GreenScreen™ Assessment for Hexamoll® DINCH®[1] (Diisononyl cyclohexanedicarboxylate) (CAS #166412-78-8, 474919-59-0)[2]

GreenScreen™ Version 1.2 Verified Assessment

Date of Verification:	**May 1, 2013**
Expiration Date:	**May 1, 2016**
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Chemical Name: Diisononyl cyclohexanedicarboxylate

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Organization: ToxServices LLC
Date: February 13, 2012; May 30, 2012; June 5, 2012; February 1, 2013; May 1, 2013

Confirm application of the *de minimus* rule[3]: Yes

Chemical Name (CAS #): Diisononyl cyclohexanedicarboxylate (CAS #166412-78-8, 474919-59-0)

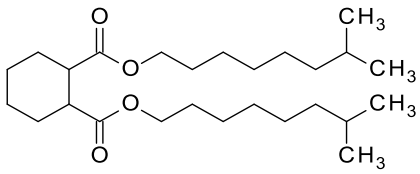
Also Called: 1,2-Cyclohexandicarbonsaurediisononyl ester; diisononyl hexahydrophthalate; 1,2-Cyclohexanedicarboxylic acid, 1,2-diisononyl ester

Chemical Surrogates, analogs or moieties used in this assessment (CASs #):
Based on the lack of data for Hexamoll® DINCH® for some endpoints, ToxServices considered the inclusion of a chemical surrogate to address data gaps. However, there are insufficient peer-reviewed mechanistic data available in the published literature to justify the selection of a surrogate to model hazards identified as data gaps.

[1] Note: Both Hexamoll® and DINCH® are registered trademarks (®) of BASF SE.
[2] Note: This chemical is associated with two CAS numbers: 474919-59-0 (inside the U.S.) and 166412-78-8 (outside the U.S.).
[3] Every chemical in a material or formulation should be assessed if it is:
1. intentionally added and/or
2. present at greater than or equal to 100 ppm.

Specifically, it is not known whether all high molecular weight cyclohexane esters share the same mechanism of action for the identified data gaps. Therefore, it is not justified to use toxicity data from surrogates to address data gaps without confirmation that the selected surrogate shares the same mechanism of action for a specific endpoint. As a result, no surrogate or analogs were selected. Modeling cannot address the remaining data gaps for neurotoxicity and respiratory sensitization; therefore, the data gaps were left unfilled.

Chemical Structure(s):



Hexamoll® DINCH®

Notes related to production specific attributes[4]: No information disclosed by manufacturer or Green Chemistry and Commerce Council (GC3).

For Inorganic Chemicals and relevant particulate organics (*if not relevant, list NA*)
Define Properties:

1. Particle size (e.g. silica of respirable size) - NA
2. Structure (e.g. amorphous vs. crystalline) - NA
3. Mobility (e.g. Water solubility, volatility) - NA
4. Bioavailability - NA

Identify Applications/Functional Uses:
(e.g. Cleaning product, TV casing)

1. Plasticizer (NICNAS 2012)
2. Food packaging (NICNAS 2012)
3. Used with PVC (NICNAS 2012)

Green Screen Rating[5]: ToxServices assigned Hexamoll® DINCH® a GreenScreen™ Benchmark Score of 2 based on Moderate Endocrine Activity (E), Skin Irritation (IrS) and Persistence (P). This corresponds to GreenScreen™ benchmark classification 2e (Moderate T) in CPA 2011a. Data gaps exist for Respiratory Sensitization (SnR); however, the remaining hazard points are sufficient to classify this chemical as a Benchmark 2 chemical. In a worst case scenario, if Hexamoll® DINCH® received a High (H) for Respiratory Sensitization (SnR), Hexamoll® DINCH® would receive a Benchmark score of 2f (High T for Group II* Human).

GreenScreen™ Hazard Ratings: Hexamoll® DINCH®																			
Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS*	SnR*	IrS	IrE	AA	CA	P	B	Rx	F
						single	repeat*	single	repeat*										
L	L	L	L	M	L	L	L	L	L	L	DG	M	L	L	L	M	L	L	L

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated values and lower confidence. Hazard levels in **BOLD** font reflect values based on test data (See Guidance).

[4] Note any composition or hazard attributes of the chemical product relevant to how it is manufactured. For example, certain synthetic pathways or processes result in typical contaminants, by-products or transformation products. Explain any differences between the manufactured chemical product and the GreenScreen assessment of the generic chemical by CAS #.
[5] For inorganic chemicals with low human and ecotoxicity across all hazard endpoints and low bioaccumulation potential, persistence alone will not be deemed problematic. Inorganic chemicals that are only persistent will be evaluated under the criteria for Benchmark 4.

Transformation Products and Ratings:
Identify relevant fate and transformation products (i.e., dissociation products, transformation products, valence states) **and/or moieties of concern**[6]

Functional Use	Life Cycle Stage	Transformation Pathway	Transformation Products	CAS #	On CPA Red List[7]?	Green Screen Rating[8]
n/a	End of Life	Hydrolysis	Isononyl alcohol	27458-94-2	Not present on the Red List of chemicals (CPA 2009)	**n/a**
n/a	End of Life	Hydrolysis	Hexahydrophthalic acid	1687-30-5	Not present on the Red List of chemicals (CPA 2009)	**n/a**

Introduction

Hexamoll® DINCH® is manufactured by BASF Corporation and is a clear colorless plasticizer that was developed for use in applications that are sensitive based on exposure and toxicological issues. Hexamoll® DINCH® is suitable for use with PVC and other polar polymers (BASF 2009). The major applications of Hexamoll® DINCH ® are use in food packaging, wire and cable, automotive, plastisols, and other related applications (NICNAS 2012).

PhysioChemical Properties of Hexamoll® DINCH ®

Table 1: Physical and Chemical Properties of Hexamoll® DINCH®

Property	Value	Reference
Molecular formula	$C_{26}H_{48}O_4$	NICNAS 2012
SMILES Notation	Not identified	
Molecular weight	424.6 g/mol	NICNAS 2012
Physical state	Liquid	NICNAS 2012
Appearance	Clear, colorless	NICNAS 2012
Melting point	Glass transition <-90ºC	NICNAS 2012
Vapor pressure	2.2x10-8 kPa at 25ºC	NICNAS 2012
Water solubility	<0.02 mg/L at 25ºC	NICNAS 2012
Dissociation constant	Not determined; no modes of dissociation are expected	NICNAS 2012
Density/specific gravity	947.2 kg/m^3 at 20ºC	NICNAS 2012
Partition coefficient	Log K_{OW} = >6.2 at 25ºC (measured)	NICNAS 2012

[6] A moiety is a discrete chemical entity that is a constituent part or component of a substance. A moiety of concern is often the parent substance itself for organic compounds. For inorganic compounds, the moiety of concern is typically a dissociated component of the substance or a transformation product.
[7] The CPA "Red List" refers to chemicals: 1). flagged as Benchmark 1 using the GreenScreen™ List Translator, or 2). flagged as Benchmark 1 or 2 using the GreenScreen™ List Translator and further assessed and assigned as Benchmark 1. The most recent version of the GreenScreen™ List Translator should be used.
[8] The way assessments are conducted for transformation products depends on the Benchmark Score of the parent chemical (See Guidance).

Hazard Classification Summary Section:
Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M or L): L
Hexamoll® DINCH® was assigned a score of Low for carcinogenicity based on negative results following a 2-year carcinogenicity study.

- Not listed as a known carcinogen by IARC, NTP, U.S. EPA, or CA Prop 65.
- REACH 2010 –
    - In the 2-year combined chronic toxicity/carcinogenicity study performed in Wistar rats according to OECD 453, male and female rats (50 per sex per dose group for the main group, 10 additional animals for the satellite group) were administered oral doses of 40, 200, or 1,000 mg/kg/day Hexamoll® DINCH® (99.6% purity) via the diet for two years for the main group or 12 months for the satellite group. Examinations included: cage side observations; detailed clinical observations; body weight; food consumption; food efficiency; ophthalmoscopic examination; hematology; clinical chemistry; urinalysis; and gross pathology. The number of thyroid massed in male rats was 3, 1, 3, and 2 for the main control, low, mid, and high dose groups, while that for female rats was 0, 1, 1, and 4 for the main control, low, mid, and high dose groups. In the satellite group, altered colloid in the thyroid gland was observed in 5, 3, and 8 females administered the low, mid and high doses, respectively compared to 0 control females displaying altered colloid. No data was presented for the incidence of altered colloid in male rats. Follicular cell hyperplasia was observed in the thyroid glands of 8, 6, 9, and 15 male rats and 3, 4, 5, and 14 female rats in the main control, low, mid, and high dose groups, respectively. Thyroid gland adenomas were observed in 3, 5, 11, and 14 male rats and 1, 3, 3, and 9 female rats of the main control, low, mid, and high dose groups, respectively. Thyroid effects have been reported to have limited relevance to human health risk assessment due to differences between rats and humans in thyroid hormone handling and in the sensitivity to thyroid-disturbing mechanisms. This conclusion is consistent with EFSA and IARC opinion on the significance of thyroid follicular cell tumors induced by chemicals with altered hormone metabolism and which demonstrate a lack of genotoxic potential. It has been well established in the rat that thyroid follicular-cell tumors are commonly associated with imbalances in TSH levels resulting in sustained stimulation of the thyroid gland. The human thyroid gland is much less susceptible to this pathological phenomenon (NICNAS 2012). No treatment related mortality or increases in malignant neoplasia up to the highest dose of 1,000 mg/kg were observed (EFSA 2006).

Mutagenicity/Genotoxicity (M) Score (H, M or L): L
Hexamoll® DINCH® was assigned a score of Low for mutagenicity based on negative mutagenicity and clastogenicity assays both *in vitro and in vivo*.

- REACH 2010 –
    - *In vitro* – A GLP-compliant Bacterial Reverse Mutation assay (OECD 471) was conducted utilizing *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, and *E. coli* tester strain WP2 uvr A with and without metabolic activation (S9-mix) at concentrations of 0, 20, 100, 500, 2,500 and 5,000 pg/plate in experiment 1 and 0, 4, 20, 100, 500, or 2,500 pg/plate in experiment 2 (99.7% purity). Hexamoll® DINCH® was found to be negative for mutagenicity under all tested conditions.
    - *In vitro* – A GLP-compliant Chromosome Aberration assay (OECD 473) was conducted utilizing Chinese Hamster Lung fibroblasts (V79) with and without metabolic activations (S9-mix) at concentrations of 0, 25, 50, 100, 200, 400, or 1,000 µg/ml of Hexamoll® DINCH® (> 99% purity). No increase in chromosomal aberrations or polyploidy were observed, as Hexamoll® DINCH® was reported to be negative for clastogenicity under the tested conditions.
    - *In vitro* – A GLP-compliant Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPRT) gene mutation assay (OECD 476) was conducted utilizing Chinese Hamster Ovary (CHO) cells with and without metabolic activation (S9-mix) at concentrations of 0, 312.5, 625, 1,250, 2,500, or 5,000 µg/ml of Hexamoll® DINCH® (99.6% purity). No increases in forward mutations were reported under the tested conditions. Hexamoll® DINCH® was reported to be negative for mutagenicity.
    - *In vivo* – A GLP-compliant Micronucleus assay (OECD 474) was conducted using male NMRI mice (5/group). Mice were administered single doses of 0, 500, 1,000, or 2,000 mg/kg of Hexamoll®

DINCH® (99.6% purity) via intraperitoneal injection. No inductions of micronuclei were observed. Hexamoll® DINCH® was considered to be non-clastogenic and non-aneugenic following the micronucleus assay.

Reproductive Toxicity (R) Score (H, M, or L): L
Hexamoll® DINCH® was assigned a score of Low for reproductive toxicity based on a study that showed this substance did not induce reproductive toxicity at doses up to 1,000 mg/kg/day.

- REACH 2010 -
    - A GLP-compliant Two Generation Reproductive Toxicity study (OECD 416) was conducting using male and female Wistar rats (25/sex/group). F0 rats were administered oral doses of 0, 100, 300, or 1,000 mg/kg of Hexamoll® DINCH® (99.6% purity) in feed daily for 38 weeks. F1 pups were selected at 21 days of age and mated at least 73 days after this selection to produce the F2 generation. The F1 offspring that were not selected as parents for the F2 generation were sacrificed at 21 days of age and subjected to postmortem evaluations. Examinations included: cage side observations; body weights; food consumption; hematology; clinical chemistry; urinalysis; estrous cyclicity; sperm parameters; histopathology; and gross necropsy. Offspring examinations included: number and sex of pups; still births; live births; postnatal mortality; presence of gross anomalies; weight gain; physical or behavioral anomalies; gross necropsy; and organ weights. No adverse effects were reported on fertility and reproductive performance of the F0 or F1 parental animals. The relative thyroid weights of high dose F1 females increased by 11.1% over controls. Minimal to slight hypertrophy/hyperplasia of the thyroid follicular epithelia was observed in 21/25 high dose F1 females, minimal to slight hypertrophy/hyperplasia was observed in 10/25 mid dose F1 females, and slight hypertrophy/hyperplasia was observed as a spontaneous finding in 3/25 control and 3/25 low dose F1 females. In 16 of the 21 cases of hypertrophy/hyperplasia observed in the high dose F1 females, minimal or slight (multi)focal accumulation of flaky colloid was observed in the lumen of the follicles. This was also observed in 12 of the mid dose F1 females (unclear if all 10 animals with hypertrophy/hyperplasia were included in this group of 12). . The effects to the thyroid are considered to be treatment-related general toxicity. A reproductive NOAEL of 1,000 mg/kg was established by the authors for this reproductive toxicity based on no adverse effects on fertility or reproduction after treatment with Hexamoll® DINCH®. A general toxicity LOAEL of 300 mg/kg/day was identified by ToxServices based on hypertrophy/hyperplasia observed in the thyroid follicular epithelia of animals in the 300 and 1,000 mg/kg/day groups.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M or L): L
Hexamoll® DINCH® was assigned a score of Low for developmental toxicity based on three studies that showed this substance did not induce developmental toxicity at doses up to 1,200 mg/kg/day.

- REACH 2010 –
    - A GLP-compliant Developmental Toxicity/Teratogenicity study (OECD 414) was conducted using female Wistar rats (25/group). Rats were administered oral doses of 0, 200, 600, or 1,200 mg/kg of Hexamoll® DINCH® (99.7% purity) via gavage on days 6 through 19 of gestation. Maternal examinations included: cage side observations; detailed clinical observations; body weight; food consumption; post-mortem examinations; ovaries and uterine content. Fetal examinations included: external examinations; soft tissue examinations; skeletal examinations; and head examinations. No adverse maternal or embryotoxic/teratogenic effects were reported by the authors. A maternal and developmental NOAEL of 1,200 mg/kg/day was reported by the study authors.
    - A GLP-compliant Developmental Toxicity/Teratogenicity study (OECD 414) was conducted using female Himalayan rabbits (25/group). Rabbits were administered oral doses of 0, 100, 300, or 1,000 mg/kg of Hexamoll® DINCH® (99.6% purity) in feed on days 6 through 29 of gestation. Maternal examinations included: cage side observations; detailed clinical observations; body weight; food consumption; post-mortem examinations; ovaries and uterine content. Fetal examinations included: external examinations; soft tissue examinations; skeletal examinations; and head examinations. No adverse maternal or embryotoxic/teratogenic effects were reported by the authors. A maternal and developmental NOAEL of 1,000 mg/kg/day was reported by the study authors.

  - A GLP-compliant pre-/postnatal developmental study performed according to OECD 414/TG 415 was completed with pregnant female Wistar rats administered oral doses Hexamoll® DINCH® (99.7% purity) of 0, 750, or 1,000 mg/kg/day in olive oil via gavage on gestational day 6 to postnatal day 20. The pups were then raised until postnatal days 100-105 without additional exposure and evaluated for sexual maturation and development including anogenital distance. No maternal toxicity was observed with treatment. No treatment-related effects were observed in clinical examinations, organ weights, sexual maturation, gross and histopathological findings, or sperm motility in the pups. A statistically significant decrease of 7-8% compared to controls was observed for anogenital distance (AGD) in the high-dose males and for anogenital index (AGI) for high dose males and females. No effects on testes descent, vaginal opening, balanopreputial separation, or sperm motility were observed. The maternal NOAEL was determined to be 1,000 mg/kg/day based on the lack of treatment-related effects at the highest dose tested and a fetal LOAEL of 1,000 mg/kg/day was established based on the decreased AGD in male offspring and decreased AGI observed in male and female offspring of dams in the high dose group.
- Additional developmental toxicity studies have been performed by BASF. Although BASF provided documentation of these reports under a Non-Disclosure Agreement, there was insufficient time to evaluate the content and quality of these studies for inclusion in this GreenScreen™ Assessment. The studies listed above are of sufficient quality to demonstrate the safety of Hexamoll® DINCH® with regards to developmental toxicity, as they were performed according to international standards (OECD 414), were GLP-compliant, and produced NOAELs equal to or greater than 1,000 mg/kg/day.

Endocrine Activity (E) Score (H, M or L): M

Hexamoll® DINCH® has been assigned a moderate hazard rating for endocrine activity based on the decreased AGD in male offspring and decreased AGI observed in male and female offspring in a developmental study and on the effects observed on the thyroid glands in several studies. A score of Moderate is assigned when there is evidence for endocrine activity (CPA 2011a). .

- Not listed as a potential endocrine disruptor on the EU Priority List of Suspected Endocrine Disruptors.
- Not listed as a potential endocrine disruptor on the OSPAR List of Chemicals of Possible Concern.
- Not listed as a potential endocrine disruptor on the Red List of Chemicals (CPA 2011b).
- REACH 2010 –
  - In the GLP-compliant pre-/postnatal developmental study performed in Wistar rats according to OECD 414/TG 415 summarized in the Developmental Toxicity section above, statistically significant decreases in the anogenital distance (AGD) of high-dose male pups and the anogenital index (AGI) of high-dose male and female pups were observed compared to controls. However, the effects on the AGI occurred in both male and female pups, contradicting any potential impaired androgen-mediated development of the male reproductive system as a result of exposure, and there were no other effects on corresponding markers of sexual development or maturation, including testes descent, vaginal opening, balanopreputial separation, and sperm motility. A LOAEL of 1,000 mg/kg/day was established for this study based on effects to the AGD and AGI on high dose offspring.
  - In the 90-day oral repeat dose toxicity study performed in Wistar rats according to OECD 408, male and female rats (20 per sex per dose group) were administered diets containing 0, 1,500, 4,500, or15,000 ppm (equivalent to 0, 107.1, 325.7, and 1,102.9 mg/kg/day for males and 0, 128.2, 389.4, and 1311.8 mg/kg/day for females, respectively) Hexamoll® DINCH® (99.6% purity) for 90-days (See the Systemic Toxicity/Organ Effects – Repeat dose section below for more details on this study). Significant increases in serum levels of thyroid stimulating hormone (TSH) were observed in high dose females after 30, 62, and 91 days of exposure. A trend of increased serum TSH concentrations was also observed in other treated males and females but these differences were not statistically significant and no dose-response relationship was observed for TSH concentrations in treated males. The absolute weights of the thyroid glands increased in males of the low and high dose groups by 12.4% and 20.8%, respectively. The relative weights of the thyroid glands increased in males of the high dose group by 20% and decreased in females of the low dose group by 14.3% compared to controls. Minimal to slight (grade 2) hypertrophy/hyperplasia of the thyroid follicular epithelia was observed in the control group and all treatment groups. The incidence for males was 2/20, 14/20, 11/20, and 16/20 for control, low, mid, and high dose groups respectively. The incidence for females was 1/20, 1/20, 3/20, and

15/20 for the control, low, mid, and high dose groups respectively. No morphological alterations were observed that could account for the decrease in relative thyroid weight in low dose females.

- In the 2-year combined chronic toxicity/carcinogenicity study performed in Wistar rats according to OECD 453, male and female rats (50 per sex per dose group for the main group, 10 additional animals for the satellite group) were administered oral doses of 40, 200, or 1,000 mg/kg/day Hexamoll® DINCH® (99.6% purity)via the diet for two years for the main group or 12 months for the satellite group (See the Systemic Toxicity/Organ Effects – Repeat dose section below and the Carcinogenicity section above for more details on this study). Absolute thyroid gland weights increased by 68.9% and 52.4% in males of the mid and high dose groups, respectively, and increased by 70.4% in females in the high dose group. Relative thyroid gland weights increased by 71.4% and 42.9% in males of the main mid and high dose groups, respectively, and increased by 55.6% in females in the main high dose group. In the satellite group, absolute thyroid gland weight decreased by 18.5% in females of the low dose group. The number of male animals with enlarged thyroid glands was 1, 0, 8, and 9 individuals in the main control, low, mid and high dose groups, respectively. The number of female animals with enlarged thyroid glands was 2, 1, 2, and 2 individuals in the main control, low, mid, and high dose groups, respectively. The number of thyroid massed in male rats was 3, 1, 3, and 2 for the main control, low, mid, and high dose groups, while that for female rats was 0, 1, 1, and 4 for the main control, low, mid, and high dose groups. In the satellite group, altered colloid in the thyroid gland was observed in 5, 3, and 8 females administered the low, mid and high doses, respectively compared to 0 control females displaying altered colloid. No data was presented for the incidence of altered colloid in male rats. Follicular cell hyperplasia was observed in the thyroid glands of 8, 6, 9, and 15 male rats and 3, 4, 5, and 14 female rats in the main control, low, mid, and high dose groups, respectively. Thyroid gland adenomas were observed in 3, 5, 11, and 14 male rats and 1, 3, 3, and 9 female rats of the main control, low, mid, and high dose groups, respectively. The effects on the thyroid observed in this study correlated with hepatic enzyme induction as demonstrated by increased serum γ–glutamyltransferase activity in high dose females on days 181 and 357 and increased alkaline phosphatases in high dose males on day 359, and by decreased bilirubin concentrations in high dose males on day 182 and 359 and high dose females on days 97, 181, and 357. Based on the negative results obtained in mutagenicity and genotoxicity tests (See the Mutagenicity/Genotoxicity section above), the adenomas and follicular cell hyperplasia of the thyroid can be attributed to indirect, non-genotoxic mechanisms of thyroid toxicity (EFSA 2006).
- In a two-generation reproductive toxicity study conducted according to OECD 416, Wistar rats (25 per sex per dose) were administered oral doses of 0, 100, 300, or 1,000 mg/kg/day Hexamoll® DINCH® via the diet for 38 weeks (See the Reproductive Toxicity section above for more details on this study). Treatment with Hexamoll® DINCH® did not produce decreased fertility or reproductive performance of the F0 or F1 parental generations. The relative thyroid weights of high dose F1 females increased by 11.1% over controls. Minimal to slight hypertrophy/hyperplasia of the thyroid follicular epithelia was observed in 21/25 high dose F1 females, minimal to slight hypertrophy/hyperplasia was observed in 10/25 mid dose F1 females, and slight hypertrophy/hyperplasia was observed as a spontaneous finding in 3/25 control and 3/25 low dose F1 females. In 16 of the 21 cases of hypertrophy/hyperplasia observed in the high dose F1 females, minimal or slight (multi)focal accumulation of flaky colloid was observed in the lumen of the follicles. This was also observed in 12 of the mid dose F1 females (unclear if all 10 animals with hypertrophy/hyperplasia were included in this group of 12). Hexamoll® DINCH® did not produce developmental toxicity in the progeny of the F0 or F1 parents and had no negative impacts on sexual maturation.

Hexamoll® DINCH™ was assigned a score of Moderate for endocrine activity based on a full review of confidential 90-day, reproductive, and developmental toxicity studies provided by BASF under a Non-Disclosure Agreement. For a discussion of this review, please refer to Appendix B.

Group II and II* Human Health Effects (Group II and II* Human)

Note: Group II and Group II* endpoints are distinguished in the v 1.2 Benchmark system. For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints and test data for single or repeated exposures may be used. If data exist for single OR repeated exposures, then the endpoint is not considered a data gap. If data are available for both single and repeated exposures, then the more conservative value is used.

Acute Mammalian Toxicity (AT) Group II Score (vH, H, M or L): L

Hexamoll® DINCH® was assigned a score of Low for acute mammalian toxicity based on oral and dermal LD_{50} values being greater than 2,000 mg/kg, the cut off for low acute mammalian toxicity (CPA 2011a).

- REACH 2010 –
  - *Oral* LD_{50} (Wistar rat) >5,000 mg/kg (GLP-Compliant, OECD 423)
  - *Dermal* LD_{50} (Wistar rat) > 2,000 mg/kg (GLP-Compliant, OECD 402)

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST)

Group II Score (single dose: vH, H, M or L): L

Hexamoll® DINCH® was assigned a score of Low for systemic toxicity/organ effects (single exposure) based on the lack of clinical signs or gross pathological findings indicting organ toxicity during the acute oral and dermal toxicity experiments.

- REACH 2010
  - No abnormal clinical signs or gross pathology were observed in Wistar rats administered an oral dose of 5,000 mg/kg in a GLP-compliant OECD 423 acute oral toxicity study or a dermal dose of 2,000 mg/kg in a GLP-compliant OECD 403 acute dermal toxicity study, although 4 males and 2 females exhibited very slight erythema and 1 male and 3 females exhibited well-defined erythema only on day 1 of the dermal toxicity study.

Group II* Score (repeated dose: H, M, L): L

Hexamoll® DINCH® was assigned a score of Low for systemic toxicity/organ effects (repeated exposure) based on reported LOAELs above 100 mg/kg/day in a 90-day study and a two-year combined chronic toxicity/carcinogenicity study and above 300 mg/kg-bw/day (after application of a 3-fold adjustment for studies less than 90 days in duration according to Section 3.9.2.9.5 of the GHS Guidance (UN 2011)) for a 28-day study. A score of Low is assigned when the LOAEL exceeds 100 mg/kg/day (CPA 2011a).

- REACH 2010 -
  - A GLP-compliant 90-Day Oral Toxicity Study (OECD 408) was conducted using male and female Wistar rats (20/sex/group). Rats were administered oral doses of 0, 107.1, 325.7, or 1,102.9 mg/kg for males, and 0, 128.2, 389.4, or 1,311.8 mg/kg (99.6% purity) for females, in feed daily for 90 days. Examinations performed include: cage side observations; clinical observations; body weight; food consumption; food efficiency; ophthalmoscopic examinations; hematology; clinical chemistry; urinalysis; neurobehavioral examination; gross pathology; and histopathology. Food consumption was significantly increased in females on days 28 (5.4%), 56 (5.5%) and 77 (6.5%). Due to the limited occurrence, and lack of a dose-response, this was not considered a treatment-related effect. Statistically increased prothrombin times were observed in mid and high dose males. However, on days 30 and 91 of the study this effect was not observed, and no female rats showed increased prothrombin times, therefore this was not considered a treatment-related effect. Enzyme examinations showed a significantly decreased aspartate aminotransferase activity in mid and high dose males on day 91. However, values were within historical ranges of the laboratory and were reported to be due to higher values in the control group. In female rats, γ-glutamyltransferase activities were found to be increased in the high dose group. This effect was considered to be treatment related. Increased blood was found in the urine specimens of mid and high dose males. An increased number of abnormal transitional epithelial cells were also detected in the urine sediments of mid and high dose males. After days 30, 62 and 91 significantly increased concentrations of thyroid stimulating hormone (TSH) were found in the serum of high dose females. No effects were found during neurobehavioral assessments. In male rats absolute weight of the thyroid gland was significantly increased in the high (20.8%) and low (12.4%) dose groups. In high dose females significantly increased (11.8%) absolute liver weights and relative kidney weights (8-10%) were observed. In high dose males mean relative liver (5.9%), kidney (9.7%),

testes (4.2%), spleen (9.3%) and thyroid gland (20%) weights were significantly increased. Mid dose males also had significantly increased relative liver (5.7%), kidney (6.7%), testes (6.8%), and spleen (6.8%) weights. Mean relative kidney (8.4%) and testes (5.6%) weights were increased in low dose males. In female rats, relative liver weights were significantly increased in the mid (5.7%) and high dose (13.2%) groups. Significantly decreased (14.3%) relative weight of the thyroid glands were observed in low dose females. Increased kidney weights and kidney effects in male rats were determined by the authors to be due to increased α2μ-globulin accumulation and therefore not relevant to human exposure. However, in the Australian chemical assessment it was determined that sufficient evidence was not provided to rule out adverse kidney effects based on findings of kidney weight changes in both sexes (NICNAS 2012). Thyroid gland hypertrophy/hyperplasia of the follicular epithelia was recorded in males (2/20, 14/20, 11/20, 16/20 in the control, low, mid, and high dose groups, respectively) and females (1/20, 1/20, 3/20, and 15/20 in the control, low, mid, and high dose groups, respectively) (no statistical significance provided). The LOAEL values were identified by the study authors as 325.7 and 1,311.8 mg/kg/day for male and female animals, respectively.

- A GLP-compliant combined chronic toxicity/carcinogenicity study (OECD 453) was conducted using male and female Wistar rats (50/sex/group). Rats were administered oral doses of 0, 40, 200, or 1,000 mg/kg of Hexamoll® DINCH® (99.6% purity) daily in the diet for 24 months. Examinations included: cage side observations; detailed clinical observations; body weight; food consumption; food efficiency; ophthalmoscopic examination; hematology; clinical chemistry; urinalysis; and gross pathology. The only adverse treatment-related effects reported were an increase in platelets in female rats of the 1000 mg/kg group, and an increase in mean absolute liver weights in the mid and high dose groups of males and high dose group of females. An increase in the mean absolute thyroid gland weights in males of the mid and high dose groups and high dose groups was measured. The LOAEL for non-carcinogenicity endpoints was determined to be 200 mg/kg/day for females and the NOAEL was 40 mg/kg/day for males.
- A GLP-compliant subacute 28-day oral toxicity study (OECD 407) was conducted using male and female Wistar rats (3/sex/dose). Animals were administered oral doses (99.7% purity) of 64, 318, or 1,585 mg/kg/day for males and 66, 342, or 1,674 mg/kg/day for females in feed daily for 28 days. Examinations performed include: cage side observations; clinical observations; body weight; food consumption; food efficiency; water consumption; hematology; clinical chemistry; urinalysis; neurobehavioral examination; gross pathology; and histopathology. No treatment related clinical or haematological effects were observed at any dose level. Body weight, food consumption, and food efficiency were normal. At the end of the administration period slightly increased γ-glutamyltransferase activities were found in the sera of the females of high dose group. After cessation of test compound administration γ-glutamyltransferase activities in the high dose females returned to normal. No treatment-related changes were observed in the other enzyme determinations, particularly in the cyanide-insensitive palmitoyl-CoA-oxidation. Blood chemistry examinations revealed significantly increased sodium concentrations in the males of mid and high dose groups and increased potassium levels in all treated males at the end of the administration period. In the females of high dose group reduced total bilirubin concentrations were detected after 4 weeks of test substance administration. After withdrawal of the test compound all changes seen in blood chemistry parameters recovered within 2 weeks. No toxicologically relevant changes were seen in the other blood chemistry parameters. Male animals of the high dose group excreted increased numbers of degenerated epithelial cells. No treatment-related changes were observed in the other urine parameters. The increase in degenerated epithelial cells in the urine specimens of the males of the high dose group normalized within 2 weeks after withdrawal of the test compound. There were no statistical significant organ weight changes however; a decrease of the heart weight was noted in the low, mid and high dose groups (approx. 8 %). The macroscopic finding "Erosion/ulcer" of the glandular stomach was recorded for two male animals of low dose group. One ovarian cyst was noted unilaterally in one female animal of mid dose group. All lesions noted are seen to be incidental or spontaneous in origin and not related to treatment. Based on these changes, the LOAEL values were determined to be 1,585 mg/kg/day for males and 1,674 mg/kg/day for females.

Neurotoxicity (N)
Group II Score (single dose: vH, H, M or L): L
Hexamoll® DINCH® has been assigned a score of Low for neurotoxicity (single dose) based on the lack of adverse clinical signs of neurotoxicity during oral and dermal acute administration of Hexamoll® DINCH® to Wistar rats. Negative neurotoxicity data for single dose exposures correspond to a low hazard score (CPA 2011a).

- Not classified as a developmental neurotoxicant (Grandjean and Landrigan 2006).
- Not listed as a potential neurotoxicant on the Red List of Chemicals (CPA 2011b).
- REACH 2010 –
    - No abnormal clinical signs were observed in Wistar rats administered an oral dose of 5,000 mg/kg or a dermal dose of 2,000 mg/kg. As these acute toxicity studies were conducted according to OECD 402 or 423, the clinical signs include observation for signs of toxicity associated with the autonomic and central nervous system, somatomotor activity and behavior patterns (OECD 1987, 2001). Therefore no, neurotoxicity was identified based on external observation of animals administered an oral dose of 5,000 mg/kg or a dermal dose of 2,000 mg/kg.

Group II* Score (repeated dose: H, M, L): L
Hexamoll® DINCH® was assigned a score of Low for neurotoxicity based on the results from a repeat dose study that reported no adverse neurobehavioral effects.

- Not classified as a developmental neurotoxicant (Grandjean and Landrigan 2006).
- Not listed as a potential neurotoxicant on the Red List of Chemicals (CPA 2011b).
- REACH 2010 –
    - A neurobehavioral analysis was conducted as part of the GLP-compliant repeated oral dose toxicity study (OECD 408) reported above. No adverse effects were reported following administrations of up to 1,102.9 or 1,311.8 mg/kg of Hexamoll® DINCH® in males and females, respectively.

Skin Sensitization (SnS) Group II* Score (H, M or L): L
Hexamoll® DINCH® was assigned a score of Low for skin sensitization based on negative results in guinea pigs.

- REACH 2010 –
    - A GLP-compliant Skin Sensitization study (OECD 406) was conducted using female Hsd Poc: DH (SPF) Guinea Pigs (n=10, 5 control). Hexamoll® DINCH® (99.7% purity) was administered in three applications: Induction of 5% in olive oil or in Freund's adjuvant/0.9% aqueous NaCl-solution 1:1 intradermal; Percutaneous induction: undiluted test substance; Challenge dose of 50% in olive oil, occlusive, epicutaneous. After the challenge dose no skin reactions were observed in either the control or tested groups after 24 and 48 hours. Hexamoll® DINCH® was determined to be non-sensitizing under the test conditions.

Respiratory Sensitization (SnR) Group II* Score (H, M or L): DG
Hexamoll® DINCH® has been assigned a data gap for respiratory sensitization.

- No relevant data identified for this compound.

Skin Irritation/Corrosivity (IrS) Group II Score (vH, H, M or L): M
Hexamoll® DINCH® was assigned a score of Moderate for skin irritation/corrosivity based on reversible, but well-defined erythema that persisted for 48 hours. Based on GHS criteria, a substance that produces mild irritation (defined as a mean value greater than 1.5 but less than 2.3 for erythema) in 2 of the 3 animals treated is classified as a Category 3 substance (UN 2011). GHS Category 3 skin irritants are assigned a hazard score of moderate (CPA 2011a).

- REACH 2010 –
    - A GLP-compliant Acute Dermal Irritation/Corrosion study (OECD 404) was conducted using New Zealand White rabbits (n=3, sex not reported). 0.5 ml of Hexamoll® DINCH® (99.94% purity) was applied under semi-occlusive coverage to the dorsolateral part of the trunk for 4 hours. Following treatment rabbits were observed for 14 days. At 48 hours following treatment, all animals showed well defined erythema, illustrated by an erythema score of 2 out of a max score of 4. 72 hours post treatment, two of the three treated animals were noted as having very slight erythema (barely perceptible), while the third had well defined erythema which improved to slight erythema by day 7.

All reactions had cleared by day 14. The mean erythema score for all 3 test animals over 14 days was 1.8. No edema was observed in any of the animals. The test substance was classified as non-irritating to the skin following EU classification.

Eye Irritation/Corrosivity (IrE) Group II Score (vH, H, M or L): L
Hexamoll® DINCH® was assigned a score of Low for eye irritation/corrosivity based on this substance not being classified as a GHS Category 1 or 2 eye irritant. Based on GHS criteria, a Category 2 compound is one with a mean conjunctival redness score greater than 2 (UN 2011).

- REACH 2010 –
    - A GLP-compliant Acute Eye Irritation/Corrosion study (OECD 405) was conducted using Himalayan rabbits (n=3, sex not reported). 0.1 ml of Hexamoll® DINCH® (99.7% purity) was instilled into one eye for 24 hours and rinsed with tap waters. Following treatment rabbits were observed for 72 hours. Twenty-four hours following administration, rabbits displayed very slight conjunctival erythema, defined as ocular vessels definitely injected above normal and illustrated by an erythema score of 1 out of a max score of 3. Symptoms cleared within 48 hours. No other signs of ocular irritation, such as chemosis or discharge, were observed. The average score for conjunctival redness was 0.3. Hexamoll® DINCH® was classified as non-irritating to the eyes following EU classification.

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M or L): L
Hexamoll® DINCH® was assigned a score of Low for acute aquatic toxicity based on L/EC_{50} values greater than 100 mg/L, the cut off for low acute aquatic toxicity (CPA 2011a).

- NICNAS 2012 –
    - Hexamoll® DINCH® has the following acute aquatic toxicity values:
        - 96-hour LC_{50} (*Brachydanio rerio*, zebrafish) >100 mg/L, performed according to EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – 96 hour, static.
        - 48-hour immobilization EC_{50} (*Daphnia magna*) >100 mg/L, performed according to OECD TG 202 Daphnia sp. Acute Immobilization Test and Reproduction Test part 1 - 48 hour, static.
        - 72-hour biomass EC_{50} (*Scenedesmus subspicatus*, algae) >100 mg/L, performed according to OECD TG 201 Alga, Growth Inhibition Test, EC Directive 92/69/EEC C.3 Algal Inhibition Test, and EPA OPPTS 850.5400 – Algal Toxicity, Tiers I and II
        - 72-hour growth EC_{50} (*Scenedesmus subspicatus*, algae) >100 mg/L, performed according to OECD TG 201 Alga, Growth Inhibition Test, EC Directive 92/69/EEC C.3 Algal Inhibition Test, and EPA OPPTS 850.5400 – Algal Toxicity, Tiers I and II
    - As these values are orders of magnitude above the water solubility of Hexamoll® DINCH® (<0.02 mg/L), no acute toxicity to aquatic organisms is expected at saturation.

Chronic Aquatic Toxicity (CA) Score (vH, H, M or L): L
Hexamoll® DINCH® was assigned a score of Low for chronic aquatic toxicity based on no effects at maximum water solubility.

- NICNAS 2012 –
    - The chronic aquatic toxicity of Hexamoll® DINCH® was evaluated in an OECD TG 211 *Daphnia magna* reproduction test. *Daphnia* were exposed to a maximum obtained water solubility of 0.021 mg/L for up to 21 days. No immobilization was observed at 14 or 21 days of treatment. Hexamoll® DINCH® has a reported NOEC value of ≥0.021 mg/L and is not expected to have any adverse effects to aquatic biota at water solubility limits.

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): M
Hexamoll® DINCH® was assigned a score of Moderate for persistence based on biodegradability tests indicating this substance has a half-life between 16 and 60 days, the range for moderate persistence (CPA 2011a).

- NICNAS 2012 –
    - A GLP-compliant ready biodegradation test (OECD 301 B "CO_2 Evolution Test") was conducted. Hexamoll® DINCH® was found not to be readily biodegradable with 60% biodegradation occurring in greater than 28 days. The amount of degradation was 4% after 7 days, 10% after 14 days, 27% after 21 days, 41% after 28 days, 64% after 38 days, 76% after 49 days, and 93% after 60 days. Hexamoll® DINCH® reaches 50% biodegradation between 28 to 38 days. It does not meet the 10-day window for biodegradation.

Bioaccumulation (B) Score (vH, H, M, L, or vL): L
Hexamoll® DINCH® has a measured bioconcentration factor (BCF) that falls between 100 and 500, the range for low bioaccumulation (CPA 2011a). This result is supported by data demonstrating rapid excretion of Hexamoll® DINCH® in rats and human volunteers

- NICNAS 2012 –
    - A GLP-compliant bioconcentration test (OECD 305) was conducted using zebra fish (*Brachydanio rerio)* exposed to 0.04-0.4 µg/L ^{14}C-radiolabeled Hexamoll® DINCH® for 14 days. Actual concentrations obtained via liquid scintillation counter measurements were 0.041-0.41 µg/L. A bioconcentration factor (BCF) of 189.3 was obtained.

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M or L): L
Hexamoll® DINCH® was assigned a score of Low for reactivity because on not having any chemicals or functional groups expected to contain high energy bonds or oxidizing species which may cause reactivity.

- Hexamoll® DINCH® would not be classified as an oxidizing chemical as it structure does not contain a halogen and oxygen atoms present are only bonded to carbon or hydrogen (UN 2011). In addition, Hexamoll® DINCH® is not expected be explosive as it does not contain structural groups that would cause concern for explosion. Furthermore, the high flashpoint (224°C) further supports that Hexamoll® DINCH® is not a reactive chemical.

Flammability (F) Score (vH, H, M or L): L
Hexamoll® DINCH® was assigned a score of Low for flammability based on not being classified as a GHS Flammable Liquid.

- NICNAS 2012 –
    - Hexamoll® DINCH® has a flash point of 224°C, which is above the 93°C cut-off criteria to be classified as a flammable liquid by GHS (UN 2011).

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APPENDIX A: Hazard Benchmark Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(Cr) Corrosion/ Irritation (Skin/ Eye)

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B: ToxServices Evaluation of Potential Endocrine Activity

ToxServices agrees that, for any phthalate ester or chemicals of similar molecular structure (Hexamoll® DINCH® has a cyclohexane ring in place of the aromatic benzene ring present in phthalate esters), evaluating anti-androgenic effects has become a key focus. ToxServices therefore appreciated the opportunity to review the completed reproductive and developmental toxicity studies conducted on Hexamoll® DINCH®. These studies are classified as "confidential business information". In reviewing these reports, we focused particularly on the following aspects:

- Specific endpoints evaluated: phthalate esters are known to affect certain endpoints, including, for example, anogenital distance (AGD), sperm parameters, male sexual maturation (e.g., testes descent and preputial separation), and male reproductive organ weights.
- Evidence of GLP compliance, such as a quality assurance statement and record of audits.
- Presentation of data: mean, standard deviation/error, and statistical analysis of critical endpoints (such as those listed above). Inclusion of historical control data, when appropriate.
- Discussion of results, specifically the degree of agreement between the text and the actual data tables and the degree to which the actual data support characterization of an effect as related or not related to test article administration.

Of particular importance in determining the possible presence of endocrine activity were the 90-day subchronic study, a two-generation reproduction study, and a developmental study. As asserted by EPA and the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), this is the most robust means by which to evaluate effects on endocrine active endpoints.

Potential Thyroid Effects

In vivo data suggest that Hexamoll® DINCH® may cause thyroid effects. In a 90-day study with Hexamoll® DINCH®, animals were treated with:

1. Hexamoll® DINCH®
2. Propylthiouracil
3. Phenobarbital

The mechanism/mode of action for thyroid effects for both phenobarbital and propylthiouracil has been well-characterized and are summarized below.

Propylthiouracil: This chemical acts directly on the thyroid by inhibiting iodine uptake. Without iodine, thyroid hormone diminishes, resulting in compensatory up-regulation of thyroid-stimulating hormone (TSH) and the subsequent mitogenic effect on the thyroid.

Phenobarbital: induces xenobiotic metabolizing enzymes in the liver, including cytochrome P450 isozymes (e.g., CYP2B) and Phase II conjugating enzymes (e.g., uridine diphosphate-glucuronosyl transferase, or UGT), which are localized to the endoplasmic reticulum in the cell. Phenobarbital-mediated induction of UGT results in increased excretion of thyroid hormone in the rat, thereby activating homeostatic compensatory mechanisms. This results in increased secretion of TSH from the pituitary. This has a mitogenic effect on the cells of the thyroid that ultimately results in a neoplastic effect in rodents. This is an example of how an effect in the liver results in an effect on a distal organ. Induction of thyroid tumors in rats by this particular mechanism is of questionable human relevance due to differences in thyroid hormone turnover and homeostasis between rats and humans (cf. Casarett and Doull's; EPA's thyroid guidance).

The state of the available data does not support the conclusion that thyroid effects are due to peroxisome proliferation. Rather, as explained for phenobarbital, thyroid effects can occur due to proliferation of the endoplasmic reticulum (i.e., microsomes) and induction of xenobiotic metabolizing enzymes therein. The specific enzymes in the liver that underlie the phenobarbital-mediated alterations in thyroid hormone status, the shifts in homeostasis, and the consequent microscopic changes in the thyroid are microsomal, not peroxisomal. Peroxisomes contain enzymes that are responsible for fatty acid oxidation and neutralization of oxidative species such as hydrogen peroxide. Although they are indeed cytochrome P450 isozymes, they are not the enzymes responsible for

xenobiotic metabolism in general or thyroid hormone metabolism specifically. Although Hexamoll® DINCH® may indeed affect peroxisomal enzymes, there is no established relationship between these types of enzymes and alterations in thyroid hormone status. Increased peroxisomal enzymes (due to organelle proliferation) would not affect thyroid hormone, based on the current state of knowledge about how thyroid hormone is metabolized. If there is a relationship between the liver effects and the thyroid effects of Hexamoll® DINCH®, it may be due to induction of thyroid hormone metabolism, as in the case of phenobarbital. It is equally possible that Hexamoll® DINCH®, and related materials directly affect the thyroid. The available data (consisting of organ weight and histopathology) cannot differentiate between a direct or indirect effect of Hexamoll® DINCH® on the thyroid. It is highly unlikely that the effect is due to peroxisome proliferation.

For these reasons, ToxServices does not agree with the conclusion that the effects seen in the thyroid are secondary to a peroxisome proliferation effect in the liver. Rather, the two findings co-occur but develop through different mechanisms.

Potential Anti-Androgenic Effects

ToxServices disagrees with the conclusion that an increase in female pup AGD as well as male pup AGD reported as part of a BASF developmental toxicity study indicates lack of anti-androgenic activity. Our review of data from a confidential BASF developmental toxicity study indicates that female AGD was not significantly affected by Hexamoll® DINCH®; rather, female anogenital index (AGI) was decreased. The magnitude of the AGI decrease among females was small, at only a few percent, and the apparent effect was likely exaggerated due to the slight increase in body weight among these female pups. Thus, the significant change in AGI is likely driven by the very slight increase in female pup body weights combined with the very slight but not significant decrease in AGD. Moreover, the reported reduction in female AGI does not reduce the validity of the male AGD findings; indeed, in 2005 Piepinbrink et al. reported that AGD was affected in neonatal female rats exposed *in utero* to di-ethylhexyl phthalate (DEHP). Unfortunately, AGD and AGI data were not included in the two-generation study report reviewed by ToxServices, so it was not possible to determine if an effect occurred in the two generation study.

Conclusion

The availability of study reports for Hexamoll® DINCH® considerably reduces ToxServices concern over data gaps. However, concern remains due to what appears to be unjustified dismissal of potentially relevant effects in reproductive/developmental and thyroid endpoints. The data for Hexamoll® DINCH® suggest that endocrine activity may be occurring; thus, a GreenScreen™ classification of Moderate is warranted for the endpoint of endocrine activity.

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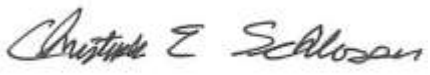
Original Hexamoll® DINCH ® (Version 1.0) GreenScreen™ Evaluation Prepared By:



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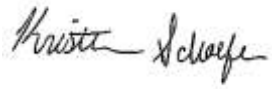
Hexamoll® DINCH® Updated to GreenScreen™ Version 1.2 By:



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