effect on the activity budget of the tortoises

The activity budgets for marked and unmarked tortoises were analysed. This analysis excluded data collected before the marking and on weigh days. These two activity budgets were compared using a G-test.

B There is no difference in activity budgets before and after the insertion of a microchip

Pre-marking activity budgets were compared with the activity budget from both marked and unmarked behaviours. Weigh days were excluded from this analysis. These two activity budgets were compared using a G-test.

C Activity budgets do not differ on weigh days

The activity budgets calculated for tortoises on weigh days and non weigh days were compared. Data collected before marking was not included. These two activity budgets were compared using a G-test.

3 Results

3.1 Application and readability of marking methods

3.1.1 Alpha•Dots™

By nature, Alpha•Dots™ are extremely small (with a diameter of 1mm), and good light conditions were needed to see them properly. The lacquer took from between 10 minutes to one hour to dry, therefore tortoises could not be put straight back in to their enclosures but had to remain in separate, empty boxes until the lacquer had dried. It could not be guaranteed that the dots were not scraped off before the lacquer had dried.

During the trial, the Alpha•Dots™ were regularly checked to see whether they had remained in place. Upon reading the chips on the final day of the trial, it was found that in those cases where the dots had fallen off, an indentation was left on the shell that could be mistaken for the dots still being present. Therefore, many were marked as present that had actually already fallen off. In addition, some of the dots had delaminated; meaning that although a dot was present, the numbers inscribed had been rubbed off.

Only one third of the Alpha•Dots™ that remained in B10 (N=2 out of 6) and the Export Room (N=1 out of 3) were readable. Although the Alpha•Dots™ were found to be easy to read on a flat surface, it was difficult to focus on them once applied to the shell. For uneven shells, the entire number on the dot was not in focus at the same time, making the number difficult to read. Despite the microscope having an inbuilt light, light was still a limiting factor when attempting to read the unique number. When exposed to water from the spraying/misting or swimming/bathing, the lacquer became opaque and made it impossible to read the dots within it. The lacquer would then peel off. The dots could also be hidden by faeces.

3.1.2 Nonatec™ microchips

It is not possible to read the chip through the metal of the needle and Nonatec™ microchips are not packed in readable packaging as with other brands of standard chips. Therefore, the chip number can only be determined once implanted. This has the advantage that the chip must be inserted into the animal before an ID number can be confirmed, eliminating the risk that a microchip number may be entered onto a permit without the animal in question necessarily being marked. However, as the chip cannot be read before insertion, a user must assume that the chip is actually present in the needle and in working order.

Nonatec™ microchips must be charged up by the reader before they are able to be read. In practice this means holding the reader in position for a few seconds. It cannot be waved over the animal as is done with current, ISO compliant readers. For some species this not a problem as the chip could be read through the shell. However, in the case of the larger specimens of Bell's hinged tortoises and the leopard tortoises, the chip could not be read through the shell and the leg had to be pulled out in order to read the chip. This may be due to the hinge or the extent to which the animal has retracted its leg into the shell.

The reader can be used charged up or plugged in, which has the advantage that it can be used at any time. The reader cannot be manually switched off, and will turn off automatically after a certain period of time. This was not found to be too much of a problem in practice. Other difficulties encountered with initial use (such as failing to locate and read the chip number) were overcome with practice.

There was some concern that the glass tubes that enclose the Nonatec™ chips were particularly delicate. Therefore, a number of Nonatec chips and standard chips were subjected to a selection of considerable stresses, including using the point of a needle as a fulcrum. None of the Nonatec™ chips broke under these pressures, unlike the standard chips, which all broke. This indicates that the Nonatec™ chips are much more robust than the standard, ISO compliant chips.

Minor haemorrhaging was caused by insertion of the microchip in 6 cases (5 Bell's hinged tortoises, 1 leopard tortoise). All of these were quickly stemmed by gentle pressure being applied with for a few seconds.

3.2 Durability of marking method

3.2.1 Alpha•Dots™

100% of the Alpha•Dots™ applied to the turtles at the SLC came off within weeks of the application. Map turtles are a predominantly aquatic species; upon coming into contact with the water, the lacquer surrounding the dots went opaque and subsequently peeled off, removing the dots with it.

At the ARC, specimens were housed in a drier environment, with room B9 kept completely dry. Rooms B10 and B11 (holding the Bell's hinged tortoises) were sprayed with water four times daily to increase humidity levels for health reasons. The difference between the humidity levels in Room B9 and Rooms B10 and B11 is likely to account for the variation in the proportion of dots remaining on the tortoises at the end of the trial (see Table 3).

Table 4 The percentage of marked tortoises with dots still present at the end of the 10 week trial

Room number	Species	% tortoises with dots present
B9	Leopard tortoise	70
B10	Bell's hinged tortoise	25
B11	Bell's hinged tortoise	12
Export	Mixture	30
SLC	Map turtles	0

3.2.2 Nonatec™ microchips

Out of the 126 marked specimens, only 3 chips could not be read after insertion into the animal (see Table 4). The chip implanted into the leopard tortoise could not be read immediately after insertion while the remaining two were initially successful but then subsequently ceased to work. It has been found that ISO standard chips can be cracked easily on insertion. This causes tissue fluid to enter the mechanics of the chip and can prevent the chip from working some time after insertion (JAK Marketing n.d.). The researchers were unsuccessful in their attempt to break the Nonatec™ chips, so it is unlikely that this was the cause of the failure. Radiography of the three tortoises with unreadable chips revealed that two still had chips present. There was no sign of the chip in the third tortoise indicating that it had fallen out after two to four weeks. It was noted that the adhesive used to seal the skin after chipping did not always appear to adhere to tortoise skin well; thus potentially allowing the chip to fall out. Moving the skin so the hole does not sit directly above the chip mitigates against the adhesive not working properly. There were other instances where chips could not be read but these were determined to be due to user error. The Nonatec™ chips require a different type of reader and the technique used to read the chips differs to that commonly used for standard ISO chips. The success rate in reading the chips improved with familiarity and practice.

Staff at the ARC Heathrow scan an average of 7,000 microchips per year. Their records show that over a 10 year period, only 15 chips of those found to be present in the animal could not be read (R. Quest. pers. com.). However, all of these chips were successfully read by the manufacturers after surgical removal from the animal. It is possible that the two chips that failed during the trial are not faulty, but are lying in such an orientation that prevents the reader from detecting them. This hypothesis is currently unable to be tested.

A further four chips failed when the plastic tag attaching the chip to the needle did not detach properly. As a result, when the needle was withdrawn the chip came too. In these cases a second chip was inserted.

Table 5 The number of tortoises chipped in relation to the number of chips that failed

Room number	Species	No. chips inserted	No. chips failed	Notes
B9	Leopard tortoise	35	1	This chip failed immediately. Radiography showed that the chip was still present (see Figure 15).
B10	Bell's hinged tortoise	24	0	
B11	Bell's hinged tortoise	31	2	One chip failed after 2 weeks, another after 4. Radiography showed that one chip was still present and one had fallen out.
Export	Mixture	30	0	

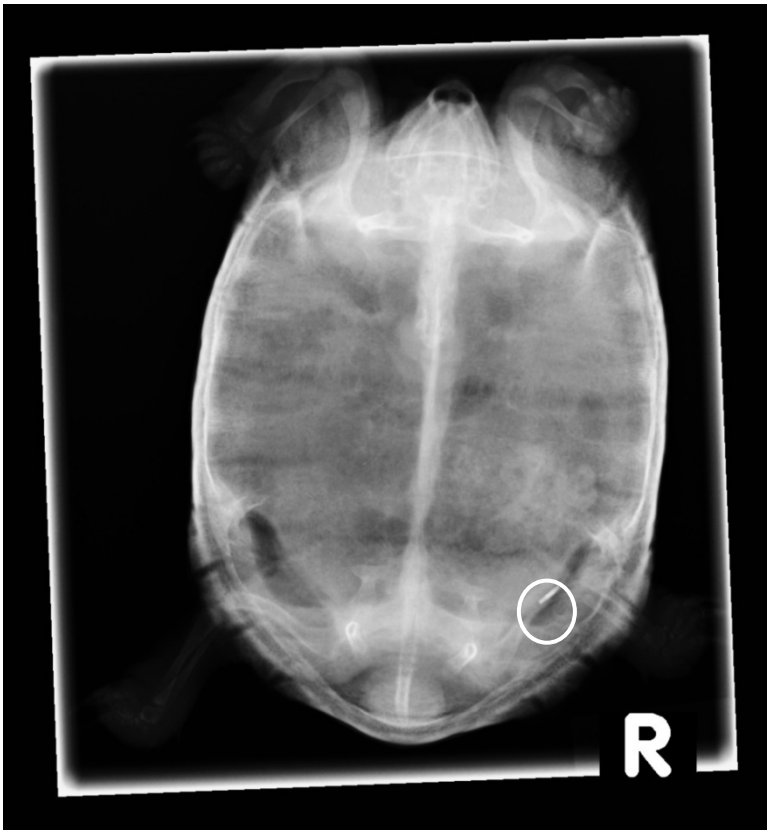

Figure 16 Minichip (circled) seen in right thigh of leopard tortoise 23 through radiograph. Credit: University of Liverpool

3.3 Physical observations

The length of the project was short in relation to the typical life span of Testudinids and some of the specimens used in the trial were almost adult size. Therefore a sizeable weight or plastron length gain would not be expected. It was not unusual for a tortoise to defecate and/or urinate whilst being handled, particularly the Bell's hinged tortoises. The negative net changes recorded all fall within the average weight of a faecal sample (14 to 28g) and/or a urine sample (14g). The veterinary advisor to the project is of the opinion that the level of weight loss shown is not a concern.

There was no significant difference between the net weight gain and increase in plastron length between marked and unmarked tortoises.

Table 6 Average net weight gain (g) and average plastron growth (mm) in each experimental group

Marked / Unmarked	Species	Number of specimens (d.f.)	Average net weight gain(g)[1] (Range)	Average plastron growth (mm)
Unmarked	Leopard tortoise (B9)	23	16.7	2.8
Marked	Leopard tortoise (B9)	37	21.2	3.4
	t-test results	(d.f. = 58)	P=0.432	P=0.594
Unmarked	Bell's hinged tortoise (B10)	15	1.9	0.9
Marked	Bell's hinged tortoise (B10)	24	10.3	1.21
	t-test results	(d.f. = 38)	P=0.130	P=0.964
Unmarked	Bell's hinged tortoise (B11)	22	-8.9	0.9
Marked	Bell's hinged tortoise (B11)	29	2.27	0.61
	t-test results	(d.f. = 53)	P=0.684	P=0.255
Marked	Home's hinged tortoise	4	1.3	1
Marked	African spurred tortoise	10	8.6	4
Marked	Leopard tortoise	6	58	6
Marked	Spur thighed tortoise	3	10.7	4
Marked	Horsfield's tortoise	3	-10	1
Marked	Red eared terrapin	3	6	3

[1] Please note, a urine sample weighed 14g and faecal samples were between 14g and 28g

3.4 Behavioural observations

The tortoises were fed and cleaned out twice a day - morning (between 0500 and 0700) and evening (between 1800 and 1900). This explains the activity patterns for the tortoises throughout the trial period (Figure 16). Active behaviours (feeding and moving) increased markedly during these periods. It was noted that the tortoises mainly ate in the morning and most of the food given in the evening was left over night.

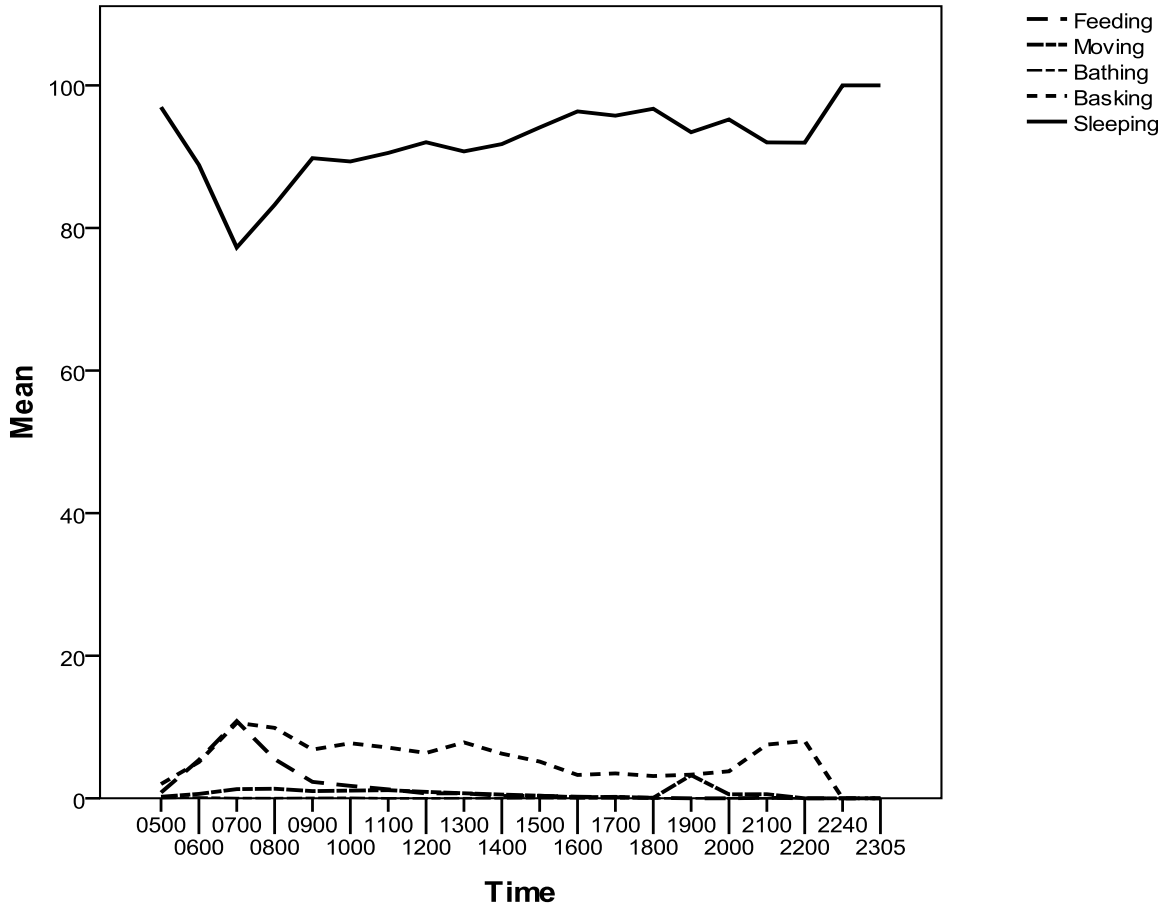

Figure 17 The proportion of tortoises performing each behaviour over the day. It is clearly seen that the dominant behaviours are sleeping and basking. Bathing was very rarely seen, as shown by the line that sits very close to the x axis

3.4.1 Marking tortoises has no effect on the activity budget of the tortoises

There was no significant difference between the activity budgets for marked and unmarked tortoises in any of the rooms (B9: G=0.039, d.f.=3, B10: G=0.051, d.f.=3, B11: G=0.051, d.f.=3).

The graph in Figure 17 depicts the similar activity budgets for the two groups of tortoises in each room.

3.4.2 There is no difference in activity budgets before and after the insertion of a microchip

No difference was found in the activity budgets before and after microchipping in any of the rooms (B9: G=3.39, d.f.=3; B10: G=3.75, d.f.=3; B11: G=2.79, d.f.=3).

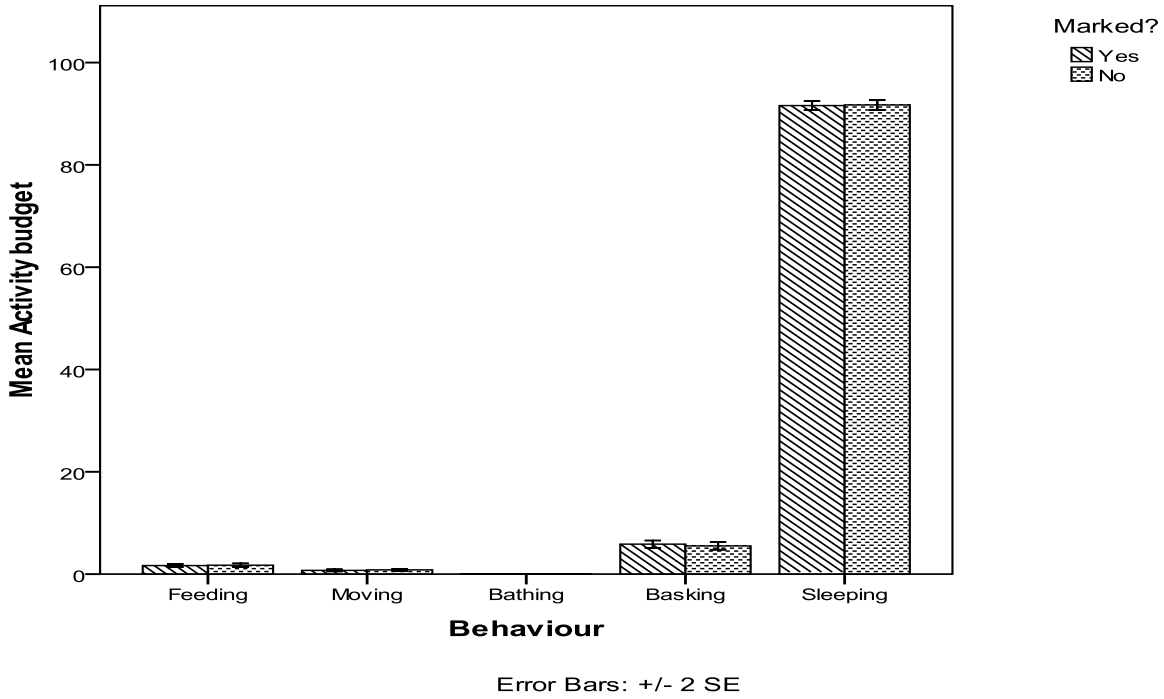

Figure 18 The activity budgets of marked and unmarked tortoises shown here are not significantly different.

3.4.3 Activity budgets do not differ on weigh days

There was no significant difference between activity budgets for tortoises on weigh days and non weigh days (B9: G=0.20, d.f.=3; B10: G=1.81, d.f.=3; B11: G=1.24, d.f.=3) despite the disruption caused by the tortoises being picked up, weighed and having their chips read.

3.4.4 Summary of behavioural observations in relation to the hypotheses

Any differences in activity budgets may be caused by changes in behavioural patterns and therefore indicate a possible compromise in welfare.

1. The Nonotec™ microchip did not make any difference to the activity budget of the tortoises in the trial, whether marked or unmarked. This hypothesis was upheld.
2. There was no difference in the activity budget of the tortoises before and after being marked. This hypothesis was upheld.
3. Activity budgets did not differ on weigh days and non weigh days and therefore this hypothesis was upheld.

4 Discussion

The Alpha•Dots™ are portrayed by the manufacturer as lasting for three years on tortoise shells or parrot beaks (N. Dearsley pers. comm. in Blay *et al,* 2008). However, our results demonstrated that the majority of dots used did not last the length of the trial (10 weeks). The lacquer appears to deteriorate in any environmental condition that contains a reasonable amount of water, either in the form of a body of water or ambient humidity. In some cases the dots delaminated and the numbers inscribed on the dots rubbed off. Additionally, the unique number on those dots applied to uneven shells could not be read in their entirety. The success rate in the leopard tortoises, a desert species with a reasonably flat shell, was only 70%. This is insufficient to be considered as a method of marking tortoises until they have reached 100mm plastron length.

The Nanotec™ microchips proved to be successful in that they did not change the activity budgets of the tortoises in the trial, but a few shortfalls remain. The microchips can safely be inserted into small tortoises, including hatchling red-eared terrapins, without compromising their behaviour or welfare. The ability to add numerical information to the chip in addition to the permanent, unique number means breeders could put in their breeder number and the hatch date if they had the appropriate software. Each tortoise would potentially carry this information around for the rest of its life and be useful to indicate its origin. However, although the unique number cannot be changed, it must be noted that anyone with the right software and equipment (available from the manufacturers) would be able to amend the additional numbers.

Out of the 126 chips inserted, only three chips could not be read once implanted. One of these was found to have fallen out. It can be concluded that 98.4% of the chips present at the end of the study worked successfully. Unfortunately, the chips cannot be read whilst in the needle so those chips with manufacturing problems cannot be eliminated prior to insertion. A comparison with the standard ISO compliant chips read by ARC indicate a lower rate of success. However, according to the ARC 0.2% of the chips scanned per year fail to read while in the animal. The rate of failure of the ability to read Nonatec™ chips while still in the animal is therefore comparable with standard chips. The two Nonatec™ chips that failed in the tortoises were not surgically removed to see if they could be read by the manufacturer.

The technique used to read the Nonatec™ chip differs from the standard ISO readers. The Nonatec™ reader charges up the microchip before it can emit a signal so the reader must be held closely over the animal. It was difficult to read the chip through the shell of some of the larger hinged tortoises. In these cases the leg had to be pulled out of the shell and the chip read directly. This was not felt to be a problem given the few times a tortoise is likely to have its chip read in a zoo or private ownership. The chips would still have to be implanted by a veterinary surgeon (RCVS 2004).

Nonatec™ microchips are not ISO compliant, a requirement of Article 66(3) of Regulation (EC) No. 865/06, therefore tortoises may need to be chipped again with an ISO compliant chip once they attain a size consider safe for insertion. Current veterinary advice indicates that this would not be a problem, given that the chip could be inserted into a different leg. However it could be avoided altogether if the Nonatec™ chip was accepted as a permanent form of marking in accordance with Chapter XVI of the above Regulation. It must be noted that there has not been any research to look at the long term effects of any microchip on the physiological and behavioural welfare of Testudinids (See Recommendations).

It was investigated to see if the Nonatec™ microchips could comply with ISO standards 11784 and 11785. However, as they comply to ISO 14443 it is not possible for the chips to also comply with 11784 and 11785 (M. Ratard pers.comm.).

The chips are guaranteed by the manufacturer (NXP) to have a unique and unchangeable number but it does not comply to the make up as stipulated by ISO 11784 at the moment. For example, ISO 11784 compliant chips include the manufacturers code and the country code in a specified order. ISO Standard 11785 states that one scanner, irrespective of type, should be able to read all transponder microchips. The Nonatec™ microchip cannot be read by an ISO compliant reader as it transmits at a different frequency. Complying to this standard is currently impossible.

It may be possible, though a lengthy process, to amend the EU CITES legislation to include non ISO compliant microchips such as Nonatec™ as a legal way of marking CITES Annex A listed Testudinid species in the EU.

5 Conclusion

This study concludes that microdots, as produced by Alpha•Dot, are not suitable for Testudinids as they don't remain on the animal for a sufficient length of time. They are particularly affected by the presence of water, either a body of water or ambient humidity. Even in the dryer desert tortoise species, only 70% of dots were present at the end of the 10 week trial. This is insufficient for the proposed use.

The Nonatec™ mini microchips were significantly more successful. The chips were easy to insert and caused no apparent physiological problems. The results showed a low rate of failure and they were difficult to break, unlike standard microchips.

Note: The fact that these chips are not currently ISO 11784 and 11785 compliant remains the only outstanding issue relating to the use of Nonatec™ microchips as a form of permanently and uniquely marking hatchling Testudinids for life.

6 Recommendations

This trial tests the short term effect of implanting tortoises with Nonatec™ mini microchips. The chips have been used predominantly in laboratory species that have a short life span relative to many tortoises. Reading the chips at six monthly intervals over the next ten years would give an indication of the longevity of the chips.

No tests have been carried out to determine the long term welfare effects of Testudinids that have been microchipped. Therefore future trials should be held with Testudinids chipped with either ISO compliant chips or the Nonatec™ mini microchip and with both chips (one in each leg).

If Nonatec™ microchips were to be recommended as a method of marking juvenile Testudinids, guidelines should be drawn up as to how best to insert and read these microchips. This information should be included in the BIAZA Recommended Code of Practice for Microchipping Zoo Animals (June 2004) and widely distributed throughout the EC and the Herpetological trade.

For mini microchips to be accepted as a permanent method of marking Testudinid species in trade (i.e. avoid the requirement to microchip a tortoise for a second time once it attains a size considered safe to microchip with standard sized transponders), EU Member States will need to address Nonatec™'s non-compliance with current ISO 11784 and 11785 standards.

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Appendix 1

A list of known animal marking methods has been compiled, and assessed as to whether the methods meet the criteria listed above. The findings of this are summarised below:

Microchip transponder: a RFID (Radio-frequency identification) tag, which has previously been discounted due to the size of the aerial required for chips to be readable. Use of new generation smaller sized transponder chips would be acceptable if judged humane for use in small animals. A number of companies now claim to make particularly small chips: Lutronic, Hitachi and AVID all manufacture a chip smaller than the one that is currently used for animal identification. We discuss this option in more detail further on. Transponder chips for tortoises may only be implanted by a veterinary surgeon, as required by the Royal College of Veterinary Surgeons in Advice Note 6 on Microchipping.

Scute marking: Often using acrylic paint, or nail varnish covered with epoxy resin, this is only a useful alternative in small research populations of animals, as marks are unlikely to be unique, nor are they likely to be unalterable, tamper proof, and they cannot easily encode the information needed. This method is recommended by the Desert Tortoise Council, for animals that are moved from building sites, or roadsides. (Desert Tortoise Council, 1994)

Shell Chipping: this has all the disadvantages of scute marking, combined with a possible risk to the animal's health, and is disfiguring.
Tattoo: currently used to mark other animals, ear tattoos are have been used in the past to mark cats and dogs, and are used in Australia by the State of Victoria to identify pigs. However, it is not unalterable, nor is it practical to implement.

Toe clipping: used historically to mark rodents in research populations, as documented in behavioural research papers (Leclerq and Rozenfield, 2001; Jacquot &Vessey, 1994; Lambin, 1994) has welfare implications, not unique, nor is it tamper proof, and is disfiguring.

Ink or dye marking: Subcutaneous ink or dye injections in small fish are used to mark reef fish in population ecology studies (Malone, Forrester & Steele; 1999). Not unique, can be carried out by anyone, does not encode information needed, is not tamper proof.

Latex Injection: An injection of latex under the skin, to create a recognisable shape. Used in research populations of fish to identify individuals (Riley, 1966; Forrester 1990). Not unique, can cause distress to the animal, cannot encode the information needed, could be disfiguring.

Freeze Branding: Is used in agriculture. Is disfiguring, not unique, is not likely to be able to encode the information, is unsightly, and is alterable. Freeze branding is also likely to generate protests from hobbyists

Shell photographs: used as a permanent marking method by the German and Austrian authorities are not considered by the UK to be a reliable marking method. Without the use of a pattern matching computer database, the ability to correctly match a photograph to a tortoise shell is limited. Staff at the Animal Reception Centre at Heathrow have found that duplicates of the same photograph have been used to identify different animals in the same shipment. Due to the fast growth rates commonly seen in young tortoises, changes in the carapace and the plastron colouration and form, photographic identification can be unreliable. Currently the shell photograph system used by the German and Austrian governments is neither computerised, nor accessible to customs in other EU countries, meaning that all matching must be done by eye. Identifying hundreds of tortoises by their photographs is time consuming and unreliable (T. Bradfield pers. com.).

The British Chelonia Group has their own, membership only, method of fingerprinting, which requires a high quality photograph of a tortoise's plastron next to a cm ruler. The photographs are entered into a pattern matching database, and also are also reviewed by a member of staff. The use of the database would seem to make the system more reliable, however it is only used by members of the British Chelonia Group (British Chelonia Group n.d.), and they declined the invitation to comment on the consultation.

More novel methods were also surveyed, with the following results.

SmartWater: is a liquid used to uniquely mark electronics and other retail products. It is a liquid polymer emulsion with a unique marker formula coded to identify the origin of the marked product. Although invisible to the naked eye, SmartWater can be detected with UV light, but must then be swabbed and the swab sent to the Smartwater lab, in order to correctly identify the marker formula (Smartwater pers.com.). Although Smartwater meets the criteria for unique identification, ease of application, and will cause no distress to the tortoise from hatching onwards, it is not easy to read, and must be swabbed and sent to a lab for identification. Smartwater has not been tested as a permanent marker on biological materials. It is not suitable due to the difficulty in reading the unique marker, and the cost of processing.

Biometrics: Theoretically, biometric information method would be a more stringent and regulated version of the photo identification method currently used in Germany. It requires that a component be unique to each individual tortoise, that it be easily assessed, and that there be a database set up tracking each of these animals when the information is recorded. It is thus likely to be a more complex and expensive way of tracking tortoises than is currently possible. Even in humans, the biometric tests that are currently used are limited to hand or fingerprints and iris scans. Powerful computers with access to the biometrics database would be required for the system to perform adequately.

DNA testing: This is neither quick nor economical, and would require the development of genetic markers allowing identification of individual tortoises for each species being "marked". Taking and running samples would require a number of days and the use of polymerase chain reaction machines, as well as gels to run the samples in order to generate a DNA "fingerprint".

Microdots: Microdots are very small dots with an identification number printed on them, which corresponds with information held in a database. They are used by the police for keeping track of car and motorcycle parts, as they are small, difficult to remove, and hard to find. Alpha-dots are the company that produce a commonly used variety. They require 40x magnification to read, and are stuck on with a lacquer adhesive, so although they would not be permanent markers, they are estimated to last 3-4 years (N. Dearsley pers. com.), or long enough for a tortoise to achieve the size necessary for use of a microchip.

Attaching barcode to the shell or plastron: possibly not tamper proof, depending on the method of attachment, as growth of the animal may stop the barcode sticking. The adhesive used could cause deformity in shell growth, depending on the rigidity of the shell and the adhesive. Barcodes could possibly be very visible. They require a specialist reader, and a database to keep track of which animals are marked with which barcode. Barcodes are used throughout the commercial world, as identifiers on passports and boarding passes. It is also possible to generate false barcodes using relatively easy computer programming techniques, rendering it unreliable as a permanent marking method.

Table 7 A summary of methods, including whether they meet the JNCC criteria

Method	**Unique**	**Permanent or semi permanent**	**Unalterable or tamper proof**	**Low cost for large numbers**	**Not affect physical tortoise negatively**	**Suitable for juveniles**	**Practical to implement**
Microchip transponder Standard size, 12 gauge needle	Yes	Yes	Yes	Yes	No	No	Yes See paragraph 4.2.1
Microchip transponder, smallest available, 18 gauge needle	Yes	Yes	Yes	Yes	Yes	Yes	Yes See paragraph 4.2.1
Scute marking with paint or epoxy	No	No	No	Yes	Yes	Yes	Yes
Shell chipping	No	No	No	Yes	No	No	Yes
Tattoo	Yes	Yes	No	Yes	No	No	No
Toe clipping	No	Yes	No	Yes	No	No	No
Ink or dye marking	No	No	No	Yes	Yes	No	Yes
Latex injection	No	No	No	Yes	Yes	No	Yes
Freeze branding	Yes	Yes	No	Yes	No	No	No
Shell photographs	Yes	No	No	Yes	Yes	No	No
SmartWater	Yes	No	Yes	No	Yes	Yes	No
Biometrics	Yes	Yes	Yes	No	Yes	Yes	No,
DNA testing	Yes	Yes	Yes	No	No	Yes	No
Micro-dots or Alpha-dots	Yes	To be tested	To be tested	Yes	Yes	Yes	Yes
Barcode	Yes	Yes	No	Yes	No	No	No, see paragraph 4.3.5

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